

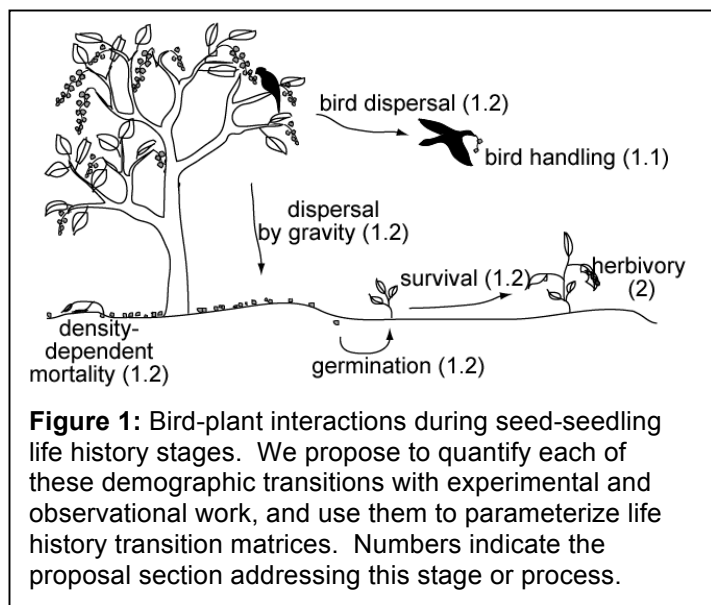
## **Project Description**

### **Background and Introduction**

One of the most important debates in ecology concerns the relative importance of mechanisms promoting coexistence in plant communities (Pianka 1966, MacArthur 1969, Levins and Culver 1971, Grime 1977, Huston 1979, Paine 1984, Menge and Sutherland 1987, Tilman 1994). Much of the contemporary discussion focuses on within-trophic level interactions (Hubbell 2001, Tilman et al. 2001); however, some have argued that this debate lacks generality, as it often takes place without explicit consideration of the role of interactions between trophic levels in regulating the abundance and diversity of plant communities (Paine 2002). Animals in upper trophic levels can exert top down control of plant recruitment in several ways, including seed dispersal (Harms et al. 2000) and consumption of herbivores or grazers (Hairston et al. 1960). For example, sea otter presence indirectly increases kelp forest productivity (Estes and Palmisano 1974), armadillos indirectly control the recruitment of trees by predating upon leaf-cutter ants (Terborgh et al. 2006) and wolves indirectly mediate diversity in cottonwood groves by consuming elk (Beschta 2005). These studies clearly show that mammals can exert strong top-down control on the structure and diversity of plant communities.

Despite the fact that birds are among the best-studied vertebrate groups in the world, disperse fruit from many tree species (Stiles 1985), and are important consumers of insect herbivores (Fig. 1), we lack flagship examples of top down control by birds analogous to the sea otter, starfish and wolf. Our current understanding of the role that birds play in structuring plant communities comes primarily from either highly focused studies examining the importance of one or a few bird species, typically in reference to a single plant species (Howe and Kerckhove 1980, Stocker and Irvine 1983, Wenny and Levey 1998), or studies that use branch or plant-level exclosures to examine the influence of the bird community on one species of plant, typically by measuring levels of herbivore damage (Greenberg et al. 2000, Van Bael et al. 2003, Mooney 2007) or plant growth rates (Strong et al. 2000, Lichtenberg and Lichtenberg 2002, Gruner 2004, Mooney and Linhart 2006). These studies, taken together, suggest that birds may play a profound top-down role in regulating plant community structure, just as sea otters, wolves and starfish do, but their full impacts on forest systems are not yet understood.

A large part of the reason we lack a synthetic understanding of the importance of birds in forest systems is the difficulty involved in conducting community level research at the appropriate scale. All studies to date have examined the role of birds in a piecemeal fashion- looking either at their importance as seed dispersers or at their effects as consumers of herbivores, and most studies have focused on one or two species of plants. This is understandable, because a truly rigorous test of the importance of birds to a forest



community would require the wholesale removal of birds from an ecosystem, not something we are willing to do deliberately. This was the case with otters on the Pacific coast (Estes and Palmisano 1974), islands created by damming a lake in Venezuela (Terborgh et al. 2001, Terborgh et al. 2006), and wolves in Yellowstone (Beschta 2005). In these examples, the loss of species precipitated major unpredicted changes at an ecosystem level and provided a window into the importance of the species or functional group under study. In this proposed research, we take advantage of a similar 'natural experiment'; the functional extirpation of birds from the Pacific Island of Guam by the invasive Brown Tree Snake (Fig. 2).

Bird loss on Guam coupled with the presence of birds on nearby islands not invaded by the snake provides a rare opportunity to determine the extent to which birds influence the structure of a tropical forest. We will take a community approach by investigating the importance of the two most widespread ecosystem services provided by birds, seed dispersal and control of herbivory, focusing on the critical seed-seedling life history stage (Fig. 1) for ten of the most common tree species on the Mariana islands. Our study will provide the first synthetic perspective on the importance of top-down control of forest community composition and diversity by birds through two major pathways, seed dispersal and consumption of herbivores.

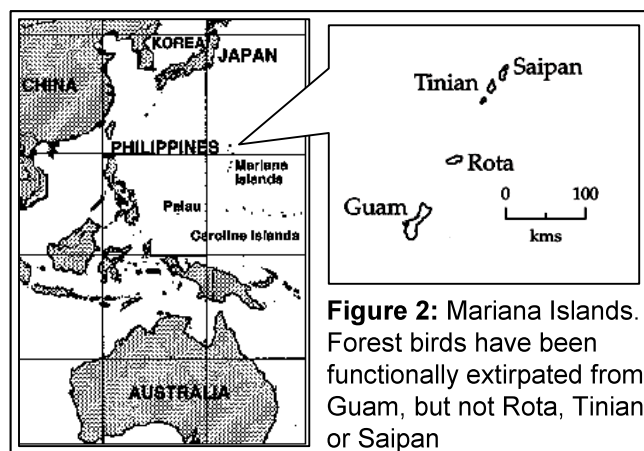
Understanding the importance of birds in terrestrial ecosystems is not simply an academic exercise: greater than 25% of bird species in the US are facing the threat of extinction (National Audubon Society 2007b), and many common species have experienced declines of 50-80% since 1967 (National Audubon Society 2007a). Current estimates suggest 7-25% of all bird species in the world and 28-56% of species on oceanic islands will be functionally extinct by the end of this century (Sekercioglu et al. 2004). Current bird declines and likely future extinctions make understanding the top-down role of birds for forest community structure more pressing now than ever.

