HOT AND BOTHERED: CHANGES IN MICROCLIMATE ALTER CHLOROPHYLL FLUORESCENCE MEASURES AND INCREASE STRESS LEVELS IN TROPICAL EPiphytic ORCHIDS

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Premise of research. Tropical epiphytes are susceptible to climatic changes, as evidenced by documented population declines, range contractions, and range shifts; however, physiological changes in individual plants may also be indicative of deteriorating climate conditions. Consequently, physiological analyses of tropical epiphytes whose natural habitats are constrained by climatic conditions are warranted to evaluate their responses to potential changes in these conditions, to assess their vulnerability, and to guide conservation actions.

Methodology. Here we investigate photosynthetic processes in Puerto Rican Lepanthes species, a group of Neotropical epiphytic orchids, as a model system to determine whether altered microclimate conditions elicit adverse physiological responses. We tested for differences in chlorophyll fluorescence, measured as $F_v/F_m$, as an indication of plant stress under modified temperature, humidity, and air vapor pressure deficit.

Pivotal results. Mean $F_v/F_m$ was positively correlated with mean relative humidity and negatively correlated with mean temperature and air vapor pressure deficit. Collectively, plants exposed to altered microclimate conditions had significantly lower mean $F_v/F_m$ than plants in unaltered conditions. Plants in altered microclimate conditions were also more likely to exhibit declines in $F_v/F_m$ over time, and they exhibited greater reductions in $F_v/F_m$ over the course of the study.

Conclusions. Epiphytic plant species such as Lepanthes could exhibit declines in $F_v/F_m$ and experience greater stress in their natural habitats if current warming and drying trends continue as anticipated in Puerto Rico and elsewhere. Declining $F_v/F_m$ is a robust indicator of plant stress, and several studies show that increased stress can promote leaf loss, limit reproduction, and lower survival rates. Thus, analyses of $F_v/F_m$ can be advantageous for monitoring epiphytic orchids and other vulnerable plant species by offering a valuable means for detecting adverse responses to climate change.

Keywords: climate change, conservation physiology, Lepanthes (Orchidaceae), $F_v/F_m$, Puerto Rico, rare plant ecology.

Introduction

Global climate change is having widespread impacts on the earth’s plant communities (Pimm and Sugden 1994; Foster 2001; Walther et al. 2002), and understanding how changing environmental conditions can affect plant species at different levels is critical for conservation efforts (Levin 1992; Lugo and Scatena 1992; Keith et al. 2008). Population studies on plants have already uncovered negative demographic trends resulting from global climate change (Zotz and Schmidt 2006; Keith et al. 2008; Allen et al. 2010). Similarly, landscape-level analyses have documented adverse geographic effects brought about by changing climatic conditions in plant communities across the planet (Foster 2001; Walther et al. 2002; Parmesan 2006; Colwell et al. 2008; Larsen et al. 2011). In addition to the findings emphasized by demographic and macroecological analyses, physiological studies have revealed that detrimental responses from plants at the individual level due to climate change are common (Zotz and Hietz 2001; Allen et al. 2010; Seaton et al. 2010). Such studies are crucial for understanding the processes that may underlie and precede larger-level changes.

Among all plant groups, epiphytes are particularly vulnerable to changes in climate because of their direct interface with the atmospheric environment and microclimate conditions, specifically temperature and moisture availability in their immediate environment.
ate surroundings (Lugo and Scatena 1992; Benzing 1998; Hietz 1998; Foster 2001; Zott and Bader 2009; Mondragon et al. 2015). For that reason, the orchid family (Orchidaceae), which is the largest family of flowering plants (~725 genera, ~25,000 species), may be especially vulnerable to climate change since an estimated 500 of its genera and almost 20,000 of its species are epiphytes (Madison 1977; Dressler 1981; Hietz 1998; Seaton et al. 2010; Mondragon et al. 2015). Considering that epiphytic orchids often exhibit specificity in microhabitat associations (Johansson 1974; Pupulin et al. 1995; Blanco 2003; Zott 2007; Adhikari et al. 2012; Crain 2012), analyses of individuals’ responses to climate change is an important undertaking for plant conservation biologists.

