**SG: Synergistic effects of forest fragmentation and droughts
on tropical plant demography**

RESULTS OF PRIOR NSF SUPPORT (Bruna). DGE-0801544: "IGERT: Spatial Ecology and Evolution: Quantitative Training in Biology, Statistics, & Mathematics" ($2,876,455; PI: M. Martcheva, Co-PIs: C. Osenberg, M. Christman, B. Bolker, E. Bruna, 2008-2014). This award supported the interdisciplinary training of doctoral students in ecology, evolution, mathematics, and statistics to address questions with a spatial dimension. **Our IGERT supported 25 trainees and 18 associates mentored by 37 faculty**. Students published >**70 articles** and gave **>140 presentations**. They also worked on interdisciplinary projects with external clients.

RESULTS OF PRIOR NSF SUPPORT FOR *HELICONIA* RESEARCH (Bruna & Uriarte).
Intellectual Merit: DDIG-INT 98-06351 ($17,600) and Collaborative Grants DEB-0614149 ($230,888) & DEB-0614339 ($251,831) have collectively resulted in **5 archived datasets and >25 papers to date, including five reviews collectively cited >1000 times**. Early studies provided the first test of the hypothesis that plant recruitment was lower in forest fragments and that thus would depress population growth rates (e.g., Bruna 1999, Bruna et al. 2002, Bruna 2003). More recent studies investigated statistical methods for modeling reproductive rates (Brooks et al. 2019), demonstrated that the growth rates of plants in fragments were chronically reduced (Gagnon et al. 2011), and assessed the effects of local environmental conditions, disperser diversity, and disperser behavior on safe-site vs. seed-limitation (Uriarte et al. 2010, Uriarte et al. 2011). Research supported by these grants has been recognized with the John L. Harper Prize from the British Ecological Society (Bruna et al. 2002) and the Outstanding Paper Award from the International Association of Landscape Ecology-US Chapter (Uriarte et al. 2011). ***Broader Impacts:*** These awards supported **27 interns** and **2 Postdocs**. The data were used in **6 PhD Theses, 2 MS Thesis**, **and 1 Honors Thesis**. Several datasets are archived at Figshare (http://tinyurl.com/y25e5mva); the core demographic dataset will be archived with funds from this proposal. Bruna used the *Heliconia* system in 4 field courses (INPA) and his tropical ecology course (UF). Uriarte's course on likelihood methods reached >50 students from 9 countries. Finally, both Bruna and Uriarte are underrepresented minorities; the products of these grants were central to their tenure and continued promotion.

RESPONSE TO REVIEWER COMMENTS: While our prior submission received enthusiastic reviews *(e.g., "an important and robust test", "tests a timely hypothesis", "the study system is fantastic, the team is highly skilled, and the proposed budget is a great deal relative to the potential impacts")*, panelists suggested we consider some alternative analytical methods for assessing lagged effects of drought on demographic vital rates. Based on this feedback, we will use the Functional Linear Models (i.e., "spline methods") suggested by Rev. 5. Not only will these streamline the construction of Integral Projection Models, they can also be used to test for density dependent effects on demography and whether they might vary among 1-ha fragments, 10-ha fragments, and continuous forest (a questions raised by Rev. 1). Finally, as to Rev. 3's assertion that insights gained from the study of a single-species: we emphasize in the proposal the features that make *H. acuminata* an excellent representative species for understory tropical plants and that detailed demographic data for tropical taxa are incredibly rare (Salguero-Gomez et al. 2015), in part because of how challenging it is to collect them. We also feel compelled to point out that some of ecology's most important advances – including those detailing the effects of both habitat fragmentation and climate change – are the result of analyzing the population dynamics of a single species. *Heliconia acuminata*'s value as a model system has been vetted for over two decades by the reviewers of our many proposal and publications, and we are confident that the results of our proposed research will have broad implications for how global change phenomena influence population dynamics and the risk of extinction.

CONCEPTUAL FRAMEWORK: Understanding the consequences of habitat fragmentation (Fig. 1) has been a central area of ecological research since this form of landscape change was identified as a threat to the integrity of ecosystems (Harris 1984, Wilcove et al. 1986), and the ongoing transformation of landscapes has kept it in the theoretical and empirical spotlight (Brudvig et al. 2017, Resasco et al. 2017). Decades of research have documented myriad biotic changes associated with fragmentation, including the local extinction of species from fragments (Harrison and Bruna 1999, Laurance et al. 2011, Haddad et al. 2015, Fletcher et al. 2018). Although the demographic mechanisms underlying these extinctions are rarely known (Bruna et al. 2009), many of them – especially in tropical forests – are thought to be driven by reduced rates of individual growth, reproduction, or survivorship in fragments (Laurance et al. 1998, Zartman et al. 2015) resulting from dramatically altered abiotic conditions (Broadbent et al. 2008). Disentangling how fragment characteristics, abiotic conditions, and demography interact to influence population dynamics has therefore become central to conceptual frameworks for studying fragmented landscapes (Didham et al. 2012, Driscoll et al. 2013, Selwood et al. 2015).

Species in fragmented landscapes must also cope with climate change (Brodie et al. 2012, Mora et al. 2013), whose effects may be particularly acute in tropical ecosystems (Corlett 2011, Brodie et al. 2012), In the last 40 years many tropical regions have experienced significant warming and declines in precipitation (Malhi and Wright 2004), resulting in increased fire activity, tree mortality, and forest degradation (Duffy et al. 2015). The most recent collection of climate models (CIMP6, Eyring et al. 2016) suggest these trends will continue; for example, increased drought frequency and intensity are predicted for much of the Amazon Basin (Duffy et al. 2015). There are two primary mechanisms for these droughts: increases in Pacific sea surface temperatures that intensify the El Niño Southern Oscillation (ENSO), and the displacement of the inter-tropical convergence zone by elevated Atlantic sea surface temperatures (ASST) (Lewis et al. 2011). ENSO droughts can have major effects on ecosystems and human well-being (Li et al. 2010), and they have historically been more frequent than ASST-driven ones. However, two of the Amazon's most recent droughts – including the 2005 'drought of the century' – were ASST-driven (Zeng et al. 2008).
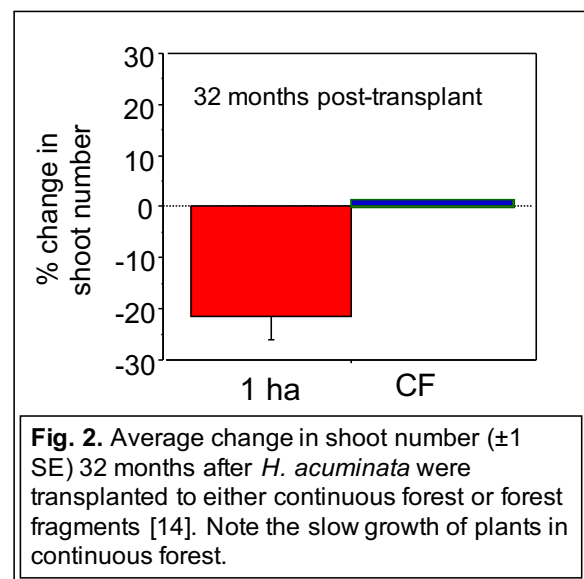

Fig. 1: Fragments at Brazil's Biological Dynamics of Forest Fragments Project after isolation. Left: 10-ha, Right: 1-ha; note nearby continuous forest (Photo: R. Bierregaard).