Our study addresses the following three questions:

- 1: Does bird dispersal enhance seed germination or seedling growth?**
- 2: Do birds regulate herbivory on seedlings?**
- 3: Do birds control forest seedling community composition through their effects on dispersal and herbivory?**

### **Study system - The Mariana Islands and the Brown Tree Snake**

The Mariana Islands are a volcanic island archipelago east of the Philippines and south of Japan (Fig. 2). We are working on the four largest islands in the archipelago: Guam - (541 km<sup>2</sup>), Saipan (115 km<sup>2</sup>), Tinian (101 km<sup>2</sup>), and Rota (85 km<sup>2</sup>). The 11 other islands are north of Saipan, smaller, uninhabited and difficult to access. Guam, Saipan, Tinian and Rota are within 120 miles of each other and experience similar temperature and rainfall. Our four islands are dominated

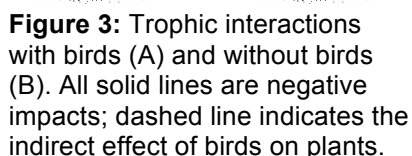


**Figure 2: Mariana Islands.** Forest birds have been functionally extirpated from Guam, but not Rota, Tinian or Saipan

We focus on the effects of birds on seed dispersal and not pollination, because less than 20% of native limestone forest tree species on Guam are bird-pollinated (Brooke 2007) while roughly 70% have fleshy fruits adapted for bird dispersal (Rogers and Tewksbury, unpublished data). These species are dispersed by six native frugivorous forest bird species (Marianas Fruit Dove, White-throated Ground Dove, Mariana Crow, Guam Rail, Micronesian Starling and Rufous Fantail). The remaining tree species are primarily dispersed by bats, gravity, or water. Wind-dispersal is rare to nonexistent in the native forest species, but at least one non-native species found in the forest is wind-dispersed (Table 1). Native top predators in the forest are primarily insectivorous birds, of which there are seven species (Bridled White-eye, Guam Rail, Mariana Crow, Island Swiftlet, Rufous Fantail, Guam Flycatcher and Micronesian Kingfisher). Forests in the Marianas, like other ecosystems with birds, have trophic webs with three to four levels; birds consume both insect herbivores and the predators and parasites of those herbivores (other insects and arachnids; Fig. 3).

Guam and Rota); we will minimize their effects by locating all experimental and observational studies in minimally-disturbed intact forests and by fencing out ungulates.

We propose to investigate the impacts of birds on forest community structure by determining the differential impact of frugivorous and insectivorous birds on the growth and recruitment of ten focal tree species, all of which occur on Guam, Saipan, Tinian and Rota. Our focal tree species include five native, bird-dispersed species, two exotic, bird-dispersed species, two native gravity-dispersed species, and one exotic, wind-dispersed species (Table 1). *Since these focal species comprise a large portion (62%) of the trees in limestone*



forests in the Mariana Islands (Rogers and Tewksbury, unpublished data), this approach allows us to speculate on the fate of the entire forest community.

We include several non-native species in our studies, because they are significant components of the forest community and may differentially respond to bird loss. We have selected focal tree species that are not pollinated by birds or bats in order to focus on avian seed dispersal and control of herbivory. Several focal species are likely dispersed by bats (*Premna*, *Neisosperma* and *Papaya*); however, bat populations have been severely reduced on all four islands (current estimates are: Guam- 40, Rota 200-400, Tinian- 25, Saipan- 25, Brooke pers. comm.), therefore we expect bat dispersal to have limited influence on all islands. The extent to which these focal species benefit from the insect control provided by birds is unknown, but this lack of knowledge largely mirrors our limited understanding of the importance of avian insect control to forests in general (Sekercioglu 2006). We focus on the seed to seedling transition because birds can affect this stage through impacts on dispersal and controlling herbivores; and plant success in this stage has a significant impact on forest community composition (Comita et al. 2007).

We will perform a series of experiments on all ten focal species on all four islands - Saipan, Tinian and Rota (with birds) and Guam (without birds). These manipulative experiments will allow us to determine the role that birds play in the seedling recruitment of focal species through their role as seed dispersers and predators of insect herbivores. We will also use comparisons of dispersal and herbivory on bird-free Guam with nearby bird-present islands to ask whether these experiments can predict the consequences of a real-life bird extinction event. Finally, we will use our data to determine the net effect of avian bird loss on the seedling recruitment of our focal species and ask whether dispersal mode, allocation to defensive compounds, and native/exotic status are predictive of the response of individual species to bird loss in the Mariana Islands. Taken together, these studies will inform conservation and management efforts on Guam and permit us to generalize results to other ecosystems experiencing bird loss.

## Methods and Preliminary Results

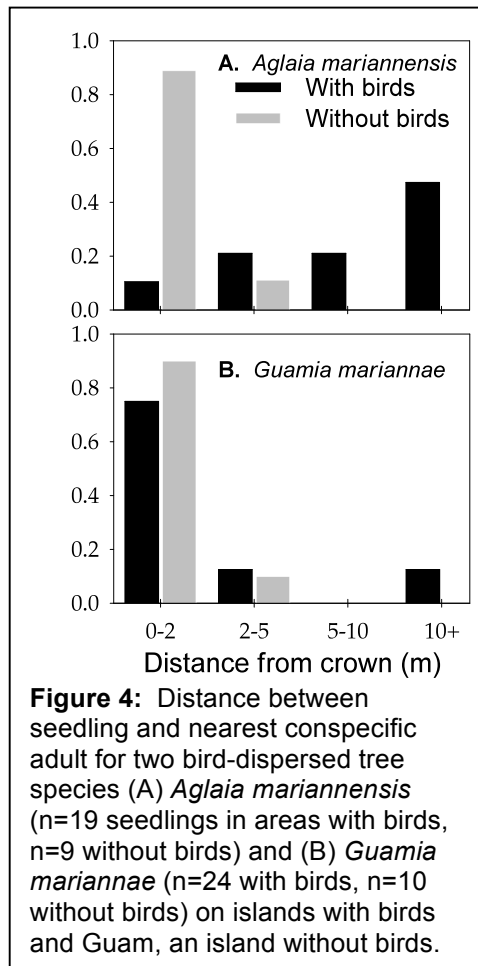
### 1: Does bird dispersal enhance seed germination or seedling survival and growth?

