

RESEARCH PAPER

# Thermal acclimation of leaf respiration is consistent in tropical and subtropical populations of two mangrove species

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## Abstract

Populations from different climates often show unique growth responses to temperature, reflecting temperature adaptation. Yet, whether populations from different climates differ in physiological temperature acclimation remains unclear. Here, we test whether populations from differing thermal environments exhibit different growth responses to temperature and differences in temperature acclimation of leaf respiration. We grew tropical and subtropical populations of two mangrove species (*Avicennia germinans* and *Rhizophora mangle*) under ambient and experimentally warmed conditions in a common garden at the species' northern range limit. We quantified growth and temperature responses of leaf respiration ( $R$ ) at seven time points over ~10 months. Warming increased productivity of tropical populations more than subtropical populations, reflecting a higher temperature optimum for growth. In both species,  $R$  measured at 25 °C declined as seasonal temperatures increased, demonstrating thermal acclimation. Contrary to our expectations, acclimation of  $R$  was consistent across populations and temperature treatments. However, populations differed in adjusting the temperature sensitivity of  $R$  ( $Q_{10}$ ) to seasonal temperatures. Following a freeze event, tropical *Avicennia* showed greater freeze damage than subtropical *Avicennia*, while both *Rhizophora* populations appeared equally susceptible. We found evidence of temperature adaptation at the whole-plant scale but little evidence for population differences in thermal acclimation of leaf physiology. Studies that examine potential costs and benefits of thermal acclimation in an evolutionary context may provide new insights into limits of thermal acclimation.

**Keywords:** Climatic adaptation, freeze tolerance, genetic differentiation, homeostasis, New World mangroves, respiration.