Several studies have demonstrated that climate change can have major effects on the population dynamics of species in the temperate zone (Doak and Morris 2010, Sletvold et al. 2013, Selwood et al. 2015, Williams et al. 2015). In contrast, almost nothing is known about the consequences of changing climates for the population dynamics of tropical species. Mild droughts have been shown to increase the growth rates of tropical trees, perhaps due to reductions in cloud cover (Condit et al. 2004). However, in severe drought years growth can be extremely low while mortality can be sharply elevated, with this increased mortality persisting for several years (Phillips et al. 2010). The observed effects of mild droughts on reproduction are more complex, but include decreases in the abundance of pollinators (Stiles 1992, Jevanandam et al. 2013) and a boom in drought-year fruit production followed by severe post-drought "famine" (Wright et al. 1999, Pau et al. 2013). In addition, while some studies have shown negligible effects of droughts on seedling recruitment and survivorship (Connell and Green 2000, Engelbrecht et al. 2002), others have shown survivorship decreases significantly during

droughts (Edwards and Krockenberger 2006). These results, coupled with evidence that the growth and survivorship of plants can be lower in fragments (Laurance et al. 1998, Bruna 2002, Zartman et al. 2015) and that droughts can exacerbate this disparity (Laurance et al. 2011), has led to the *hypothesis that as drought frequency or intensity increases, the viability of plant populations in fragments will decrease relative to that of populations in continuous forest* (Laurance and Williamson 2001, Opdam and Wascher 2004, Selwood et al. 2015).

This *"Fragmentation-Drought Hypothesis (FDH)"* **remains untested for four principal reasons. First**, while studies assessing the demographic responses of plants to droughts are increasingly common (Williamson et al. 2000, Slik 2004, Wright and Calderon 2006, Meir et al. 2009, Meir and Woodward 2010, Phillips et al. 2010, Feldpausch et al. 2016), almost none have been conducted in fragmented landscapes. Those that have (e.g., Laurance et al. 2001) assessed the consequences of only a single drought, so it is unknown if the observed results are typical or an artefact of drought severity. **Second**, studies of how tropical plants respond to fragmentation or drought typically focus on either 'adults' (e.g., trees >10 cm dbh) or 'juveniles' (e.g., seedlings, saplings) but rarely on both (Bruna et al. 2009, Corlett 2011, Laurance et al. 2014). The same is true of the limited number of studies to have investigated potential synergies between climate and human alterations of landscapes (but see Maza-Villalobos et al. 2013, Sletvold et al. 2013, Uriarte et al. 2016a). However, without considering multiple life-history stages simultaneously it is challenging to predict how disturbances, either independently or in concert, influence population dynamics and extinction risk (reviewed in Bruna et al. 2009, Ehrlen et al. 2016). **Third**, the long-term and multi-site demographic data needed to test the FDH are very rare: Salguero-Gomez et al. (2015) found that the modal length of data collection in the demographic studies they reviewed was <4 years, while Crone et al. (2011) found that 48% of the plant matrix models they reviewed used data from only one site. Data with which to test population-level hypotheses about climate-fragmentation synergies are even more scant (Ehrlen et al. 2016), especially for tropical systems (Corlett 2011). Tropical species make up only 25% of the 782 plant species in the COMPADRE demographic database (Salguero-Gomez et al. 2015), but demographic data were collected in habitat fragments for only four of these. Even more striking is only one of these species was studied in both continuous habitat and fragments: *Heliconia acuminata*. Long-term and multi-site data that span the organismal life-cycle are challenging to collect, but they are essential – while some of the dramatic demographic impacts of drought are short-lived (Laurance et al. 2001), others can persist for several years (Martinez-Ramos et al. 2009, Anderegg et al. 2015). Still other effects can be subtle and take years to accumulate and detect (Gagnon et al. 2011, Anderegg et al. 2015). Furthermore, tropical plants are typically long-lived and slow-growing (Fig. 2), so multi-year data are essential for estimating the spatio-temporal variation in vital rates required for robust demographic models (Morris and Doak 2002). **Finally**, all of these limitations are compounded by the need for precipitation data collected close to focal populations and at a level of temporal resolution with which one can identify droughts, quantify their intensity, and test for temporal lags ranging from weekly to yearly in demographic responses (van de Pol et al. 2016).

Fig. 2. Average change in shoot number (±1 SE) 32 months after *H. acuminata* were transplanted to either continuous forest or forest fragments [14]. Note the slow growth of plants in continuous forest.

Most studies investigating the effects of drought or fragmentation on tropical plants focus on trees (Laurance et al. 1998, Wright et al. 2005, Uriarte et al. 2016b), in part because they are reservoirs of carbon (Pan et al. 2011). ***However, herbaceous species can comprise up to 30% of the plant species in lowland tropical forests*** (Gentry and Dodson 1987, Gentry and Emmons 1987), where they are habitat and food for myriad animal taxa and economically and culturally vital non-timber forest products (Shackleton et al. 2018). Despite their biocultural importance, however, the way in which global change phenomena influences their population dynamics remains conspicuously understudied. In 1997 we began censusing thirteen populations of the understory herb *Heliconia acuminata* (Heliconiaceae) in an experimentally fragmented landscape in the central Amazon (>9500 plants to date). These demographic surveys and related data collection are the core of >25 publications that collectively provide one of the most comprehensive pictures available of how tropical forest fragmentation influences plants, including population and genetic structure (Bruna and Kress 2002, Bruna and Nogueira Ribeiro 2005, Cortes et al. 2013), seed dispersal, seedling recruitment (Bruna 1999, 2002, Uriarte et al. 2010, Uriarte et al. 2011), growth, survivorship (Bruna et al. 2002, Bruna and de Andrade 2011, Gagnon et al. 2011), and population dynamics (Bruna 2003, Bruna and Oli 2005). **We will use these data, along with high-resolution data on precipitation collected across our study landscape and a suite of advanced quantitative tools, to provide the first comprehensive test of the Fragmentation-Drought Hypothesis.** To do so we will quantify droughts and their magnitude, determine how droughts alter vital rates, and use these results to parameterize demographic models. **We will address these questions: (1)** Do droughts decrease post-seedling growth and survivorship, is this influenced by plant size, and are these effects exacerbated in fragments relative to continuous forest? **(2)** Do fragmentation and drought interact to influence plant reproduction, seedling establishment, and seedling survivorship? **(3)** Do changes in demographic vital rates interact to increase the probability of extinction in fragments relative to that in continuous forest under projected drought scenarios?
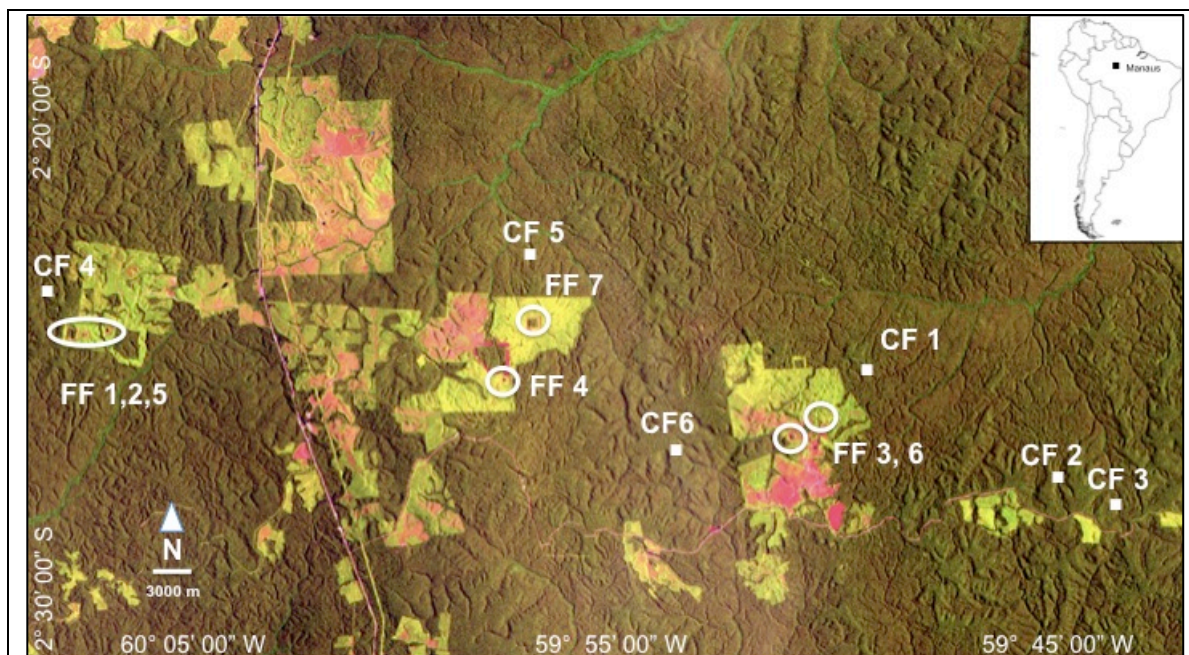