Several studies have linked alterations in climatic variables to adverse geographic, demographic, ecological, and biological trends observed in epiphytic orchid species. Liu et al. (2010) warn that dozens of epiphytic orchid species in just a single reserve could experience range contractions or be extinguished as a consequence of climate change. Additional studies show that specific variations—such as higher temperatures and lower moisture availability—are negatively correlated with growth, reproduction, and recruitment in certain orchids (Sanford 1971; Zott and Schmidt 2006; Oliva-Arenas et al. 2011; Williams et al. 2015). Tremblay and Salguero-Faría (2001) documented increased mortality in an epiphytic orchid population exposed to warmer and drier conditions within its natural habitat. Moreover, several horticultural experiments demonstrate that higher temperatures can inhibit spike formation and flower production (Sakanishi et al. 1980; Lopez and Runkle 2005; Blanchard and Runkle 2006; Lopez and Runkle 2006; Chen et al. 2008) and decrease flower and inflorescence longevity in certain orchids (Lopez and Runkle 2004). Other studies reveal that increased temperatures and low relative humidity can alter photosynthetic processes in some species (Zott and Tyree 1996; Stancato et al. 2001; Ali et al. 2003; Pollet et al. 2009, 2010). Thus, it is plausible that many epiphytic orchids could be highly susceptible to climate change, and detailed analyses are needed to detect threats at early stages.

Here we explore how anticipated changes in local climate conditions may affect a group of tropical epiphytic orchids from the genus *Lepanthes* Sw. (Orchidaceae) on the island of Puerto Rico. In recent decades, the climate in Puerto Rico and much of the Caribbean has generally become warmer and drier, and several models predict that these trends in temperature and moisture availability will endure for the next 50 yr (Scatena 1998; Neelin et al. 2006; Hayhoe 2012; PRCCC 2013). Furthermore, projections indicate that climate change in Puerto Rico is expected to outpace global averages (Hayhoe 2012). Over the past century, mean monthly and annual temperatures in Puerto Rico have increased by more than 0.01°C per year (PRCCC 2013). Moreover, annually Puerto Rico has had a greater number of days with maximum temperatures ≥32.2°C and fewer days with temperatures ≤23.9°C. Mean annual temperatures on the island are expected to increase by an additional 2°–5°C by the end of this century (PRCCC 2013). In terms of moisture availability, multiple analyses have indicated that rainfall is decreasing in Puerto Rico (Hayhoe 2012; PRCCC 2013). Furthermore, projections suggest that precipitation will continue to decrease on the island and that there will be more dry days and longer time spans without rainfall (Hayhoe 2012), potentially lengthening periods of stress for plant species. Confounding these consequences is the evidence that climate conditions will also exhibit more extremes in terms of temperature and precipitation patterns in the future (Hayhoe 2012). Thus, it is likely that epiphytic orchids such as *Lepanthes* spp. will be exposed to altered microclimate conditions in Puerto Rico for the foreseeable future, and it will be critical for conservation managers to understand how these plants might respond if they are to be preserved in their natural habitat.

Even though each of the focal species in this study occurs largely on protected land (Tremblay 2000), recent surveys demonstrate that their populations have declined and their local ranges have contracted (Tremblay 1997, 2000; Crain and Tremblay 2012). Climate data from the area indicates that conditions in the Cayey Mountains are becoming warmer and drier (SERCC 2007; Hayhoe 2012; PRCCC 2013). Because *Lepanthes* spp. are diminutive in size and they exhibit a high degree of environmental specialization, it is conceivable that physiological responses to changing microclimate conditions could be contributing to negative trends in population and range sizes (Tremblay and Salguero-Faría 2001; Zott et al. 2001; Fernández et al. 2003; Ruiz-Canino et al. 2007; Tremblay and Castro 2009; Crain 2012).

Hence, in this analysis we examined the effects of microclimate variables on the physiology of *Lepanthes* orchids to evaluate potential consequences of altered climate conditions. The aim of this study was to determine whether changes to microclimate variables (such as average temperature and moisture availability in a plant’s immediate surroundings) affect photosynthetic processes in these orchids. Specifically, we tested the hypotheses that chlorophyll fluorescence and plant stress— as indicated by measures of variable fluorescence to maximum fluorescence (Fv/Fm)—are significantly affected by changes in temperature, relative humidity (RH), and air vapor pressure deficit (VPD). The ultimate goal was to determine how *Lepanthes* spp. will respond physiologically to changing microclimate conditions so that appropriate conservation strategies may be developed.