Frugivorous birds can impact the early life history stages of trees through handling fruit, which may increase germination rates (Traveset and Willson 1997, Traveset et al. 2001), and by transporting seeds to new areas, which allows these seeds to escape high density-dependent mortality near parent trees (Janzen 1970, Packer and Clay 2000, Hille Ris Lambers and Clark 2003, Freckleton and Lewis 2006). Studies focused on one (Bleher and Bohning-Gaese 2001, Cordeiro and Howe 2003) or two (Bleher and Bohning-Gaese 2001)

**Table 1:** Density (# adult trees/ha) of the 10 focal species. Data are based on 25x25 m plots on Guam (4) and in the Northern Mariana Islands (9). Stars (\*) indicate the presence of juveniles in at least one plot without a conspecific adult.

Status, Dispersal mode	Species	Adult Density	
		Guam	Northern Mariana Islands
Native, Bird.	<i>Guamia</i> ,	696	425
	<i>Aglaia</i>	916	289*
	<i>Psychotria</i>	8	228
	<i>Maytenus</i>	56	36*
	<i>Premna</i>	40	43*
Exotic, Bird	<i>Papaya</i> ,	101	75*
	<i>Triphasia</i>	320	0 <sup>a</sup>
Native, Gravity	<i>Cynometra</i> ,	232	1150
	<i>Neisosperma</i>	116	139*
Exotic, Wind	<i>Leucena</i>	235	80
All other species		2232	908*

<sup>a</sup> *Triphasia* occurs in the Northern Marianas, but was not found in the 9 plots sampled there.



**Figure 4:** Distance between seedling and nearest conspecific adult for two bird-dispersed tree species (A) *Aglaia mariannensis* (n=19 seedlings in areas with birds, n=9 without birds) and (B) *Guamia mariannae* (n=24 with birds, n=10 without birds) on islands with birds and Guam, an island without birds.

plant species have shown significant impacts caused by loss of frugivorous birds, including clumped spatial distributions and lower recruitment. Our preliminary results from the Marianas reveal a similar pattern (Fig. 4). On Guam, where birds have been extirpated, randomly selected seedlings of *Aglaia mariannensis*, a native bird-dispersed focal species, were found exclusively within 5 meters and primarily within 2 meters of a conspecific tree (likely parent), while on islands with birds, seedlings were often two to three times further from the nearest parent trees (t-test:  $p < 0.001$ , Fig. 4A). For *Guamia mariannae*, one of the most common tree species in limestone forest, seedlings are often no more than 2m from an adult tree on all islands due to sampling constraints (Fig. 4B). However, the only seedlings found more than 5 meters away were on islands with birds. Both examples are consistent with the theory that bird loss leads to shorter dispersal distances and more clumped spatial patterns. To determine the importance of avian seed handling and seed dispersal for the recruitment of all 10 focal species, we will first quantify the impacts of bird handling on seed germination, and then combine estimates of bird-dependent seed dispersal with density-dependent seed and seedling survival.

#### 1.1: Effects of bird handling on germination rates

We will determine the effect of bird handling on germination on Saipan, where plant growing facilities and the presence of birds make germination trials possible. We will conduct these experiments only on bird-dispersed species (probably 7 species), after confirming the primary dispersal mode of all species through video observation of ripe fruits on focal trees (Levey et al. 2006). There are three potential fates for a fruit developing on a bird-dispersed tree species: 1) fall from tree untouched by birds, 2) be removed from tree by birds, and 3) remain on tree. We compare germination of seeds in category 1 to category 2 to determine bird impacts on germination. Seeds in category 3 are not handled by birds and are likely unripe. We assume that the proportion of seeds that fall into this category does not depend on the presence of birds, and therefore do not include seeds from this category in our experiment.

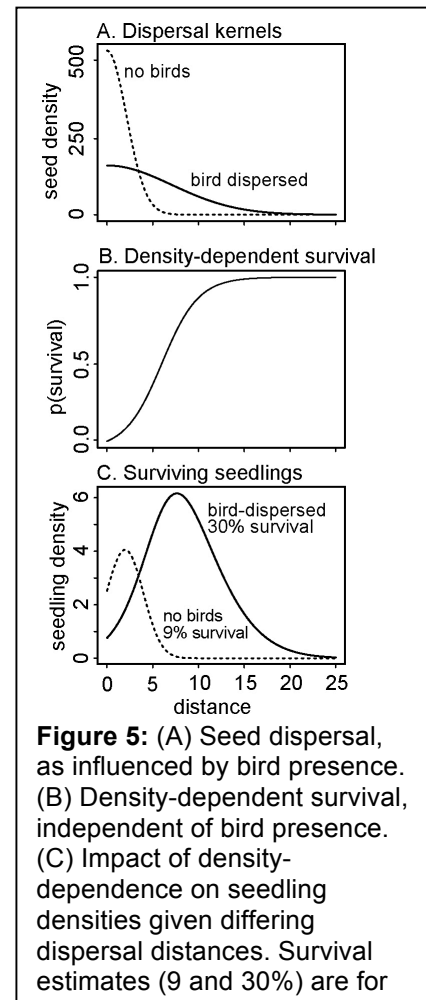
To collect seeds, we will put netting under fruiting trees of each bird-dispersed species and collect all seeds that fall into the net. Seeds that show evidence of handling by birds (i.e. beak marks, flesh removal or obvious regurgitation or excrement) will be considered category 2 seeds; the remaining will be considered unhandled by birds (category 1). We tested the efficacy of this approach for seed collection in the previous 2 field seasons in the Mariana Islands, and we are confident we can collect sufficient seed for these experiments and separate handled from unhandled seed. We will plant 50 ripe but unhandled (category 1) and 50 ripe and handled (category 2) seeds from each species (100 seeds X 10 species=1000 seeds) in potting soil under controlled conditions and monitor them for germination. The effect of handling on the proportion of seeds that germinate will be analyzed using a general linear model with a binomial distribution.

**Predictions:** Bird-dispersed seeds handled by birds will have higher germination rates than those not handled by birds. We expect this to hold for native and non-native bird-dispersed species.