Fig. 3. Satellite image of the BDFFP. *Heliconia* demography plots shown as white points (Continuous Forest, CF) or circles (Forest Fragments, FF). Dark green is primary forest, light green & red are regenerating forest & pasture. See Table 1 for plant abundance in plots.

STUDY SYSTEM: The Biological Dynamics of Forest Fragments Project (BDFFP) was initiated in 1979 approximately 70 km north of Manaus, Brazil (2°30'S, 60°W). It is collaboratively funded and administered by the Smithsonian Tropical Research Institute and Brazil's National Institute for Amazonian Research (INPA). In addition to large expanses of continuous forest, the BDFFP has forest fragment reserves ranging in size from 1-100 ha (Fig. 1,3). These fragments were isolated from 1980-1984 by felling the trees surrounding the patch chosen for isolation and, in most cases, burning the downed trees once they dried. The vegetation regenerating around fragments is cleared regularly to ensure their isolation (Bierregaard et al. 2001). ***The BDFFP is an ideal location in which to conduct long-term research on habitat fragmentation and tropical plant demography.*** It is one of the few sites where focal fragments are of similar size and shape, where researchers collected pre-isolation data, and where there are no confounding effects of fire, hunting, and harvesting (Laurance et al. 2011). The changes in this landscape have been documented in >600 publications, and the BDFFP reserves have been designated protected areas by Brazil's federal government (Poder Executivo Federal 1985), ensuring their long-term integrity.

Heliconia acuminata (Fig. 4) is a perennial monocot native to Amazonia (Kress 1990) and is the most abundant understory herb at the BDFFP (Ribeiro et al. 2010). The >200 species of *Heliconia* and their hummingbird pollinators represent one of the most widely studied plant-pollinator associations in the Neotropics (Linhart 1973, Kress 1983, Kress 1990). Some *Heliconia* grow in large patches along forest edges or in disturbed areas and are pollinated by territorial hummingbirds. Others are found in the shaded forest understory at lower density, produce fewer flowers with less nectar, and are pollinated by hummingbirds that "trapline" from one plant to the next (Berry and Kress 1991). *Heliconia acuminata* is in this

Fig. 4. **(A)** *H. acuminata* flowers are open one day & pollinated by 2 species of *Phaethornis* (Bruna et al 2004a). **(B)** H. acuminata on edge of 1-ha fragment. Note dried leaves, likely a response to water stress (Bruna et al. 2003)

'understory' group; in our sites it is pollinated by *Phaeothornis superciliosus* and *P. bourcieri* (Bruna et al. 2004) and its seeds (1-3 per fruit, $\bar{x}$=2) and are dispersed primarily by a thrush and several species of manakin (Uriarte et al. 2011). Seeds germinate ~6 months after dispersal at the onset of the rainy season. While rates of seed mortality from burial under leaf litter can be high, seed predation is rare (Bruna 1999, 2002). This – along with the absence of a seed bank and minimal foliar herbivory – greatly facilitates efforts to quantify reproductive effort, interpret patterns of seedling emergence, and construct demographic models (Bruna 2003).

Demographic Data: In 1997-1998 we established a series of 5000 m^2 plots (50x100 m) in the BDFFP reserves in which we marked and measured all *H. acuminata*. The plots are located in continuous forest (n=6), 10-ha fragments (n=3), and 1-ha fragments (n=4), with distance between plots ranging from 500 m-41 km (Fig. 3, Table 1). Plots in 1-ha fragments were on one randomly selected half of the fragment, plots in 10-ha fragments were in the fragment center,

and plots in continuous forest were placed in locations 500-4000 m from the borders of secondary and mature forest (Fig. 3). Each plot is subdivided into 50 quadrats (10 x 10 m) marked with PVC pipe to simplify surveys and to facilitate the collection of complementary data all reproductive plants. We record plant shoot number and height as proxies for plant size; Both are correlated with leaf area, flowering, and survivorship (Bruna and Kress 2002, Bruna

Table 1. Summary of thirteen plots in which annual demographic surveys are carried out. Plots in forest fragments (FF) were established in 1997, as were Continuous Forest (CF) plots 1-3. CF-4-6 were established in 1998 (Bruna and Kress 2002).

Habitat type	Plot No.	Years Isolated	*H. acuminata in 2014 survey*
1-ha Fragments	FF 1-4	1980-1984	338 312 405 404 established 1997
10-ha Fragments	FF 5-7	1980-1984	293 624 826 *established 1997*
Continuous Forest	CF 1-6	- -	1581 1228 1341 *established 1997* 209 307 428 *established 1998*

et al. 2002). In 2008 we mapped the location of each plant to the nearest 10 cm; in subsequent years we did the same with all new seedlings. The plots and populations are far larger than those in most plant demographic studies (Fiske et al. 2008); this allows us to overcome potential biases in vital rates due to sampling bias (Fiske et al. 2008) and correct for any underlying gradients (Fiske and Bruna 2010). Finally, there is a seven-fold range in *H. acuminata* density in the forest plots, with density in several of the plots comparable to that in low-density fragments (Table 1). The existing variability, along with the statistical tests described below, will help to disentangle any effects of local density from those of fragmentation *per se*.