**Material and Methods**

**Study Group: Lepanthes Sw. (Orchidaceae)**

The genus *Lepanthes* is one of the most diverse groups of Neotropical orchids, with more than 1100 species in Latin America and the Caribbean, and it includes a large number of threatened species (Luer 1986; Tremblay and Hutchings 2002; Crain and Tremblay 2012, 2014). We focused our analysis on four well-studied species of *Lepanthes* that are endemic to the island of Puerto Rico: *Lepanthes caritensis* Tremblay & Ackerman, *Lepanthes rubripetala* Stimson, *Lepanthes veleziana* Stimson, and *Lepanthes woodburyana* Stimson (Ackerman 1995, 2014). These species are sympatrically distributed within the Cayey Mountains of Patillas at approximately 18.09°N, −66.03°W. At this site, these orchids occur in the same habitats and often occupy the same zones of individual host trees, specifically on trunks and lower branches. Furthermore, each of the study species is believed to employ the same C3 photosynthetic pathway, as multiple studies have yet to reveal evidence of crassulacean acid me-
Assessing Variation in $F_v/F_m$ and Plant Stress

The effects of microclimate conditions on photosynthetic processes and stress in *Lepanthes* were assessed by measuring variable fluorescence to maximum fluorescence ratios ($F_v/F_m$) collected from the leaves of 102 individuals over the course of 3 yr. All sampled plants were found on six host trees located in the same forest patch in the Cayey Mountains. Plants chosen for the study were selected on the basis of their overall size and health. Sample plants were fully mature and had a vigorous general appearance; immature, damaged, and desiccated plants were not included. Measurements were performed on a single leaf from each plant, and only healthy mature leaves were selected, whereas leaves with visible yellowing or excessive moss or lichen cover were not used.

Sample plants were randomly divided into separate groups with approximately equal proportions of each species. Plants in the first group (baseline group) were left in place at their original field sites to collect baseline readings from individuals in their natural setting. These baseline readings were used for comparisons with the experimental groups (treatment groups 1 and 2). The experimental plants were collected and housed in plant propagation facilities at the University of Puerto Rico. All the plants in the experimental groups were collected by retrieving accessible sections of lower branches that were occupied by *Lepanthes* spp. Plants were not removed from their original bark or branches to minimize any potential stress or root damage from the translocation process (Tremblay 2008) and to control for any effects of substrate characteristics. Plants were maintained intact on these branches for the duration of the study.

Experimental plants were initially housed in the laboratory in closed terrariums that were approximately 27 cm × 17 cm × 20 cm with about a 5-L capacity. The bottoms of the terrariums were lined with mulch to hold in moisture and to keep the branches with orchids in place. Temperature, humidity, and light measurements were taken at field sites from where the orchids were collected, and microclimate conditions in the terrariums were maintained at similar levels. Climate control kept temperatures in the terrariums at approximately 23.0°C, and plants were watered whenever rainfall was recorded at the field site, approximately twice per week. Differences in light conditions can affect photosynthesis and $F_v/F_m$, so all plants were exposed to natural as opposed to artificial light. The terrariums were positioned in the laboratory so that they received suitable amounts of sunlight, approximately 12 h of indirect southern exposure daily. Light levels ($\mu$mol photons m$^{-2}$ s$^{-1}$) were similar at the field site and at the experimental site over the course of the study on the basis of monthly averages km$^{-2}$ ($t = -0.03$, $P > 0.05$; PRAGWATER 2015). All experimental plants were left in these conditions for a period of 2 mo to acclimatize after the translocation process.

Although we took care not to remove plants from their woody substrates and they were taken to a site not far from the natural population and with similar conditions, it is possible that the translocation process had some effect. In future studies, tests for potential effects of translocation could be conducted by maintaining a control group in the laboratory under natural climate conditions. Examination of such a treatment would enable one to distinguish the effects of removal. Nevertheless, we did not observe any noticeable effects of the translocation process.