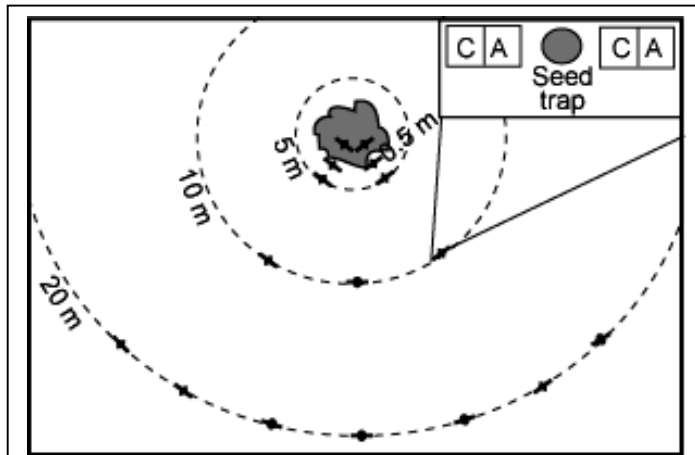
### 1.2: Implications of bird dispersal for density-dependent mortality of seeds and seedlings

Bird-dispersal should enable seeds to travel further from their parent tree (Fig. 5A). Thus, in the absence of birds, seeds will reach higher densities because a greater proportion of seeds will fall close to the parent tree. Since host-specific predators and pathogens generally lead to disproportionately high mortality at high densities (Fig. 5B), more seeds should succumb to density-dependent mortality when birds are absent (Fig. 5C). To determine whether focal tree species experience disproportionate mortality when seeds are in high densities (i.e. near parent tree), and thus, benefit from bird-dispersal by getting away from the parent tree, we will use experiments and observations to i) quantify seed dispersal distances of focal species in the presence (Saipan, Rota, Tinian) and absence (Guam) of birds and ii) determine whether seeds and seedlings experience density-dependent mortality.

We will select three focal trees per species per island on the three islands that have birds, and five focal trees per species on Guam, as it is the only island without birds. Conspecific focal trees will be selected by randomly choosing a GPS point within limestone forest (we have mapped the location of suitable forests on all islands), tagging the first 10 reproductive adults of each species encountered and selecting the individual with the largest trunk diameter. We use the largest local individual for each species so that we can capture sufficient seeds with our trapping array to fit robust dispersal kernels across species and islands. We will repeat this process until all focal trees are chosen, re-sampling only when a selected individual is <200m from a previously selected conspecific individual. At each tree, we will place an array of 16 seed traps at a range of distances from the focal tree along a 90° arc extending in a random direction from the canopy of the tree (Fig. 6). If there are conspecific trees within 40 meters, we will note their distance and size and incorporate that info into dispersal kernel fitting and estimates of density-dependent survival and growth. Seed traps will be suspended to avoid damage from ungulates and emptied regularly, and seeds of focal species will be separated and counted. These data allow us to quantify dispersal distances for each focal species on each island.



**Figure 5:** (A) Seed dispersal, as influenced by bird presence. (B) Density-dependent survival, independent of bird presence. (C) Impact of density-dependence on seedling densities given differing dispersal distances. Survival estimates (9 and 30%) are for



**Figure 6:** Experimental design for measuring seed rain, seed and seedling growth, survival and herbivory around focal trees (canopy in gray). Black points = seed traps (ST). Traps are located at 0.5m underneath canopy edge (2 traps) and at 0.5m (2 traps), 5m (2 traps), 10m (3 traps) and 20m (7 traps) away from the canopy edge. 1m x 1m seed and seedling addition (A) and control (C) plots will be established around two seed traps per distance to study seed and seedling density dependent mortality patterns.

Next, we will plant and monitor seeds and seedlings adjacent to each seed trap to determine whether our focal species experience density-dependent mortality. We will plant seeds and seedlings rather than relying on natural seed rain to supply us with seeds and seedlings because we expect that few individuals will be dispersed to the low densities far from focal trees; thus, planting gives us more power to detect density-dependent mortality. We will gather ripe seeds from trees on each island by putting netting under the tree canopy (see section 1.1). For bird-dispersed species, we will only plant seeds handled by birds, because seeds with pulp remaining on them are likely to arrive only near the parent tree, while pulp-less seeds are likely to arrive at all distances. Seedlings for

planting will be grown for two months at facilities on Guam (University of Guam), Saipan (Division of Fish and Wildlife), and Rota (Division of Fish and Wildlife) prior to establishment in the field. We expect little trouble in growing these seedlings, as germination protocols for all 10 species have been developed by the Guam Department of Agriculture Forestry Division. Seedlings will be transported from Saipan to Tinian.

We will establish two 2m x 1m plots at each seed trap (Fig. 6) for seed and seedling plantings. Ungulates will be fenced out of these plots on the two islands with ungulate populations (Guam and Rota). We will plant 100 seeds and 10 seedlings in a 1m x 1m subplot within each plot, with the second subplot serving as an un-manipulated control. All added seeds and seedlings will be tagged to distinguish them from natural recruitment into the plots. All conspecific seedlings in each 2m x 1m plot present at the start of the experiment or emerging during the experiment will be marked and monitored along with the planted seedlings. We will monitor plots for seedling emergence, seedling survival, and herbivory (see section 2) after 1 day, 3 days, 1 week, 1 month, 2 months, 4 months, 6 months, 12 months, 18 months and 24 months. We will also harvest two planted seedlings per species per plot at the end of the experiment and quantify seedling dry weight to test for density-dependence in seedling growth.

We will use maximum likelihood to fit seed dispersal kernels to seed trap data, testing for differences between dispersal kernels on Guam, which lacks birds, and Saipan, Tinian, and Rota, all of which have intact avifauna. We will compare kernels using maximum likelihood model fitting and a number of different dispersal kernels (e.g. normal, exponential, 2dt) following previous studies (Ribbens et al. 1994, Clark et al. 1998, Hille Ris Lambers and Clark 2003). This will allow us to determine how strongly bird loss impacts seed dispersal distances (Fig. 5A).