Complementary data: We have intensively studied the biology of *H. acuminata* and its responses to environmental variation, and can use these studies as needed to construct demographic models (e.g., Bruna 2003). For instance, we have studied the diversity of *H. acuminata*'s seed dispersers and how their movement and foraging is influenced by local resource availability and matrix composition (Uriarte et al. 2011). We also have data on rates of pollinator visitation (Bruna et al. 2004) and fruit production (Bruna and Kress 2002), fruit nutritional quality, seed germination rates, and seedling recruitment (Bruna 1999, 2002). We have data on soil chemistry and texture in plots, as well as canopy cover, topography, and the transmission of photosynthetically active radiation (PAR) to the understory (Uriarte et al. 2010). We also have access to data from multi-decadal studies at the BDFFP on bird and tree communities, with which we can determine the identity, location, and history of all trees > 10 cm dbh in our plots (Laurance et al. 1998) and the abundance and movement *H. acuminata*'s pollinators and dispersers (Stouffer and Bierregaard 1995, Stouffer et al. 2006, Powell et al. 2013). Finally, since its inception the BDFFP has recorded daily precipitation at multiple sites across the study landscape, including where our plots are located.

Our datasets overcome the four primary hurdles that have impeded tests of the Fragmentation-Drought Hypothesis, thereby providing an unparalleled opportunity to elucidate the independent *and* synergistic effects of these phenomena on population dynamics. Since we began sampling there have been two weak droughts (2004, 2006, both ENSO), three moderate ones (2002, 2009, both ENSO), and three severe ones (1997 ENSO, 2005 & 2010 ASST), and planned sampling will likely include the effects of at least 1 additional ENSO (National Weather Service 2014, 2015). This is sufficient replication for an unprecedented analysis of how droughts (in general) and their severity (in particular) influence the long-term demography of understory plants, including lagged effects. Our dataset also comprises sufficient years to detect the different *types* of effects that drought and fragmentation

can have on demography. This includes intense and immediate effects (e.g., Bruna 1999, Bruna et al. 2002) as well as subtle but chronic ones (Gagnon et al. 2011). For example, we previously compared the annual growth rates of *H. acuminata* in fragments and continuous forest from 1998-2007 and found that while average yearly growth was lower in fragments in 7 of 9 years,
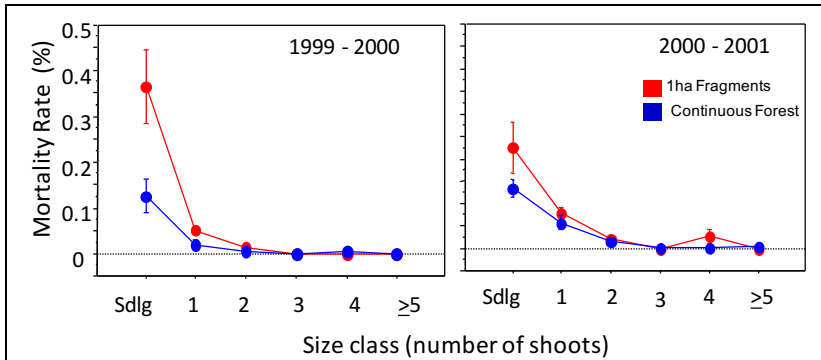

Fig. 5. Probability of *H. acuminata* survival in two non-drought years as a function of plant size.

none of these annual differences were statistically significant (Gagnon et al. 2011). After nine years of chronically reduced growth, however, plants in fragments were significantly smaller than those in continuous forest. This resulted in much lower cumulative reproduction (Gagnon et al. 2011). If the effects of drought on growth, survivorship, or reproduction have a similar time-lag, our dataset is one of the few whose duration is sufficient to detect them. ***Finally, the biology and life-history of H. acuminata make it an excellent model system with which to study the demography of tropical plants, especially herbaceous species.*** Like most such species it is long-lived, slow growing, and found primarily in the shaded understory. *Heliconia acuminata* is self-incompatible (Darrigo & Bruna, *unpubl data*) and requires animal vectors for pollination and seed dispersal (Howe and Smallwood 1982), which is also typical of most species in lowland tropical forests (Bawa 1990); hummingbird pollination and avian seed dispersal are both ubiquitous in the tropics (Blake and Loiselle 1992, Kress and Beach 1994).

TESTING THE FRAGMENTATION-DROUGHT HYPOTHESIS: ANALYTICAL METHODS

While we know the years in which there were major regional drought events, our first step will be to confirm their duration and quantify their intensity with the BDFFP precipitation data. To do so we will calculate the Standardized Precipitation Index (SPI) for each demographic transition year by first fitting three-month moving precipitation totals to a gamma distribution function; this distribution provides a probability distribution for 3-month totals that we can then use to derive SPI values in units of standard deviation (McKee et al. 1993). We will then use the NASA-JAXA 3B43v6 Tropical Rainfall Measuring Mission (TRMM) monthly dataset (1998-2015) to calculate the years of precipitation anomalies and qualitatively compare them to our regression-model estimates of SPI. This is the foundation for identifying drought years and estimating their magnitude (McKee et al. 1993). To identify post-2015 anomalies we will use data from the Global Precipitation Measurement Mission (GPM) launched in 2014 as a follow-up to TRMM. As per Duffy et al.'s (2015) analysis and projections of droughts in the Amazon, transition years will be provisionally classified as "severe drought" (SPI $\leq$ -2), "moderate drought" (SP1 = -1.0 to -1.99), or "non-drought" (SPI > -1). It is important to note that because SPI is an index of 'meteorological drought', it is a conservative estimate of the potential impact of reduced precipitation on plants (i.e., 'agricultural drought'; Duffy et al. 2015).

Question 1: Do droughts decrease post-seedling growth and survivorship, how is this influenced by plant size, and are these effects exacerbated in fragments relative to forest? Drought- and fragmentation-related changes in the growth of tropical trees can vary as a function of individual size (Condit et al. 2004, Phillips et al. 2010), but mortality appears to increase sharply in drought years (Williamson et al. 2000, Slik 2004) and in forest fragments. In contrast, our prior work indicates post-seedling *H. acuminata* stressed by abiotic conditions

shrink by shedding shoots and leaves (Figs. 1, 4b) but have high survival rates (Fig. 5, Bruna et al. 2002). Furthermore, we have shown with both experiments (Bruna et al. 2002) and analyses of our long-term survey data that *Heliconia* growth is reduced in fragments (Gagnon et al. 2011, Fig. 2) and that this reduced growth – especially of large plants – is the primary contributor to lower population growth rates there (Bruna and Oli 2005, Fig. 6). These reductions are even more dramatic on fragment or forest edges (Bruna et al. 2002), where abiotic conditions are the most severely altered in fragmented systems (Broadbent et al. 2008). This suggests the potential for drought related declines in growth throughout the BDFFP landscape, including in forest, but that the declines could be exacerbated in fragments.

Methods: We will use the powerful 'functional smoothing' approach recently developed by Teller et al. (2016) to test for synergistic effects of fragmentation and drought on *H. acuminata* growth and survivorship, determine if there are time-lags in the appearance of these effects, and if they are influenced by local conspecific density. Until recently, most of the statistical approaches used to address "high-dimensional problems" such as these required assumptions without which the number of candidate models to be compared with model selection procedures quickly became unwieldy (e.g., a limited suite of options for competition kernels, restricting the number of climate variables included, aggregating continuous climatic data into an *ad hoc* time interval). Teller et al.'s spline methods, which estimate smooth functions over continuous domains such as space or time, overcome these shortcomings while outperforming other approaches for assessing temporal lags (Teller et al. 2016). They also have one other important advantage over other methods of testing for density dependence or changes in demographic vital rates: the resulting models are also the growth and survivorship functions used to construct the Integral Projection Models we will use in our Population Viability Analysis (Question 3).