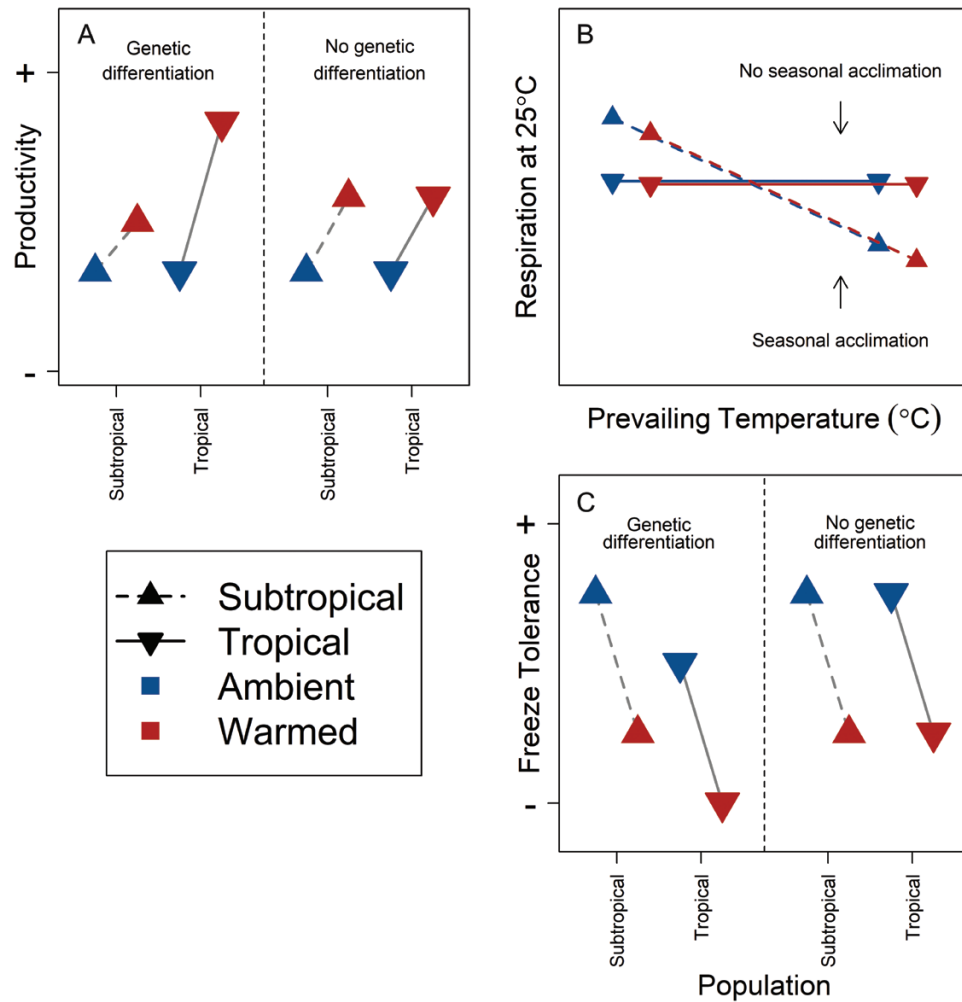
## Introduction

Respiration ( $R$ ) is a key regulator of plant growth and function, providing energy and substrates for growth and maintenance of metabolic function. Leaf  $R$  and photosynthesis are linked in that  $R$  fuels processes that maintain photosynthesis including nitrate reduction, phloem loading, and protein turnover, while photosynthesis provides substrates for leaf  $R$  as well as growth (Wang *et al.*, 2020). Respiration (typically measured in darkness) is enzymatically driven and therefore temperature dependent. Over short time periods (i.e. seconds to minutes), leaf  $R$  increases quasi-exponentially with increasing temperature before reaching a peak of ~50–60 °C (O'Sullivan *et al.*, 2013, 2017; Aspinwall *et al.*, 2019). However, some plants acclimate to changes in growth temperature by adjusting the short-term temperature response of  $R$ . Such acclimation can occur within several days (Bolstad *et al.*, 2003; Lee *et al.*, 2005; Aspinwall *et al.*, 2016). Acclimatory responses include (Type I) a reduction in the temperature sensitivity (i.e.  $Q_{10}$ , activation energy) and/or (Type II) a downward shift in the overall temperature response of leaf  $R$  (Atkin and Tjoelker, 2003). Thermal acclimation of plant  $R$  has clear fitness implications for plants (Körner and Larcher, 1988; Atkin *et al.*, 2006; O'Sullivan *et al.*, 2013, 2017). Stronger or weaker acclimation results in varying degrees of respiratory homeostasis (Slot and Kitajima, 2015) with potential consequences for plant growth rates and fitness (Xiong *et al.*, 2000; Kurimoto *et al.*, 2004).

Plant growth is also a temperature-dependent process, and species or populations from different thermal environments often show different growth responses to changing growth temperature. For instance, cold climate plant populations (boreal, temperate) grown in their native environment often increase growth when experimentally warmed; warm climate populations (e.g. tropical) show neutral to negative growth responses to warming (Rustad *et al.*, 2001; Norby and Luo, 2004; Lin *et al.*, 2010; Drake *et al.*, 2015). Warm climate populations have also shown evidence of temperature-limited growth when grown in cooler climates (Byars *et al.*, 2007; Mimura and Aitken, 2010; Molina-Montenegro *et al.*, 2012). Thus, population growth responses to warming or cooling reflect temperature adaptation. Given linkages between plant growth, photosynthesis, and leaf  $R$  (Thornley, 1970; De Vries, 1975; Amthor, 1984; Wang *et al.*, 2020), it is informative to assess whether population differences in temperature adaptation, assessed at the whole-plant scale (i.e. growth), correspond with population differences in thermal acclimation (plasticity) of leaf  $R$ . Understanding such differences could provide insight into the evolution of adaptive plasticity (Via, 1993; Van Kleunen and Fischer, 2005; Ghalambor *et al.*, 2007; Arnold *et al.*, 2019), and the impacts of temperature change on population growth, survival, and function. Importantly, it is also possible that temperatures during sporophyte embryo development may override patterns of temperature adaptation through epigenetic mechanisms (Kvaalen and Johnsen, 2008)