Next we will test for the presence of density-dependent survival and growth (Fig. 5B). To do

so, we will calculate germination probabilities at each seedling plot as the number of germinants divided by the number of seeds dispersed into the plot (seed rain + 100 seeds added). Seedling survival is simply the number of surviving seedlings divided by the number of planted seedlings. For each species, we will apply a generalized linear mixed-effect model with a binomial distribution to survival and germination data, with species identity, distance, and island location as the main effects, and tree (nested within island) as a random effect. We will similarly determine the effects of species identity, distance, and island on seedling growth using a linear mixed-effect model. Testing for an interaction between species identity and distance allows us to ask whether the strength of density-dependence varies between species. Although we do not expect the relationship between density and survival (or growth) to vary between islands, we can determine this by testing for an interaction between island (i.e. bird presence) and distance.

**Predictions:** We predict that dispersal kernels of bird-dispersed trees will have shorter mean dispersal distances on Guam than those on islands with birds. We predict that there will be no difference in dispersal kernel shape between Guam and other islands for non bird-dispersed species. We predict that seed germination, survival, and growth should be lower closer to focal trees. We expect that density-dependent growth and survival will not differ between islands with and without birds for any species. Finally, we expect that non-native species will experience weaker density-dependent mortality than native species because fewer specialized agents of density-dependent mortality should affect non-native species in their exotic ranges.

### **1.3: Net impacts of seed dispersal and density-dependence on seedling distributions**

One consequence of limited dispersal, combined with density-dependent germination and survival, is a change in the spatial distribution of juveniles relative to parent trees (Fig. 5A vs. 5C) over time (Hille Ris Lambers and Clark 2003). As an independent test of the existence of density-dependent survival, we will use counts of naturally occurring seedlings in plots around focal trees (Fig. 6) to estimate the ‘seedling shadow’; analogous to a seedling dispersal kernel for each focal species. Then, we will compare seedling to seed dispersal kernels to ask whether density-dependent survival causes seedling densities to be less clustered around parent trees than predicted from seed dispersal distances. As with seed dispersal estimation (1.2), we will compare seedling kernels using maximum likelihood model fitting and a number of different dispersal kernels (e.g. normal, exponential, 2dt) following previous studies (Ribbens et al. 1994, Clark et al. 1998, Hille Ris Lambers and Clark 2003).

**Predictions:** We predict that seedling dispersal kernels of species experiencing low density-dependent mortality will be similar in shape to seed dispersal kernels (from section 1.2), while seedling dispersal kernels of species experiencing high density-dependent mortality will be much less clumped around trees than seed dispersal kernels. Overall fecundity will also be lower on Guam for species that experience reduced dispersal and high density dependent mortality.

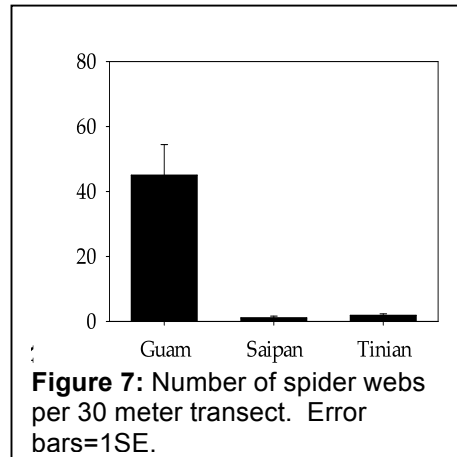
## **2: Do birds regulate herbivory on seedlings?**

Seven insectivorous bird species form the top of the terrestrial forest food web in the Mariana Islands (all are now functionally absent from Guam) and consume a variety of insect prey. The Bridled White-eye, for example, often sits on branches, gleaning insects and spiders from the leaves and branches, while the Rufous Fantail captures insects while flying, and the Mariana Crow has been observed eating web-building spiders.

Theory on top-down trophic control predicts that bird predation limits the abundance of prey



populations, which cascades down to impact lower trophic levels – i.e. forest trees. Although many studies have shown that birds can reduce arthropod abundance (Greenberg et al. 2000, Murakami and Nakano 2000, Strong et al. 2000, Hooks et al. 2003, Gruner 2004, Van Bael and Brawn 2005, Mooney 2007), the indirect impacts on plant damage have been equivocal. Studies have not found that birds consistently control insect herbivory, but rather that avian control of herbivory depends on position in the canopy (Van Bael et al. 2003, Van Bael and Brawn 2005), productivity (Mazia et al. 2004), plant species identity (Mooney and Linhart 2006), or time (Lichtenberg and Lichtenberg 2002). One study (Gruner 2004) even found no effect of birds on leaf damage. Thus, it would be premature to conclude either that birds have no impact or that they have a strong impact on plant performance through their control of insect herbivores. Since birds prey upon both predatory and herbivorous arthropods, the indirect impact of bird loss on plants may be attenuated by the opposing pressures (decreased predation by birds, increased predation by predatory arthropods) on herbivores.

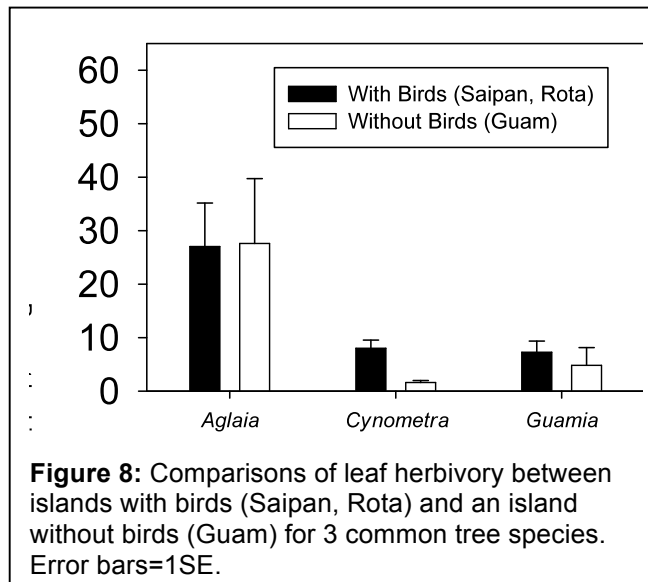


Following the predictions of top-down trophic control, web-building spiders were observed to be more abundant on Guam relative to Saipan, Tinian and Rota (Jaffe 1994, Quammen 1996, Fritts and Rodda 1998), although this pattern had not been quantified until our study in 2007, where we found spider web densities 20-40 times higher on Guam than on nearby islands (ANOVA  $p < 0.001$ ; Fig. 7). However, a preliminary study of three tree species in 2006 did not find greater leaf herbivory on Guam (Fig. 8) indicating that a difference in arthropods may not be affecting herbivory on adult trees of these species. In addition, a preliminary bird exclosure experiment in 2007 on Saipan, Tinian, and Guam found no difference in herbivory rates on an annual crop plant between plants grown inside versus outside of exclosures on any island (with or without birds), nor a greater overall level of herbivory on Guam than on Saipan or Tinian. In all, existing studies have not reached a consensus on whether birds have strong top-down effects on forest communities, and our preliminary data suggest that birds do not have strong top-down effects in the Mariana Islands.