After the initial 2-mo acclimation period, each terrarium was transferred to one of two experimental microclimate settings. In the first setting, plants were kept in terrariums where they were exposed to slightly drier microclimate conditions than those typifying their natural settings (treatment 1). This was achieved by reducing the frequency in which plants were watered to approximately once per week. Conditions in this setting simulated reduced rainfall and moisture availability, as predicted by climate models for Puerto Rico (Hayhoe 2012; PRCCC 2013). In the second setting, terrariums were kept in a shade house where plants were exposed to warmer and drier conditions than those typifying their natural settings (treatment 2). Temperatures were naturally warmer in the shade house, and the frequency with which plants were watered was reduced to once per week. Conditions in this setting simulated model predictions of increased temperatures as well as reduced moisture availability (Hayhoe 2012; PRCCC 2013). In all settings, the terrariums received sufficient indirect and diffuse sunlight similar to the largely shaded conditions at the field site where the plants were collected (>75% canopy cover at all sites). We used HOBO data loggers to measure light conditions (lumens ft$^{-2}$) once per hour in each of the three treatment settings over the course of three sunny days. No significant differences in light levels were detected ($F_{2,63} = 3.14$, $P > 0.05$). All plants were kept in their experimental microclimate conditions for a full 24 h before any measurements were taken to allow for an initial adjustment period. Overall, our experimental design allowed us to assess the responses of individual plants specifically to variation in microclimate conditions.

We measured chlorophyll fluorescence to quantify variable fluorescence to maximal fluorescence ratios ($F_v/F_m$) from plants in each of the three microclimate settings. $F_v/F_m$ is a relative measure of the maximum quantum efficiency of photosystem II photochemistry, and it is a parameter that is commonly used in chlorophyll fluorescence studies because it is a sensitive indicator of plant stress (Maxwell and Johnson 2000; Logan et al. 2007; Baker 2008; Pardow and Lakatos 2013). Generally, unstressed plants will exhibit $F_v/F_m$ values of about 0.83, whereas decreases in these values occur as plants are exposed to environmental stressors that inhibit photosynthetic efficiency (Zott and Tyree 1996; Maxwell and Johnson 2000; Logan et al. 2007; Baker 2008).

To obtain $F_v/F_m$ measurements from the study plants, we used a Hansatech Instruments FMS2 field portable pulse modulated chlorophyll fluorometer (Hansatech Instruments 2014). We followed standard protocols (Hansatech Instruments 2014) and the applicable recommendations of Logan et al. (2007). All measurements were taken at approximately the same time, near dawn (~8:00 a.m.), because $F_v/F_m$ can vary throughout the day (Pollet et al. 2009). Plants measured at this time have also been exposed to the longest period of natural darkness, which is the optimal time for measuring $F_v/F_m$ (Logan et al. 2007). Additionally, purposely designed leaf clips were used to dark acclimate each sample leaf for a period of at least 30 min to insure that all photosystem II reaction centers were open and capable of performing photochemistry. This controlled for any effects of
light conditions before sampling (Ali et al. 2005; Casanova-Katny et al. 2006; Baker 2008; Coopman et al. 2008). After leaves were dark acclimated, minimal fluorescence ($F_o$) and maximal fluorescence ($F_m$) were measured with the fluorometer’s built-in program settings (Maxwell and Johnson 2000; Baker 2008; Hansatech Instruments 2014). These measurements were then used to calculate variable fluorescence ($F_v$) as the difference between $F_m$ and $F_o$ and, subsequently, $F_v/F_m$ (Hansatech Instruments 2014). Plants were remeasured approximately once per week, and $F_v/F_m$ was monitored through time.

While $F_v/F_m$ was measured, we used a Kestrel 3000 weather meter to simultaneously record microclimate conditions next to each plant. Along with each measurement of $F_v/F_m$, we recorded the corresponding temperature (°C) and relative humidity (%) next to each leaf. With these measurements, we calculated air VPD (Pa) as the difference between saturation vapor pressure (SVP) and actual vapor pressure (AVP), where SVP was calculated as $610.7 \times 10^{(0.075 \times 237.3 - T)}$, AVP was calculated as $(RH \times SVP)/100$, RH was relative humidity, and $T$ was the recorded temperature (Murray 1967; Monteith and Unsworth 2013). Air VPD was specifically used to characterize microclimate conditions because this variable integrates temperature and RH, and it accounts for the changing impact of RH at given temperatures. Air VPD is also a driving force for water movement in plants, which affects CO$_2$ diffusion into leaves and thus photosynthesis. Once all sampling events were completed, we calculated mean temperature, RH, and air VPD associated with each individual to characterize the overall microclimate conditions surrounding each plant.