such that signatures of temperature adaptation and acclimation across populations from different thermal environments may not be solely attributed to genetic effects (Bossdorf *et al.*, 2008). Average temperature and temperature seasonality vary with latitude such that low-latitude regions experience hotter and more uniform (aseasonal) temperatures while higher latitude subtropical or warm-temperate regions experience somewhat cooler and more variable (seasonal) temperatures (Lutz *et al.*, 2007). Species that span tropical and subtropical or warm-temperate regions have populations experiencing disparate thermal environments, which could result in distinct patterns of thermal acclimation shaped by temperature adaptation (Janzen, 1967; Ghalambor *et al.*, 2006). Plants adapted to more thermally variable environments might have evolved greater capacity for acclimation of leaf physiology than plants from thermally uniform environments (Arnold and Körner, 1997; Bolstad *et al.*, 2003; Tjoelker *et al.*, 2008; Silim *et al.*, 2010; Tjoelker, 2018). Large-scale studies using multiple species have found convergent patterns in thermal acclimation of leaf  $R$  (Slot and Kitajima, 2015; Heskell *et al.*, 2016; Zhu *et al.*, 2021). However, relatively few studies have explored within-species (population-level) variation in thermal acclimation of leaf physiology. Among those studies, few have found evidence for differences in thermal acclimation among populations (Bolstad *et al.*, 2003; Lee *et al.*, 2005; Tjoelker *et al.*, 2008; Gunderson *et al.*, 2010; Silim *et al.*, 2010; Aspinwall *et al.*, 2017; Tjoelker, 2018). Yet almost all these studies have focused on populations that span temperate and boreal regions. Very few studies include species with ranges spanning tropical and subtropical or warm-temperature environments (Drake *et al.*, 2015; Crous *et al.*, 2018).

Mangroves are an ideal group to study genetic differentiation in plant growth responses to temperature and thermal acclimation of leaf  $R$ . These halophytic coastal wetland trees are common in the tropics and subtropics. Northern and southern hemisphere range limits for broadly distributed mangrove species are largely determined by the severity and frequency of freezing temperatures (Pickens and Hester, 2011; Cook-Patton *et al.*, 2015; Osland *et al.*, 2017, 2019). Among New World mangroves, *Avicennia germinans* (L.) L. and *Rhizophora mangle* L. are the most broadly distributed (latitudinal range from 22°S to 30°N for both species) and possess the most poleward populations. These populations are expanding poleward as freezing events become less frequent (Cavanaugh *et al.*, 2015; Dangremond and Feller, 2016). As broadleaved evergreens, deciduousness does not provide phenological escape from cold temperatures. Thus, evergreen leaves of mangroves are subject to the full range of seasonal temperature variation. Both species contain populations across their latitudinal extent (tropical to subtropical/warm-temperate) with unique genetic and functional adaptations (Pil *et al.*, 2011; Sandoval-Castro *et al.*, 2014; Kennedy *et al.*, 2016, 2017, 2020). More broadly,



**Fig. 1.** Theoretical diagram outlining questions and hypotheses related to tropical and subtropical/warm-temperate mangrove populations and their leaf-scale and whole-plant responses to temperature. In (A) we illustrate expected responses of tropical and subtropical populations that exhibit genetic differentiation in productivity responses to warming (left side) or respond similarly to warming (right side). We expected that tropical populations would show larger productivity responses to warming than subtropical populations at our subtropical common garden site. In (B) we expected that tropical populations (solid lines) would show little or no thermal acclimation of leaf respiration to warming and prevailing temperature conditions across seasons. Conversely, we hypothesized that populations from subtropical/warm-temperate regions (dashed line) would exhibit stronger thermal acclimation of  $R$  over seasons and across treatments due to adaptation to strong temperature seasonality, promoting homeostasis in  $R$  *in situ* across a wide range of temperatures. If both tropical and subtropical populations exhibit similar relationships between prevailing conditions and leaf respiration, this will indicate no genetic differentiation in thermal acclimation. In (C) we illustrate expected lower cold tolerance of tropical than subtropical populations (left side) and reduced cold tolerance with experimental warming across all species and populations.

mangrove-dominated systems are highly productive and make large contributions to the global carbon cycle despite being limited to coastal environments (Donato *et al.*, 2011; Duarte *et al.*, 2013; Alongi, 2014; Duarte, 2017).