Despite equivocal support for top-down trophic control by birds, additional studies are still worth performing. We are not aware of bird exclusion studies that compare the response of a variety of plant species and use a metric and life history stage that definitively measures an impact on plant fitness (i.e. seedling survival rather than adult leaf damage). Ultimately, we lack an ecosystem-level example comparable to those in aquatic or carnivore-ungulate-terrestrial plant systems. Thus, our study will clarify the role of trophic control by birds by addressing each of these points through a focus on growth and survival in the seedling stage of ten focal species and a comparison of experimental results to a real-world natural experiment (Guam).

## **2.1: Impact of insectivorous birds on seedling survival, growth and herbivory**

We will use bird netting to create four large bird exclosures and paired control areas per island (16 total) in native forests. At each site, we will establish a 10m x 10 m (100m<sup>2</sup>) plot in limestone forest (excluding ungulates with fencing if necessary) and randomly select half to be the bird exclosure treatment. We will use existing trees and bird netting



(BirdNet2000™) to build the bird exclosure. The top of the netting will be six feet high, sufficiently tall to exclude birds from the seedlings and the netting will be cut to encircle trees within the exclosure. By conducting this experiment on Guam in addition to the three islands with birds, we can determine whether there is an exclosure effect, as there should be no difference between treatment and control on Guam. We can also compare seedling survival, growth, and herbivory of plants grown in the control areas of all four islands to determine whether the differences between bird exclosure treatment and control found in the manipulative

exclosure experiments scale up to island-level differences between Guam (no birds) and Saipan, Tinian, and Rota (with birds).

We will plant 10 seedlings of each focal species (1 seedling/m<sup>2</sup>, total=50m<sup>2</sup>) in each treatment (exclosure and control). We will monitor seedling survival and sample arthropods on a subset of plants at 1 month, 2 months, 4 months, 6 months, 12 months, 18 months and 24 months. We will also quantify herbivory (% leaf area missing on pre-marked select leaves) at each sampling period and harvest remaining seedlings at 24 months. We will dry and weigh these seedlings to determine exclosure effects on seedling growth. To sample arthropods, we will place a bag over the plant and shake to dislodge arthropods, and then physically remove any remaining arthropods. An undergraduate student from the University of Guam will work with PI Miller and senior personnel Moore to sort and identify all arthropods to family (usually sufficient to identify individuals as predatory vs. herbivorous). Any unknown specimens will be sent to the appropriate expert for identification. A voucher collection will be maintained at the University of Guam. Studies that have found an influence of birds on arthropod populations have ranged from as short as 2 months (Holmes et al. 1979, Hooks et al. 2003) to as long as 3 years (Bock et al. 1992, Mazia et al. 2004, Mooney and Linhart 2006), placing our study well within the time frame necessary to capture a response. By collecting all arthropods (predatory and herbivorous), we will be able to assess whether leaf damage and spider and herbivore abundances increase with bird exclusion.

To determine the level of defense (chemical and physical) for each focal species, we can take advantage of the tradeoff between growth and defense in seedlings (Coley et al. 1985) and conduct assays for common chemical defense compounds. We will grow 10 individual seedlings from seeds collected at each site on all four islands (10 seedlings X 4 sites X 4 islands = 160 seedlings) in a common garden under controlled growing conditions at the University of Washington greenhouse. We will spray insecticide (Rotenone, a non-synthetic pesticide commonly used in agriculture) on all seedlings to prevent insects from damaging the plants and will measure growth rate on all plants at the end of four months. Since growth rate is generally inversely correlated with level of defense (Coley et al. 1985), we will rank the ten focal species from least defended (highest growth rate) to most defended (lowest growth rate). We will conduct assays of common extractable terpenes, phenolics,

other primary alkaloids and tannins on all seedlings for additional rankings of species by level of defense. These extractions will be performed using established techniques in Dr. Tewksbury's laboratory at the University of Washington.

The effect of bird presence on survival, growth, herbivory, and arthropod abundance will be analyzed using generalized linear mixed models (with appropriate probability distributions – binomial for survival; Poisson for arthropod counts; lognormal for biomass), with treatment (exclosure, control), island, and level of defense as main effects and site as a random effect.

**Predictions- exclosure-to-control comparison:** We predict that bird exclusion will lead to an increase in the abundance of large predatory arthropods, including spiders, as well as a lesser but still positive increase in the abundance of herbivores (Fig. 3B). We predict that less defended plants will be strongly affected by the no bird treatment whereas more defended plants will be less affected by the no bird treatment. We also predict that native species will experience greater benefits from exclosures than non-native species, because non-native species should be less affected by host-specific insect herbivores. We predict no difference in herbivory between control and exclosure areas on Guam.

**Predictions- island-to-island comparison:** We predict that overall herbivory will be higher on Guam than on nearby islands, but that species with high chemical or physical defenses and non-native species that have escaped their specialist predators will be less affected by bird loss than undefended native species. We also predict that on Guam there will be more predatory arthropods, more herbivores on less defended plant species, and no difference in herbivores on highly defended plant species compared to islands with birds.

### **3: Do birds control forest seedling community composition through their effects on dispersal and herbivory?**

What are the consequences of bird extinctions for plant community structure, given the large but species-specific role that birds may play in seed dispersal and insect herbivore population dynamics? Predicting these consequences has been difficult because existing studies rarely focus on multiple species or the multiple roles that birds play in affecting plant demography. Here, we take the first such step by using our extensive experimental and observational data to estimate the combined impact of frugivorous and insectivorous bird loss on seedling recruitment for our ten focal species. Next, we generalize about possible changes to forest structure by determining the relative impact of bird loss on seedling recruitment for species with similar functional traits (e.g. dispersal mode, allocation to defensive compounds, native/exotic status). Finally, we use comparisons of actual seedling recruitment on Guam with that on Tinian, Saipan and Rota to validate our estimated predictions of bird impacts on seedling recruitment for our focal species. Although we cannot estimate the effects of bird loss on the entire life history of our focal species, we believe that effects on seedling recruitment are indicative of effects on population growth rates because forest structure is often determined by early life history stages (Comita et al. 2007).