### Statistical Analysis

Mean $F_v/F_m$ was calculated for each individual plant as an estimate of its overall stress during the 3-yr study period. Mean $F_v/F_m$ was plotted as a dependent variable against mean temperature, RH, and air VPD as independent variables. We generated linear models to determine whether there were significant relationships between the mean microclimate variables and mean $F_v/F_m$. We also used the mean $F_v/F_m$ of the individual plants to compare the average responses from plants in each of the three different microclimate settings. We used a one-way ANOVA to test for significant differences in mean $F_v/F_m$ between the three groups.

Linear regression models were used to evaluate temporal trends in $F_v/F_m$ for each plant in the study. We plotted $F_v/F_m$ against time for each plant and determined the direction of the slope ($\beta$) of the linear model. The linear models allowed us to determine whether individual plants exhibited significant changes in $F_v/F_m$ through time. We also quantified the proportion of plants in each of the three treatment groups that exhibited negative temporal trends in $F_v/F_m$ and calculated the mean of the individual model slopes for each group. We used a one-way ANOVA to test for significant differences in the mean slopes for each group. These analyses were valuable for comparing the temporal effects of microclimate change on $F_v/F_m$ in the three study groups.

Last, we calculated mean $F_v/F_m$ from plants in each of the three study groups during the initial survey and again during the final survey. We used paired $t$-tests to look for differences between mean $F_v/F_m$ during the initial surveys and the final surveys for each group. This test permitted us to determine whether mean $F_v/F_m$ in each group changed significantly over the course of the study.

All statistical analyses were performed with the R 3.0.1 statistical package (R Development Core Team 2013). Collectively, these analyses allowed us to determine whether $F_v/F_m$ and stress in Lepanthes are affected by various microclimatic variables. This information is critical for understanding how epiphytic orchids such as Lepanthes respond to altered microclimate conditions.

### Results

#### Microclimate Conditions

Throughout the study, we took measurements from the leaves of 102 individual plants under varying microclimate conditions. In total, we amassed 1547 $F_v/F_m$ readings along with the corresponding temperature, RH, and air VPD ($X = 15.2$ samples per plant, SD = 19.1) that were used to calculate mean microclimate conditions and mean $F_v/F_m$.

Collectively, the study plants were exposed to temperatures ranging between 17.8° and 31.7°C during sampling events. The mean temperatures associated with each individual plant ranged between 23.1° and 27.0°C. Values of RH ranged between 52.7% and 100% during sampling events, whereas mean values associated with each individual plant ranged between 75.8% and 99.9%. Values of air VPD ranged between 0.0 and 2210.6 Pa during the study, while means associated with each plant ranged between 0.9 and 801.1 Pa.

The average microclimate conditions—in terms of temperature, RH, and air VPD—surrounding baseline plants in the field and plants at each of the two treatment settings varied significantly ($P < 0.01$ for $t$-tests between each variable for all groups). At the field site (baseline), mean temperature during sampling was 23.6°C (range = 19.9°–27.6°, SD = 1.3), mean RH was 98.1% (range = 76.5%–100.0%, SD = 4.3), and mean air VPD was 54.4 Pa (range = 0.0–837.6, SD = 131.6) over the course of the study period. In the first microclimate setting (treatment 1), which was characterized by drier conditions, mean temperature during sampling was 22.5°C (range = 17.8°–27.4°, SD = 1.5), mean RH was 87.4% (range = 63.0%–100.0%, SD = 9.5), and mean air VPD was 338.2 Pa (range = 0.0–886.0, SD = 241.0) during the analysis. In the second microclimate setting, which was characterized by warmer and drier conditions (treatment 2), mean temperature was 27.9°C (range = 23.0°–31.7°, SD = 1.7), mean RH was 83.1% (range = 52.7%–100.0%, SD = 9.5), and mean air VPD was 653.3 Pa (range = 0.0–2210.6, SD = 410.6) during the study.
Mean $F_v/F_m$ for individual plants ranged between 0.55 and 0.83 (median = 0.73). ANOVA tests comparing the individual species’ initial $F_v/F_m$ and mean $F_v/F_m$ revealed no significant differences among species (initial values $F_{3.57} = 1.64, P = 0.19$; mean values $F_{3.98} = 1.10, P = 0.35$); consequently, species were analyzed collectively for all statistical analyses. A negative relationship between mean temperature and mean $F_v/F_m$ ($P < 0.001$, df = 100, $r^2 = 0.12$) and a positive relationship between mean RH and mean $F_v/F_m$ ($P < 0.001$, df = 100, $r^2 = 0.32$) was revealed by simple linear models (fig. 1A, 1B). Furthermore, a negative relationship between air VPD and $F_v/F_m$ was observed (fig. 1C; $P < 0.001$, df = 100, $r^2 = 0.30$). A multiple regression model using all three independent variables and the associated interaction terms was also significant ($P < 0.001$, df = 94, $r^2 = 0.31$); however, temperature and RH were correlated, air VPD was the only significant variable in the model, and overall it performed similarly to the simple linear model for air VPD in terms of explanatory power. Since air VPD inherently accounts for the interaction between temperature and RH, and the simple linear model for air VPD was more parsimonious, it was considered the superior model. In both cases, however, the results supported rejection of the null hypothesis that temperature, RH, and air VPD have no effect on $F_v/F_m$ in Lepanthes orchids.