In this study, we addressed three questions. (i) Do tropical and subtropical mangrove populations exhibit different growth responses to experimental warming at the species' northern range limit, reflecting temperature adaptation? (ii) Does thermal acclimation of leaf  $R$  over time (seasons) and across temperature treatments differ between tropical and subtropical mangrove populations? (iii) To what degree does thermal acclimation of leaf  $R$  promote homeostasis of leaf  $R$  over time? In this case, ho-

meostasis is defined as the degree to which leaf  $R$  remains stable as growth temperature changes. An unplanned freeze event occurred at the end of the experiment which provided an opportunity to ask a fourth question. (iv) Do tropical and subtropical mangrove populations differ in cold tolerance? We hypothesized (Qi) that tropical populations would be more temperature limited at our northern site compared with subtropical populations such that warming would increase productivity in tropical populations more than subtropical populations (Fig. 1A). Next, we hypothesized (Qii) that subtropical populations would show stronger thermal acclimation of leaf  $R$  across seasons than tropical populations, demonstrated by a steeper negative relationship

between prevailing  $T_{\text{air}}$  and leaf  $R$  at 25 °C or the  $Q_{10}$  of  $R$ . However, in each population, seasonal acclimation would be consistent across treatments (Fig. 1B). Stronger thermal acclimation of leaf  $R$  in subtropical populations would result in greater homeostasis of leaf  $R$  across seasons and temperature treatments (Qiii). Similar to previous studies (e.g. Markley *et al.*, 1982), we expected (Qiv) subtropical populations would show greater cold tolerance (less freeze injury) than tropical populations. In addition, we expected that experimental warming would limit cold acclimation (Pagter and Arora, 2013) and increase freeze damage across species and populations (Fig. 1C).

## Materials and methods

### Experimental design and growth conditions

*Avicennia germinans* (black mangrove) and *Rhizophora mangle* (red mangrove) are broadly distributed mangrove species native to the Atlantic and Pacific coasts of the Americas and the western African coast. Both species occur from warm-temperate to tropical regions along coastlines, with range expansion being strongly limited by winter severity and freeze events (Dodd *et al.*, 2002; Zomlefer *et al.*, 2006). Seedlings from Belize were collected in October 2019 from Twin Cayes (16.8024°N, -88.0820°S). Seedlings from Florida were purchased from Sandhill Growers (Arcadia, FL, USA) with seeds sourced from southwest Florida (approximately 26°N, approximately -81°W). The regions where populations of each species were collected differ in temperature seasonality with some difference in precipitation (Supplementary Fig. S1). Specifically, Belize is a tropical climate with small differences in daily mean and maximum temperatures, and little seasonal variation in temperature. Florida is subtropical with greater greater diurnal temperature variation and a few freeze events each year (Supplementary Fig. S1). Total precipitation is similar (~1300 mm year<sup>-1</sup>) at the two sites, with precipitation skewed towards autumn in Belize. Seedlings were transported to the University of North Florida and planted in 164 ml cone-tainers (on 23 October 2019) filled with commercial garden soil containing a slow-release fertilizer (N-P-K of 500, 500, and 30 mg kg<sup>-1</sup>) (Sta-Green Garden Soil, Rowlett, TX, USA). The exact environmental conditions (e.g. temperature and humidity) in each collection location during seed development and germination are not known, such that potential epigenetic effects during seed development are impossible to determine. Nonetheless, seedlings were grown together for ~3 months in a common greenhouse environment (full sun, common temperature, and partial submergence in fresh water) until the experiment commenced.