Although functional smoothing methods are tractable and efficient, simulations suggest that model performance is sensitive to the number of observations of climate history and the variance in the climate response (Teller et al. 2016). We will therefore initially conduct our analyses and models with the suggested space-for-time substitution (i.e., pooling annual data from the multiple plots in a habitat type; Doak and Morris 2010, Teller et al. 2016, Tenhumberg et al. 2018), which will result in a number of data-years in excess of those at which Teller et al. observed peak model performance. We will also assess the performance of all our models using the recommended semi-parametric power analyses with simulated data (Teller et al. 2016). These power analyses will also help us determine if we can follow-up with analyses of plot-level data (i.e., comparing the different plots within each habitat class).

Density Dependence: Our evaluation of how drought and habitat type shape individual growth and survivorship will begin by testing for density dependence. We will model survival probability and the expected size of surviving plants as functions of plant size (z, log[size]), an index of competition (crowding, w), the habitat type in which plants are located (a categorical variable used to identify the surrounding subplots), and – if necessary – habitat type (H, i.e., 1-ha fragment, 10-ha fragment, or continuous forest). Although we have previously found shoot number to be the best predictor of vital rates, we will test for potential density dependence using three different metrics of *H. acuminata* size – height, shoot number, and height+shoot (while our previous matrix models used categories based on stem number, IPMs can be constructed with measurements that are continuous, categorical, or a combination of both, Ellner and Rees 2006). The general equation for the crowding experienced by a *H. acuminata* individual *i* is the sum of conspecific neighbor sizes across a set of concentric rings around the plant,

$$w_{i,k} = F(d_k)A_{i,k} \hspace{3cm} \text{(eqn 1)}$$

where F is the competition kernel, d_K is the average of the inner and outer radii of ring k, and $A_{i,k}$ is the total size of conspecific plants in ring k around plant i. The total crowding on plant i is

$$W_i = \sum_k w_{i,k}. \qquad\qquad \text{(eqn 2)}$$

W_i, which can be used (if necessary) as a covariate in demographic models. For example, the survival probability of a plant i in a forest fragment might be modelled as:

$$\text{logit}(s_i) = \alpha_0 + \alpha_1 z_i + \gamma_q + \delta W_i \qquad\qquad \text{(eqn 3)}$$

where a_0 and a_1 are the intercept and slope parameters for size z, and c is the coefficient for subplot group q. Between-year variation can be incorporated by fitting a_0 or a_1 as fixed or random effect; we will initially use fixed effects in light of recent simulations suggesting fixed- and random-effect demographic models perform equally well (Metcalf et al. 2015). The coefficient d gives the effect of density on survival ($d<0$ = competition). The procedures for making the competition kernel F a smooth function of spline coefficients $\vec{b}$ and then fitting Eq. 3 will follow Teller et al. (2016). If there is evidence for density dependence, we will determine if its magnitude differs between habitat types (i.e., forest and the two fragment size classes), then follow-up with models including year as a factor to test for how it changes over time.

Testing for effects of drought and fragmentation on growth and survivorship: Following our test for density dependence, we will test for effects of drought on growth and survivorship, and if these differ by habitat, with Functional Linear Models (FLMs) of lagged precipitation data. FLMs are smooth splines $f(x)$ whose values are multiplied by a vector z_x and then summed. If z_x is a time series of monthly data, the resulting estimated spline function will reveal the contribution of each month to the observed demographic response. The model takes the form:

$$\eta(y_{i,t}) = \sum_{j=0}^{k} \beta_j x_{i,t,j} + \sum_{m=0}^{n} f(m) z_{i,t,m} \qquad \text{(eqn 4)}$$

where $y_{i,t}$ is the response at site i in year t. The first term is a vector of k linear predictors (e.g., habitat type) as in a general linear model. The second one is the functional linear term of precipitation $z_{i,t,m}$ for each site i, time t and m months before year t; n is the maximum number of lags considered. We will fit two FLMs: one for precipitation (total monthly rainfall) and one for drought intensity (the monthly Standardized Precipitation Index, SPI). We will fit models with linear terms for habitat type and a habitat type x year interaction (sensu Tenhumberg et al. 2018). The habitat x year terms allow for differences in responses between forest and fragments and for linear trends across years other than predicted by variation in precipitation.

We will estimate the FLMs using the procedures and code in Teller et al. (2016). Briefly, we will evaluate how vital rates in year t are influenced by precipitation up to m months prior (m = -1,-2,-3…N); our initial models will consider a lag of N = 48 months. We will then model the relationship between the annual rates and each metric by fitting smoothing splines and assess which model (i.e., driver) best fits the data and if model fit is improved when including habitat type and habitat x year interactions with AIC. Note that equation 4 only considers linear growth responses; if our results suggest responses that are non-linear (i.e., that growth responses to drought are size dependent), then we will extend our FLMs *sensu* McLean et al. (2014).

Alternative methods: While we are confident in the suitability of FLMs to disentangle climate x fragmentation effects on demography, there are alternatives should spline-methods appear to

be data-limited. Our first is to use mixed-models to estimate demographic functions (Rees and Ellner 2009). These can also be used to the construct stochastic IPMs for Question 3, though with some important limitations (see *"Demographic methods"*, below). Alternatively, Wu and Huang's (2009) ensemble empirical mode decomposition method (EEMD) is a highly efficient Monte Carlo method for processing non-linear and non-stationary signals (Guan et al. 2012). It has the major advantage of not requiring the imposition of a subjectively selected trend to remove the intrinsic growth trend, something common in dendrochronology and other analyses of annual growth and climate. Finally, we can explore the use of Stochastic Antecedent Models (SAMs, Ogle et al. 2015), which evaluate the exogenous (e.g., droughts, fragmentation) and endogenous (e.g., past size, flowering) components of 'memory' in ecological systems.