#### **3.1: Quantifying the effects of bird loss on seedling recruitment:**

We estimate the impact of bird loss on seedling recruitment, the probability that a seed ( $s$ ) produced by each of our ten focal species ( $i$ ) survives to become a 24 month old seedling ( $l$ ) by integrating the effects of bird handling on seed germination, density-dependent survival and mortality due to herbivory. In the presence of birds, the probability of seedling survival is:

$$p\left(\frac{I_i}{S_i}\right)_b = [g_b p_b + g_n (1 - p_b)] d_b h_b \quad 1$$

in the absence of birds, this probability is:

$$p\left(\frac{I_i}{S_i}\right)_n = g_n d_n h_n \quad 2$$

$g_b$  is seed germination after handling by birds (i.e. the proportion of handled seeds that germinate), while  $g_n$  is seed germination when not handled by birds – these parameters come directly from our germination trials (section 1.1). Both  $g_b$  and  $g_n$  are relevant to average germination in the presence of birds, because not all seeds are handled by birds. The parameter  $p_b$ , which is the probability of each seed being handled by a bird, weights the contribution of the two germination parameters, and will be determined from 12-hour video recordings of fruit-laden branches on each of our focal trees.

$d_b$  is the average probability of survival in the presence of birds given density-dependent mortality, while  $d_n$  is the average probability of survival in the absence of birds given density-dependent mortality. We do not observe these parameters directly, but instead, calculate them using two functions, the dispersal kernel and the relationship between distance from the parent tree and survival (both parameterized in section 1.2). We will randomly sample 1000 dispersal distances from each dispersal kernel, and calculate expected survival probabilities given these distances using density-dependent survival functions. Averaging these 1000 samples gives us the average probability of survival from seed to 24 month seedling. Scaling these estimates by density-independent mortality (i.e. survival at  $\infty$  distance from the parent tree) gives us  $d_b$  and  $d_n$ , the reduction in survival due to density-dependent mortality alone.

Parameters  $h_b$  and  $h_n$  represent the reduction in survival due to herbivory, in the presence and absence of birds. These estimates come directly from our exclosure studies (experiment 2.1) on Saipan, Tinian, and Rota, where we have quantified the 24 month survival of planted seedlings outside (with birds) and inside (without birds) of exclosures. Note that the probability that a seed for wind and gravity-dispersed focal species will become a 24-month old seedling is simply  $h_b$  on islands with birds and  $h_n$  on islands without birds, since the parameters describing germination and survival probabilities relative to bird handling or dispersal do not apply.

The effects of bird loss on seedling recruitment for species  $i$  ( $r_i$ ) can be calculated from the two seedling recruitment probabilities, specifically:

$$r_i = p\left(\frac{I_i}{S_i}\right)_n / p\left(\frac{I_i}{S_i}\right)_b \quad 3$$

represents the factor by which seedling recruitment decreases (or increases) for each species  $i$ , once birds are extinct and stop dispersing seeds and consuming insect herbivores.

Assessing whether impacts of bird loss on seedling growth and survival (the  $r_i$ 's) depend on the traits of each species, e.g. dispersal mode, allocation to herbivore defense, native/non-native status, also allows us to predict how forest community structure will and perhaps is already changing on Guam with the loss of birds. For example, if the  $r_i$ 's of bird-dispersed

species are uniformly lower than those of wind- or gravity-dispersed species, we predict that forests of the future Guam will contain lower densities of these functional groups than do Saipan, Tinian and Rota.

**Predictions:** We predict that recruitment of wind- and gravity-dispersed taxa will be less impacted by bird loss than bird-dispersed taxa, because these taxa are only influenced by changes in herbivory, not in the amount of density-dependent mortality experienced. We predict that bird loss impacts on seedling survival will be negatively correlated with allocation to defense, with more heavily defended species showing smaller impacts of bird loss. Finally, we predict that seedling growth and recruitment of native taxa will be more negatively influenced by bird loss than non-native species, because native species are more likely to be controlled by specialist insect herbivores.

### **3.2: Comparing model predictions to the effects of an actual bird extinction**

The advantage of performing these experiments in the Marianas is that the functional extirpation of avifauna from Guam allows us to verify our estimates of bird impacts on seedling survival with a 'natural experiment' of bird removal. We will use additional data, independent of that used to estimate impacts of bird loss on seedling survival, to compare these predictions with actual differences in seedling densities on Guam (no birds) vs. comparable islands with birds (Saipan, Tinian and Rota).

We will use naturally recruited seedlings in our seedling plots (section 1.2) to determine how seedling densities of our ten focal species differ between Guam (with birds) and the islands without birds (Saipan, Tinian, and Rota). For each individual species, we will only use counts of naturally occurring seedlings in plots surrounding focal trees, to ensure that adult densities are comparable. We can use this data to estimate the factor by which seedling densities for each species on Guam differ from seedling densities on Saipan, Tinian, and Rota. It is possible that bird loss also affects the fecundity of our focal species (through insect herbivory of flowers and developing seeds), complicating this comparison. However, we can use fecundity parameters from fitted dispersal kernels to explore this possibility and scale estimates of seedling density differences between the islands.

**Predictions:** Across all species, we expect that the factor by which seedling densities on Guam are greater or lesser than on islands with birds will be correlated with  $r_i$ 's, our predictions of bird loss impacts on seedling recruitment. Thus, we predict that Guam will have greater seedling densities of non bird-dispersed, highly defended non-native species and lower seedling densities native, bird-dispersed species than Saipan, Tinian and Rota.

### **Broader Impacts**

*High School education:* We are collaborating with Jennifer Farley from the Guam Environmental Education Committee to develop a volunteer program for students from Simon Sanchez High School (a public high school primarily composed of Pacific Islanders), where students can assist with collection of seeds from seed traps and video review of fruiting tree visitors. We will continue to speak with area high school classes on all islands as opportunities arise.