Considering the three different treatment groups, mean $F_v/F_m$ was substantially higher for plants kept in the original baseline field site (baseline: $X = 0.74$, SD = 0.04) than for plants in either of the treatment settings (treatment 1: $X = 0.70$, SD = 0.04; treatment 2: $X = 0.66$, SD = 0.04; fig. 2). Results of an ANOVA confirmed significant differences in mean $F_v/F_m$ among the three treatment groups ($F_{2,125} = 29.76$, $P < 0.01$), indicating that these plants are affected physiologically in altered microclimate conditions. Consequently, these results also support rejection of the null hypothesis that there is no effect of microclimate conditions on $F_v/F_m$ in Lepanthes orchids.

Temporal differences in $F_v/F_m$ in each of the treatment groups were also observed over the 3-yr duration of the study. Simple linear regression models of $F_v/F_m$ over time revealed that 36% of the individuals in the control group at the original field site (baseline) showed declines in $F_v/F_m$ ($b < 0$) over the course of the study. The mean of the individual model slopes ($b$) from plants in this group was positive ($X = 0.01$, SD = 0.01), however, indicating that on average, $F_v/F_m$ increased in this group over time. In the group exposed to drier microclimate conditions (treatment 1), 56% of the individual plants showed declines in $F_v/F_m$ ($b < 0$) over the study period. The mean of the individual model slopes ($b$) from plants in this group was negative ($X = -0.003$, SD = 0.01), suggesting a collective decline in $F_v/F_m$ over time in this group. In the group exposed to warmer and drier microclimate conditions (treatment 2), 64% of the individual plants showed declines in $F_v/F_m$ ($b < 0$) during the course of the study. The mean slope value ($b$) from plants in this treatment group was also negative ($X = -0.003$, SD = 0.01) and indicative of a collective decline in $F_v/F_m$ in this group over time. Results from an ANOVA comparing mean slope values from the three study groups demonstrated significant differences ($F_{2,125} = 10.72$, $P < 0.01$). Accordingly, individual plants in either of the treatment groups were more likely to exhibit declines in $F_v/F_m$ over the course of the study, and collectively they showed greater declines in $F_v/F_m$ on average than the baseline control group over time.

The results of the paired $t$-tests comparing mean $F_v/F_m$ in each treatment group during the initial surveys and during the final surveys further highlighted temporal changes (fig. 3) and indicated differences between the responses of plants in each of the groups. For the group in the baseline field conditions, the results of a paired $t$-test demonstrated no difference in initial mean $F_v/F_m$ ($X = 0.73$, SD = 0.05) and final mean $F_v/F_m$ ($X = 0.74$, SD = 0.06) for the group ($t = -1.11, P = 0.27$). The opposite was true for the two treatment groups in altered microclimate conditions. For plants in the first treatment group exposed to drier conditions, the paired $t$-test indicated...
Physiological Effects and Potential Consequences