Question 2: How do fragmentation and drought interact to influence reproduction, seedling establishment, and seedling survivorship? Trees often produce bumper crops of fruit in ENSO years (Wright et al. 1999, Curran and Leighton 2000), perhaps due to bet-hedging or thinner canopies reducing light limitation. There is also high seedling mortality during droughts (Gilbert et al. 2001), though seedlings that persist may grow quickly due to high light resulting from canopy dieback (Delissio and Primack 2003). *Heliconia acuminata* individuals of the same size have similar probabilities of flowering in fragments and forest in non-drought years (Fig. 7), and fruit production in fragments and forest is similarly variable (Bruna and Kress 2002). However, there are far fewer reproductive *H. acuminata* in fragments than continuous forest because populations in fragments are generally smaller and skewed towards pre-reproductive size classes (Bruna and Kress 2002, Gagnon et al. 2011). A fragmentation-drought synergism could further depress flowering in fragments if adult survivorship or growth are proportionately lower in fragments following droughts (Question 1). This would likely also translate into lower seedling abundance there, as the number of seedlings emerging in plots is strongly correlated with the number of flowering plants in them the previous flowering season (Bruna 2002). There could also be fragmentation-related differences in recruitment if there were any time-lagged effects of droughts on flowering and individuals in fragments were more susceptible to such effects. Finally, droughts could lead to lower seedling abundance in fragments irrespective of any effects on flowering or fruiting by further reducing the already
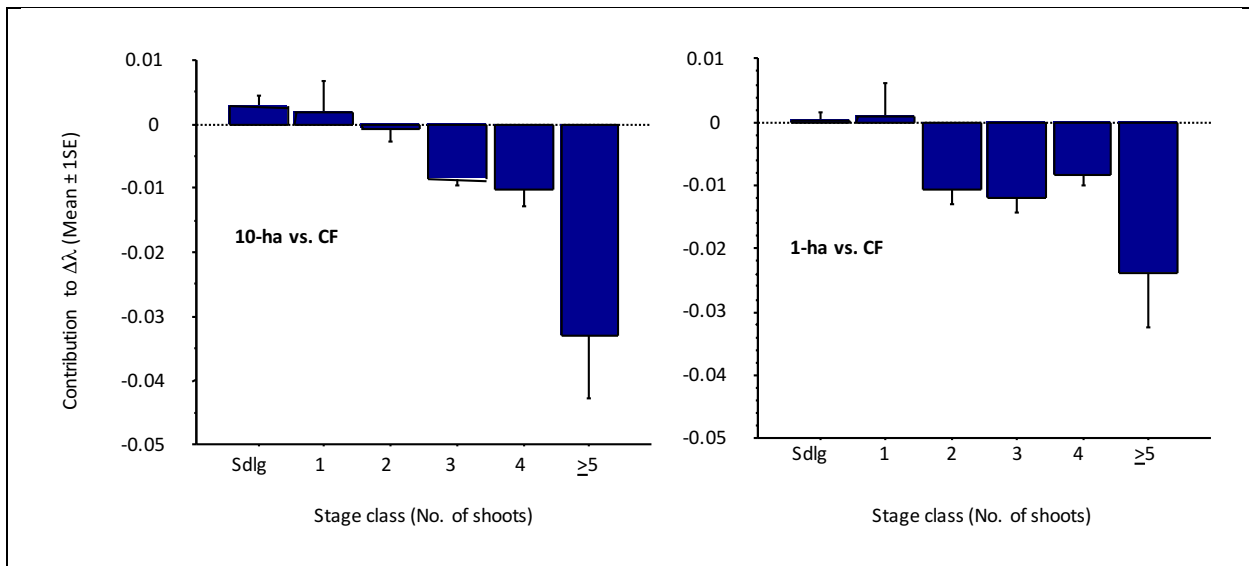

Fig. 6. From 1998-2003 λ of *H. acuminata* populations was lower in 1-ha and 10-ha fragments than continuous forest (CF). This shows the contribution (avg. over 5 transition yrs) of different stage classes to the difference in λ between these habitats (i.e., $\Delta\lambda$). Because CF was used as the 'control' matrix in the LTRE, negative contributions indicate a stage lowers λ in fragments; the bar represents by how much (Bruna & Oli 2005).

lower rates of seed germination (Bruna 1999, 2002) and seedling survivorship (Bruna 2003) in fragments. Because *H. acuminata* seeds are dispersed <25 m (Uriarte et al. 2011), any reductions in fragments are unlikely to be offset by dispersal from populations in nearby forest.

Methods: To determine how drought and habitat type influence the different components of reproduction, and test for potential lags in these effects, we will use the 'functional smoothing' framework described above for Question 1. We will again begin by testing for density dependence all three vital rates: probability of flowering, seedling establishment, seedling survivorship. We will then follow-up with FLMs including habitat type and habitat x year interactions along with an initial lag of N = 48 months since drought. As before mixed-models, EEMD, and SAMs are again the alternative frameworks for analysis

Question 3: Do the observed changes in demographic vital rates interact to increase the probability of extinction in fragments relative to that in continuous forest? The FDH does not merely predict that droughts and fragmentation interact to alter the demography of *H. acuminata* – they undoubtedly do, and to determine how we will use Life-Table Response Experiments (Caswell 1989). *Rather, the central prediction of the FDH is that drought-driven changes in demography will make populations in fragments far more likely to go extinct than those in continuous habitat.* To test this hypothesis, we will use Population Viability Analyses (PVA, Morris and Doak 2002) to estimate the likelihood of *H. acuminata* extinction from 1-ha fragments, 10-ha fragments, and continuous forest after 50, 100, and 150 years of **four climate scenarios: (1) no change in the historical frequency or severity of droughts, (2) increased drought frequency**, **(3) increased drought severity**, and **(4) increases in both drought frequency and severity**. These scenarios reflect the most advanced projections to date of Amazonian precipitation and droughts through 2100 (Duffy et al. 2015), and our approach to testing for synergistic effects of drought and habitat fragmentation on population viability builds on recent studies investigating how population dynamics are altered by climatic variability (e.g., Doak and Morris 2010, Dalgleish et al. 2011, Nicole et al. 2011, Williams et al. 2015, Molowny-Horas et al. 2017) and multiple disturbances (e.g., Mandle et al. 2015, Tye et al. 2016).

Demographic Methods. Our PVA will be constructed with Integral Projection Models (IPMs, Easterling et al. 2000, Ellner and Rees 2006, Merow et al. 2014). Below we describe the general structure of our IPMs and the modeling framework simulating the independent and synergistic effects of fragmentation and drought on population viability. We then describe the Life-Table Response Experiments (LTRE) with which we will investigate the demographic mechanisms underlying changed in population growth rate in the different scenarios. For both the PVA and LTRE we focus on transient rather than asymptotic dynamics for two reasons. First, our prior studies with asymptotic models indicate populations will not have reached stable size distributions in the time frame encompassed by our simulations (Bruna 2003). Second, transient dynamics appear to be a superior means of assessing population-level responses to perturbations – such as drought – whose long-term magnitude and potential for synergistic effects with other disturbances are unclear (Stott et al. 2011).

Briefly, an IPM describes the change in the number of individuals of different sizes from year *t* to year *t+1.* It does so with a 'kernel' composed of functions that describe size-based survivorship, growth and reproduction from one year to the next. Our full model takes the form:

$$n(y,t+1) = \int_{\Omega} [p(x,y) + f(x,y)]n(x,t)dx \qquad \text{(eqn. 1)}$$

where $p(x,y)$ represents transitions of an individual of size x to size y attributable to survival and growth: $p(x,y) = s(x,y)g(x,y)f(x,y)$ describes per-capita production of y sized individuals in the next census by reproductive individuals of size x (i.e., the recruit density function at the next census): $f(x,y) = s(x,y)f_n(x)p_Ef_d(y)$, where $s(x)$ is size specific survival, $f_n(x)$ is the number of fruits produced by a plant of size x, p_E is the number of seedlings resulting per fruit, and $f_d(y)$ is the size distribution of seedlings. The vital rate functions underlying $P(y,x)$ and $F(y,x)$ come from tests of Question 1 & 2; should these analyses indicate there is density dependence in vital rates, then we will modify our IPMs

Fig. 7. Probability of *H. acuminata* flowering as a function of how many shoots they have in fragments vs. continuous forest (Bruna and Kress 1999).

using approaches similar to those described in Childs et al. (2011) and Ellner et al. (2016). Note there are no terms for seed banks, seed predation, or the effects of herbivores as we have shown these are negligible (Bruna 2002, Bruna and Kress 2002). We are also able to assume no immigration or emigration (sensu Doak and Morris 2010) because we have shown seed dispersal is spatially restricted (Uriarte et al. 2011, Cortes et al. 2013). Our IPMs will be built and analyzed with the R statistical programming language (R Core Team 2018) using the code from Ellner et al. (2016) and package IPMpack (Metcalf et al. 2013), which we have previously used to construct IPMs for other plant species at the BDFFP (Bruna 2014, Bruna et al. 2014).