*University-level education and training:* A cornerstone of this project is the involvement of young scientists with a dedication to ecology, conservation, and the Pacific Islands. This is particularly important in this region, as many of the students who pursue higher education in specialized fields such as biology leave the region to pursue careers outside of the Mariana Islands. We will involve these young scientists in several ways. First, we will mentor students from the University of Guam through paid field assistant positions. There are few

such positions available in the Marianas for interested students. Second, PI's Tewksbury and Hille Ris Lambers will give guest lectures in biology classes at the University of Guam and the Northern Marianas College. Third, we will run a 3-week comparative island ecology course in Year 3 of the grant. This course will involve 8 students from the University of Washington through the UW Exploration Seminar opportunity along with 12 students from the University of Guam. The University of Guam students will be fully funded by this grant. The course will be run by PI Tewksbury, PI Miller, and the graduate student on this project (Rogers). The class will combine guest lectures from experts in the region with hypothesis development and testing and will culminate in a symposium where students present their research findings to local biologists and government officials.

*Conservation and Management:* This study provides basic information on the natural history of the Mariana Islands that is of interest to the general public. We will continue to work closely with employees from the Guam Division of Forestry, Northern Mariana Islands Division of Fish and Wildlife, and the Tinian and Rota Departments of Lands and Natural Resources and submit yearly summaries of our research to these organizations. We have shared information through guest lectures at the National Wildlife Refuge at Ritidian Point and will continue to speak to public and professional audiences in the Marianas.

*Contributions to Infrastructure:* This study marks the beginning of a partnership between the University of Washington, a research institution, and the University of Guam, a land-grant institution that serves a student body primarily composed of Pacific Islanders. We are collaborating with Dr. Aubrey Moore, an entomologist at the University of Guam, to test a question related to our research proposal and further the development of a valuable new insect identification tool. Dr. Moore is one of the principal investigators on an NSF grant (#0552086) testing an instrument called the Flight Activity Signature Technology for Identification (FAST-ID), which uses a solar cell to collect insect wingbeat waveforms from unidentified individual flying insects. The number of waveforms collected will be a measure of insect abundance. We will use the FAST-ID instrument for two full days at four locations in native forest on Guam, Saipan, Tinian, and Rota, twice during the wet season and twice during the dry season to test the hypothesis that Guam has more flying insects than Saipan, Tinian or Rota. We will collect a sample of living insects each survey day using aerial malaise traps to act as an alternate measure of flight activity. This would be the first application of this tool to an ecosystem-level study.

## **Conclusion**

Guam is at a unique and dangerous crossroads: the removal of birds from the island has likely initiated a complex series of impacts on a regionally endemic flora in one of the world's biodiversity hotspots (Myers et al. 2000). Understanding the nature and magnitude of these effects will be critical for the effective management of Guam's forests in the absence of birds. And with the possibility of large-scale avian extinction looming on many islands, understanding the consequences of such whole-island loss may prove critical for proactive conservation decision-making. The ecological lessons learned from this research can be applied even more broadly than the geographic scope addressed in this proposal, since bird species are threatened around the world. Understanding the roles birds play in maintaining forest health may be critical for preventing widespread ecosystem decay.

The complete loss of birds from a large island cannot be replicated experimentally. Scientists must take advantage of this anthropogenic experiment to gain information at a much larger scale than otherwise possible. We have already lost more than 20 years of data on plant community change on Guam since the loss of birds; it is imperative that we do

not lose any more. The timing of this research is particularly crucial, as we have many factors in place that will enable this to be a successful project. Tewksbury is advising a graduate student (Rogers) in her third year of graduate school with 5 years of experience and extensive contacts in the region. She provides a link to PI Miller and senior personnel Moore at the University of Guam. She will carry out a scaled down form of this project for her PhD without this grant, but with it, our team would be able to conduct a much broader and more definitive examination of the impacts of bird loss. We must seize upon this opportunity to gain insight into the ecological impacts of bird loss on an ecosystem-scale, which will be important not only to people living on Guam, but also to the conservationists concerned with bird loss and ecologists interested in the functional role of birds.

### Timeline of Activities

The extensive data we are proposing to gather, in combination with the logistical difficulties imposed by collecting data on four different islands, result in a project that is clearly ambitious. We believe that we can meet the challenges inherent to implementing the proposed research because of our combined experience in the study system and the techniques we will utilize. Below, we provide the timeline we will follow to complete these projects within the timeframe of the grant.

		2008		2009				2010				2011		
		Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su
1. Seed Dispersal	1.1 Seed dispersal video/observations													
	1.1 Bird Handling													
	1.2 Seed rain		set up											
	1.2 Density-dependent mortality plantings		set up											
	1.3 Seedling shadow													
2. Control of Herbivory	2.1 Bird exclosure experiment			set up										
3. Forest diversity	3.1 Model													
	3.2 Model-to-forest comparison													
Broader Impacts	Island Ecology course													
	Technology development: FAST-ID													

### Results from previous NSF Support

Hille Ris Lambers has one collaborative NSF grant (DEB 0743183) that was funded in December of 2007. Tewksbury has one current collaborative NSF grant (DEB - 613975) and one recently completed SGER grant (DEB 0636630) – in both cases, products are still forthcoming. Previous to these awards, Tewksbury was PI on an international grant examining the importance of secondary compounds in the fruits of wild chilies to the process of seed dispersal (DEB – 0129168, 2002-2005). This grant was conducted primarily in Bolivia, where Tewksbury set up international collaborations with local NGOs and museums. To date, Tewksbury has published 7 papers from this grant (Tewksbury 2002, Filardi and Tewksbury 2005, Tewksbury et al. 2006, Levey et al. 2006, 2007, Bronstein et al. 2007, Tewksbury et al. 2007). Six of these have graduate or undergraduate co-authors, and 2 additional manuscripts are in the final stages of completion. This grant also resulted in 2 Bolivian students with completed undergraduate thesis projects (one of which is working at a leading NGO in Bolivia, the second one starting graduate work at the University of Florida). Previous to this work, Tewksbury was post-doctoral coordinator for a

second Collaborative NSF grant (DEB-9815834, 1999-2004), examining the importance of landscape connectivity on plant-animal interactions – focusing primarily on pollination and seed dispersal. Tewksbury has authored or co-authored seven papers based on this work (Two in *Science*, one in *PNAS*).