Overall, the results from our analysis highlight significant relationships between mean \( F_v/F_m \), temperatures, RH, and air VPD. Decreases in mean \( F_v/F_m \) and increased stress occur in _Lepanthes_ in response to warmer and drier conditions with increased air VPD. This finding is in accord with other physiological analyses of orchid species that document negative relationships between \( F_v/F_m \) and increasing temperatures or decreasing RH (Zotz and Tyree 1996; Stancato et al. 2001; Ali et al. 2005; Jeon et al. 2006; Hsu 2007; Pollet et al. 2009, 2010). Additionally, our results demonstrate that \( F_v/F_m \) is collectively lower in plants exposed to microclimate conditions with higher mean air VPD than those in their typical natural conditions. Likewise, reductions in \( F_v/F_m \) occur over time in plants exposed to altered conditions, unlike those in their existing natural settings. Similar trends have been observed in studies of other orchid species (Stancato et al. 2001). Interestingly, the temporal trends in \( F_v/F_m \) from the initial measurements to the final measurements in both experimental treatment groups were similar (mean model slopes were both \(-0.003\)). One might expect that the drier experimental microclimate setting (treatment 1) would be significantly less stressful than the warmer and drier experimental microclimate setting (treatment 2). While this may not seem to be the case on the basis of the regression model slopes, final mean \( F_v/F_m \) for plants in the warmer and drier microclimate conditions was substantially lower than for those in only drier conditions (0.62 vs. 0.67). This information suggests that warmer, drier microclimates were actually more stressful than only drier microclimates and that a good portion of the effect occurred rapidly (within 1 d) after exposure to these conditions. Overall, if anticipated climate change led to warmer and drier microclimate conditions in habitats where _Lepanthes_ are present in Puerto Rico (Scatena 1998; Neelin et al. 2006; SERCC 2007; Hayhoe 2012), these species are likely to have chronically reduced \( F_v/F_m \).

Although declines in \( F_v/F_m \) could provide an adaptive benefit to plants in terms of protection from light-induced damage and oxidative stress (Maxwell and Johnson 2000; Demmig-Adams and Adams 2006; Logan et al. 2007), this benefit could still come at a cost. Studies have shown that reduced \( F_v/F_m \) from photoinhibition is also correlated with reduced leaf sizes and plant growth (Ali et al. 2005; Jeon et al. 2006; Hsu 2007). Thus, reductions in mean \( F_v/F_m \) could limit processes contributing...
to leaf growth, a known correlate of flower production in *Lepanthes* (Fernández et al. 2003), despite the added protective benefits provided by photoinhibition.

Accordingly, the physiological effects of climate change have the potential to translate to larger consequences for *Lepanthes* populations over time. Studies on a variety of orchids suggest that suboptimal $F_v/F_m$, photosynthetic activity, and stress can lead to slower growth rates, loss of leaves, and reduced flowering (Zotz and Tyree 1996; Willems and Dorland 2000; Hsu 2007). Although we did not specifically measure leaf growth in this study, the generation of new leaves on experimental plants was uncommon, and it occurred on only a few plants over the course of the study. Furthermore, we observed leaf loss on several individuals, and although we could not distinguish this from natural leaf turnover, leaf mortality usually occurred during extended warm and dry spells when $F_v/F_m$ was reduced. Average air VPD associated with sample plants before leaf loss (340.97 Pa) was above the average for the species’ natural setting and average $F_v/F_m$ (0.61) before leaf loss was well below the collective average for plants in their natural setting. Research on *Lepanthes* spp. demonstrates that measures of plant growth or size (e.g., leaf sizes, number of leaves, and number of stems) are correlated with both survival and reproductive rates (Tremblay 1997; Agosto-Pedroza and Tremblay 2003; Fernández et al. 2003; Rosa-Fuentes and Tremblay 2007). While we did not track overall rates of reproduction in this study, plants in the field produced flowers on a regular basis because they can bloom year round. Experimental plants also produced flowers on occasion, but it was uncommon and did not occur at all on many individuals. Reductions in mean $F_v/F_m$ affect growth, reproduction, and survival in *Lepanthes* populations as it does in other plant species (e.g., Arntz et al. 2000; Ali et al. 2005; Hsu 2007), it is plausible that the anticipated climate change in Puerto Rico will have adverse effects on the population dynamics of these orchids.