Including interannual variation in environmental conditions in IPMs can be challenging (Metcalf et al. 2015), with methods for doing so developing rapidly. There are currently two primary methods for modeling the effects of environmental stochasticity on vital rates. The first is *Parameter Selection* ("random effects", sensu Rees and Ellner 2009), where one constructs a completely new kernel for each year of the IPM by drawing values of vital rates from distributions. The second is *Kernel Selection* ("fixed effects", sensu Rees and Ellner 2009), where one instead uses the annual survey data to create a kernel for each transition year, and then selects one of these kernels each time step of the IPM (analogous to resampled projection matrices; Boyce et al. 2006). While there are some important advantages to Parameter Selection (reviewed in Rees and Ellner 2009, Metcalf et al. 2015), recent simulations suggest the simpler and faster Kernel Selection performs at least as well when estimating population growth rates (Metcalf et al. 2015); it also has the advantage of maintaining the correlations between different vital rates (Metcalf et al. 2015, Williams et al. 2015). We will therefore use Kernel Selection to build IPMs, unless our analyses for Questions 1 & 2 indicate mixed-effects models are superior to spline-methods for characterizing environmental variation in vital rates. Note, however, that quasi-extinction risk can be sensitive to correlation between vital rates (Ellner et al. 2016), making it very important to assess the sensitivity of model projections to correlations when building IPMs with Parameter Selection (Rees and Ellner 2009).

Habitat-specific population viability in alternative drought scenarios: Before assessing population viability under different drought scenarios, we will first isolate the effect of habitat fragmentation *per se.* To do so we will use IPMs to project population dynamics in 1-ha fragments, 10-ha fragments, and continuous forest for 50, 100, and 150 years using *only* kernels from "non-drought" years. The initial population vectors will be based on the range in population size and structure observed in each habitat in the first census, and each habitat's
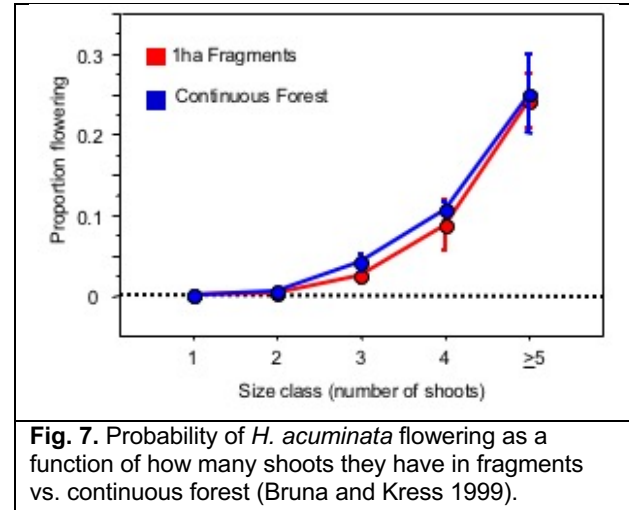
non-drought kernels will have equal probability of being selected in each time step. We will use the projected population sizes in each year to calculate λ_s (as the geometric mean of the annual growth rates), the final population size, and if the population size fell below the quasi-extinction threshold of 25 individuals at the end of the simulation. The simulations of each time interval and initial population size will be repeated 1000 times, after which we will calculate the mean and 95% confidence intervals of λ_s and final population size and the probability of quasi-extinction. We will use randomization tests (Caswell 2001) to compare λ_s of forest and fragment populations, and determine how the simulation length, initial population size, and habitat influenced final population size and quasi-extinction with mixed effects models (Bolker et al. 2009) in which simulation number is included as a random effect (Metcalf et al. 2015).

We will then use a similar procedure to quantify the effect of historical and predicted patterns of drought frequency. We will first conduct PVAs for each habitat assuming **pre-1980 drought frequencies**. To select the kernel for each year of the PVA we will (1) select a value of the Standardized Precipitation Index for the Amazon from the probability density function of 1950-1979 SPI values (Fig. 6 of Duffy et al. 2015), (2) classify this SPI value as 'severe drought' (SPI $\leq$ -2), 'moderate drought' (SP1 = -1.0 to -1.99), or 'non-drought' (SP1 > -1), and then (3) randomly select a habitat-specific *H. acuminata* kernel from the same drought category. To simulate the effect of **increases in drought frequency**, we will use the probability density function of SPI values predicted for 2071-2100. To simulate increased frequency of droughts without increased drought intensity, we will truncate the SPI function such that the maximum drought intensity is the same as for 1950-1979. To account for the fact that probability of having a drought year in Amazonia increases almost four-fold from 2020 to 2100 (Duffy et al. 2015), we will shift the mode of the truncated distribution to more negative SPI values as simulation length increases. To simulate **increased drought intensity**, we will shift the SPI probability density function to more negative values reflecting the increased likelihood of severe droughts at each time period (Fig. 7 in Duffy et al. 2015). Finally, we will model the effects of **simultaneous increases in drought frequency and intensity** by shifting both the mode and lower range of the predicted SPI function. We will again use randomization tests (Caswell 2001) to compare λ_s in 1-ha fragments, 10-ha fragments, and forest under different drought scenarios, and mixed models to compare the frequency of quasi-extinctions and final population sizes.

As for Questions 1 and 2, we will conduct the above analyses using 'summary populations' created by pooling the data from the multiple plots in each habitat type. This is an robust means of synthesizing the demography of multiple populations – it corrects for the disproportionate weight that low plant numbers in some size classes can give to estimates of vital rates in some locations (Horvitz and Schemske 1995, Caswell 2001) and ensures that IPMs are based on the full range of vital rates observed in a habitat (Bruna 2003, Bruna et al. 2014). However, we will also conduct plot-level analyses of population viability using the framework above if analyses for Q1 and Q2 indicate we have sufficient power. This will provide insights into the magnitude of inter-fragment demographic variation, and the role of other factors thought to shape plant demography (e.g., population size, location in the landscape, canopy cover, soil properties).