The experiment was conducted outdoors on the campus of the University of North Florida in Jacksonville, FL (30.2619°N, 81.5165°W). This site represents the northern range limit of *Avicennia* and *Rhizophora* (Kennedy *et al.*, 2017). Climate is warm-temperate to humid-subtropical. Mean annual temperature is 20.4 °C and mean annual precipitation is 1332 mm. The study was comprised of six blocks, each containing two plots. Blocks were randomly positioned in a 2 m×4 m shade-free, flat location. Plots within each block were assigned to one of two treatments: ambient temperature or experimental warming. On 27 February 2020 seedlings were transplanted into pots (11 liter volume) filled with a 50:50 mixture of sand and the same commercial garden soil used in the cone-tainers (Sta-Green Garden Soil). A single potted replicate of each species and population was randomly assigned to a treatment plot (i.e. four seedlings per plot). In total, the study included 48 seedlings (2 species×2 populations×2 treatment plots×6 blocks). The four seedlings in each plot were placed together into one 56 liter fabric pouch (Root Pouch, Hillsboro, OR, USA). Pouches were placed in large (121 liter) plastic containers filled with tap water

to the soil line of the pots. Over 1 week, we gradually increased salinity (Instant Ocean Sea Salt, Blacksburg, VA, USA) from 0 ppt to 18 ppt (simulating brackish conditions) to help reduce the potential for salt shock. A similar approach was used by Suárez and Medina (2005) and Aspinwall *et al.* (2021). Salinity levels were measured weekly or after significant rainfall using a handheld pore water salinity sensor (YSI Model 85, YSI Incorporated, Yellow Springs, OH, USA) to ensure deviations from the target pore water salinity (18 ppt) were never more than 3 ppt. Following rainfall, excess water was siphoned off to keep the water level in the containers near the soil line.

Warming, which started the day seedlings were transplanted, was achieved passively using PVC-framed chambers (2 m tall×1 m×1 m) wrapped in 6 mm polyethylene greenhouse film that excludes <10% of photosynthetically active radiation (Greenhouse Megastore, Danville, IL, USA). A 0.75 m diameter hole was cut into chamber roofs to allow adequate air flow, gas exchange, and natural rainfall. Chambers passively warm by trapping radiation. Consequently, daytime warming is achieved using this design, with minimal warming occurring during cloudy weather or at night, when radiation is limited. However, unlike infrared heaters, chambers warm air temperatures rather than canopy surface temperatures (e.g. De Boeck *et al.*, 2012; Kimball *et al.*, 2020) and do not inhibit dew formation (Feng *et al.*, 2021).

Air temperature ( $T_{\text{air}}$ , °C) and relative humidity (RH, %) were recorded every 15 min using three HOBO data loggers (HOBO U12 Pro V2; Onset Computer Corporation, Bourne, MA, USA). Two loggers were placed in warmed plots with one placed in an ambient plot (50 cm above the soil line). The average ambient daytime  $T_{\text{air}}$  (07.00–19.00 h) during the experiment was 26.8 °C, with warming causing a 3.5 °C increase in average daytime  $T_{\text{air}}$  (Supplementary Figs S2, S3). The average ambient night-time  $T_{\text{air}}$  was 20.8 °C, with warming causing a 0.6 °C increase in average night-time  $T_{\text{air}}$ . Warming caused a modest decline in daytime RH (-6%) and a small increase (+0.7 kPa) in average daytime vapor pressure deficit (VPD; Supplementary Fig. S2). During the warmest period of the experiment (95th quantile of  $T_{\text{air}}$  and VPD) daytime VPD in the ambient and warmed treatments was 2.8 kPa and 4.4 kPa, respectively. A natural freeze event occurred near the end of the experiment (Supplementary Fig. S2, 2 December) where overnight  $T_{\text{air}}$  declined to -2.1 °C and -1.0 °C in the ambient and warmed treatments, respectively.