Furthermore, population dynamics regulate the geographic distributions of *Lepanthes* spp. (Schemske et al. 1994; Gaston 2003; Tremblay et al. 2006; Crain and Tremblay 2012). Therefore, reduced $F_v/F_m$ and increased physiological stress from altered climate conditions could have eventual consequences in the form of range contractions or shifts via extirpation of subpopulations with reduced growth or reproductive rates (Larsen et al. 2011). This could be particularly important in scenarios where orchid populations are already restricted because of resource limitations (Otero et al. 2007; Pauw and Bond 2011; McCormick and Jacquemyn 2014). Considering the already limited range of many *Lepanthes* spp., added or sustained range contractions would greatly increase the overall vulnerability of these orchids. Thus, it is apparent that *Lepanthes* in Puerto Rico and conceivably elsewhere (Richter et al. 2009; Larsen et al. 2011) may be highly vulnerable to climate change as a consequence of mounting effects of physiological stress.

### Physiological Analyses and Conservation

From a conservation standpoint, physiological analyses on individual plants may facilitate detection of nascent effects of climate change (Lugo and Scatena 1992; Zotz and Hietz 2001; Nadkarni and Solano 2002; Ali et al. 2005; Hsu 2007; Liu et al. 2010; Pardow and Lakatos 2013). These types of analyses could greatly enhance our ability to forecast and prevent greater consequences before they occur (Seaton et al. 2010; Pardow and Lakatos 2013). Accordingly, reductions or gradients in $F_v/F_m$ among individuals or populations at different elevations or in different parts of the species’ overall range as a consequence of environmental variability may provide a potential means to identify threatened populations or species of *Lepanthes* before they exhibit symptoms at the population or landscape level. For example, analyses of $F_v/F_m$ and stress among populations where microclimate conditions may vary (e.g., at low, middle, and high elevations or along the margins and cores of a species’ distribution) may be especially useful for recognizing afflicted populations, forecasting potential responses to changing conditions, and pinpointing more hospitable sites (Channell and Lomolino 2000; Colwell et al. 2008; Kelly and Goulden 2008; Larsen et al. 2011). Such information could greatly enhance restoration or translocation efforts for species of concern (Swarts and Dixon 2009; Schwartz and Martin 2013). Although there are various additional measures of photosynthetic performance and stress that may provide unique information on plants’ responses to climate conditions (Maxwell and Johnson 2000; Baker 2008), the methods applied here are nondestructive, easily implemented, and particularly suitable for detecting changes that may be currently undetectable by larger-scale demographic or geographic analyses. Thus, monitoring plant physiology and specifically $F_v/F_m$ as an indicator of plant stress will enhance conservation practitioners’ ability to detect and respond to threats from climate change at an early stage.

### Conclusions

If climate changes follow predictions for Puerto Rico and the Caribbean region (Scatena 1998; Neelin et al. 2006; SERCC 2007; Hayhoe 2012), *Lepanthes* spp. on the island are likely to have increasing stress and adverse physiological responses. Although the effects of changing $F_v/F_m$ and increased stress may not be observable at larger scales initially, it is feasible that they could contribute to eventual demographic declines through reduced growth and reproductive success and, subsequently, geographic declines. The analysis presented here demonstrates that *Lepanthes* spp. show distinct signs of physiological stress under altered microclimate conditions and that conservation practitioners can test for impacts of climate change before larger responses occur. Considering that global climate change is a worldwide phenomenon that is likely to affect countless orchids and other plant species across the planet, we encourage the use of photosynthetic performance and chlorophyll fluorescence analyses that include $F_v/F_m$ as a means to identify affected populations or species so that proactive plant conservation measures may be taken.

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Literature Cited


Fernández DS, RL Tremblay, JD Ackerman, E Rodríguez, LN López 2003 Reproductive potential, growth rate, and light environment in Lepanthes napetris Stimson. Lankesterriana 7:75–78.


Hansatech Instruments 2014 Hansatech instruments fluorescence monitoring system fits 2 user manual. Hansatech Instruments, Norfolk.

Hayhoe K 2012 Quantifying key drivers of climate variability and change for Puerto Rico and the Caribbean. Texas Tech University, Lubbock.


Southeast Regional Climate Center (SERCC) 2007 Historical climate summaries for Puerto Rico and the U.S. Virgin Islands. University of North Carolina, Chapel Hill.