Identifying the demographic mechanisms behind changes in λ_s using LTRE: Our prior simulations suggest extreme reductions in recruitment are necessary for population declines, and empirical estimates of seedling establishment in fragments were frequently below these thresholds in non-drought years (Bruna 2003). This suggests a demographic mechanism by which increasing droughts could reduce λ in fragments: by exacerbating the already lower probability of seedling establishment in these sites. However, our initial simulations were based on asymptotic matrix models and did not take into account inter-annual variability in vital rates. Furthermore, in subsequent analyses we found that the lower values of λ projected for
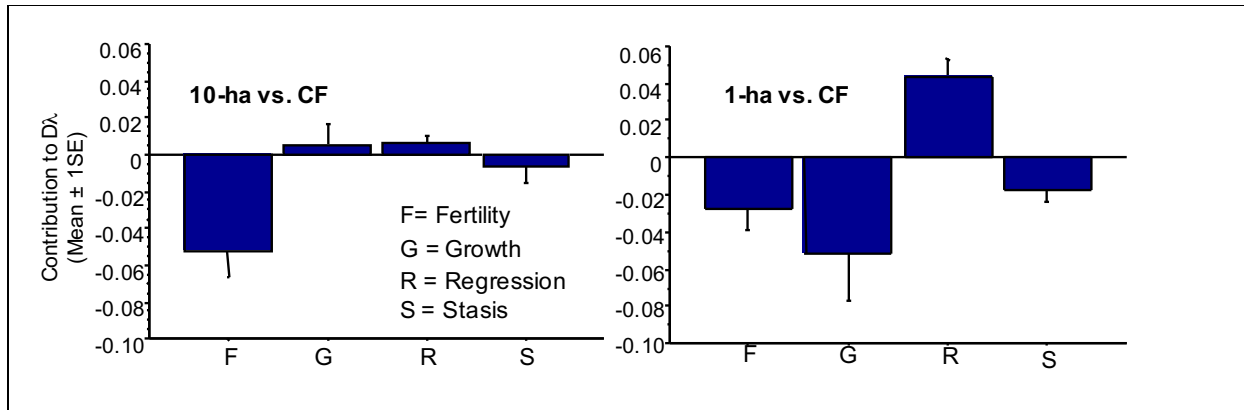
Fig. 8. From 1998-2003 λ of *H. acuminata* populations was lower in 1 and 10-ha fragments than continuous forest (CF). This shows the contribution (avg. over 5 transition years) of different demographic vital rates to the difference in l between these habitat types (i.e., Δλ). Because CF was used as the 'control' matrix in the LTRE, negative contributions indicate a rate lowers λ in fragments and the value of the bar represents how much (Bruna & Oli 2005).

populations in 1-ha fragments were due primarily to changes in vital rates related to growth (i.e., more shrinking due to shoot loss, lower growth rates than in continuous forest, lower likelihood of stasis in a stage class, Fig. 8). We also found that large and long-lived plants make the greatest contribution to growth rates in both fragments and forest (Bruna and Oli 2005). Taken together, this suggests alternative demographic pathways by which increases in drought severity/frequency could elevate extinction risk – via reduced growth or increased rates of regression to smaller size classes, both of which can lead to reduced likely hood of flowering. While the longevity of established *H. acuminata* could theoretically buffer populations from the effects of climatic variability (Morris et al. 2008), we predict that the changes in growth- and regression- related vital rates resulting from more frequent or intense droughts will overwhelm this buffering, reducing population viability in fragments relative to continuous habitat.

We will test this hypothesis using ***life-table response experiments* (LTRE)**, with which we will identify which demographic stages (Fig. 6) and vital rates (Fig. 8) that are the primary contributors to differences in population growth rate between the different habitat x drought scenario combinations. LTRE is a retrospective analyses that decompose the observed differences in projected values of λ between different populations into the contributions from different demographic variables (Caswell 1989). Rees and Ellner (2009) have developed an elegant method for conducting IPM-based LTREs for populations in temporally varying environments (Rees and Ellner 2009). However, this method assumes focal populations are close to the stable size distribution, and hence is best suited to analyses of asymptotic growth rates. We will therefore conduct our LTRE using Maldonado-Chaparro et al.'s (2018) recently developed method for Transient LTRE, which partitions the variance of the realized annual population growth rate into contributions from model parameters at different time lags. In addition to being well-suited to decomposing the effects of environmental drivers, this method also has regression-based diagnostics similar to those put forward by Rees and Ellner (2009).

INTELLECTUAL MERIT: Climate-Land Use Interactions are posited as an emergent and powerful driver of changes in ecological processes, including extinctions (Laurance et al. 2014, Seidl et al. 2017). However, few studies have evaluated how they interact to influence population dynamics and extinction risk (Selwood et al. 2015, Ehrlen et al. 2016). In part this is because the long-term demographic datasets needed to do so are rare, especially outside of the temperate zone (Selwood et al. 2015). Our project overcomes this limitation, allowing us to use

a cutting-edge set of quantitative tools to address questions central to conceptual models of fragmentation-climate interactions (Didham et al. 2012, Selwood et al. 2015). In doing so we will advance our understanding of how a widespread species – one characteristic of a speciose and important but understudied group of tropical plants – responds to global change. Our long-term census data will be added to datasets we have already made publicly available for use by the ecological community; these data can be used to advance scholarship in areas ranging from demographic methods to life-history theory to spatially explicit population dynamics (Ehrlen and Morris 2015). Finally, the results of our project will indicate what life-history stages or demographic vital rates – and therefore ecological interactions or conditions – should be the focus of future data collection. *Our results will be the foundation of an NSF-LTREB proposal in to identify how the precise causal mechanisms by which drought alters demography in fragmented landscapes (e.g., changes in soil moisture, light levels, temperature, competition).*

BROADER IMPACTS: Our project will address three of NSF's Broader Impact criteria. **First,** we *"advance discovery while promoting teaching, training, and learning"* by recruiting a postdoctoral scholar to conduct the proposed research and working with them as they prepare to present the results at meetings and publish manuscripts for describing the results. We have also requested funding for the postdoc to visit the field sites, which will improve their understanding of the system and provide unique insights into the collection of demographic data in tropical forests. We believe this will in turn improve their models, presentations, papers, and independent research and teaching programs. **Second**, we will *"enhance infrastructure for research and education"* by making our annual surveys, associated datasets, and code for their analysis publicly available for (re)use by archiving them in data repositories. These repositories are critical infrastructure for research and education – not only can they be used in courses or for testing new hypotheses, they also allow students and other researchers at institutions with limited financial resources to gain access to long-term data or data collected at remote locations (Bruna 2010). **Third,** we will *"broaden dissemination to enhance scientific understanding"* by working in collaboration with Dr. Eric Segal, who is the Director of Education at UF's Harn Museum of Art. Bruna has previously collaborated with Dr. Seagal on an educational project using materials from the Harn Collection – an analysis of the plants and animals in chromolithographs from the *Ornithologie Bresilienne ou Histoire des Oiseaux du Bresil* by Jean Theodore Descourtilz (1796-1855; https://descourtilz.wordpress.com). This time we will use the Harn's collection to develop an exhibit for the Lockhart Faculty Gallery on how tropical landscapes have been reshaped by human activities, including climate change. This will be accompanied by a description of our project and results. Admission to the Harn is free; it receives >100K visitors per year, including large numbers of K-12 and university students.

RESEARCH TEAM: This project continues the productive collaboration between Bruna and Uriarte. Bruna has over 20 years of experience at the BDFFP, as well as skills in the development and application of demographic models. Uriarte uses a diverse array of advanced quantitative methods to understand how tropical forests are shaped by factors such as climate, land-use, and local biotic and abiotic conditions (Uriarte et al. 2004, 2005, Schwartz et al. 2015, Uriarte et al. 2016a). Together we will select and mentor a Postdoctoral Scholar to conduct the proposed research. The proposed analyses are complex, challenging, and time-intensive; hiring of a postdoctoral researcher is an efficient and cost-effective means of successfully completing them in the proposed time-frame that also provides an opportunity for training an early-career scientist in advanced statistical and modeling techniques. While the postdoc will be based with Bruna at the University of Florida, they will have the opportunity and funds to travel to Columbia University to work with Uriarte. Bruna will be responsible for data management and archiving, as well as implementing the exhibit at the Harn Museum with Dr. Segal.