### Plant growth and biomass

Stem diameter (at 5 cm above the soil line) and height were measured on seven dates to coincide with measurements of leaf  $R$  (details below). The volume of the main stem (cm<sup>3</sup>) was estimated based on the volume of a cone [volume= $\pi \times (\text{diameter}/2)^2 \times (\text{height}/3)$ ]. The number of fully expanded leaves was also counted on each plant. Volume growth rate was calculated by dividing the change in volume (cm<sup>3</sup>) by the number of days between measurements (cm<sup>3</sup> d<sup>-1</sup>). Seedlings were harvested 244 d after the experiment began and 12 d after the freeze event. Prior to harvests, we assessed the severity of freeze injury by visibly scoring (0–2 scale) the proportion of leaves that appeared brownish-gray (Osland *et al.*, 2020). No injury was visible prior to this assessment. Plants that showed no injury received a score of '0', while plants with <25% of leaves showing freeze injury received a score of '1'. Plants with >25% of leaves exhibiting freeze injury received a score of '2'.

Entire seedlings were lifted from the soil and washed (2 mm sieve). Roots and pneumatophores (herein roots), stems and branches (herein stems), and leaves were separated. All mass was dried at 70 °C for 5 d for dry mass determination.

### Leaf respiration

Leaf dark respiration per unit area ( $R_{\text{area}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was measured at seven time points between 17 April and 13 December 2020. One or two



recent fully expanded leaves were excised from each seedling ~3 h before sunrise and taken to the laboratory. Leaf area ( $\text{cm}^2$ ) of each sample leaf was measured using a leaf area meter (LI-3000C, Li-COR Inc., Lincoln, NE, USA).  $R_{\text{area}}$  was measured using three cross-calibrated portable infrared gas analyzers (one LI-6400XT, two LI-6800s, Li-COR Inc.) fitted with large chambers (LI-6400-22L or LI-6800-24, Li-COR Inc.) to prevent leak artifacts. Reference  $\text{CO}_2$  was  $410 \mu\text{mol mol}^{-1}$ . Analyzers were placed inside a dark temperature-controlled growth cabinet (E41L1, Percival Scientific Inc., Perry, IA, USA).  $R_{\text{area}}$  was measured at 15, 20, 27, 35, and 40 °C by setting the cabinet temperature and gas analyzer block temperature in a stepwise fashion (i.e. all leaves measured at 15 °C, then all leaves 20 °C, etc.). Plants were kept in sealed plastic bags with a moist paper towel between measurements to prevent desiccation. After measuring  $R_{\text{area}}$  at all temperatures, leaves were dried at 70 °C for 72 h for dry mass determination. Leaf mass per area (LMA,  $\text{g m}^{-2}$ ) was used to convert  $R_{\text{area}}$  to  $R$  per unit mass leaf dry mass ( $R_{\text{mass}}$ ,  $\text{nmol g}^{-1} \text{s}^{-1}$ ).

Non-linear least-squares regression was used to model the temperature response of  $R$  for each leaf at each time point. We compared the suitability of three different algorithms: (i) a second-order log-polynomial function; (ii) an exponential function with a single  $Q_{10}$  value (Ryan, 1991); and (iii) a modified Arrhenius function. The log-polynomial function is written as:

$$\ln R = a + bT_{\text{leaf}} + cT_{\text{leaf}}^2 \quad (1)$$

where  $a$  is an estimate of  $\ln R$  when  $T_{\text{leaf}}=0$  °C,  $b$  is the slope of the  $\ln R$  versus  $T_{\text{leaf}}$  relationship at 0 °C, and  $c$  represents the quadratic non-linearity in the  $\ln R$  versus  $T_{\text{leaf}}$  relationship (Heskel et al., 2016; Zhu et al., 2021). Using the coefficients  $b$  and  $c$ , the  $Q_{10}$  of leaf  $R$  can be estimated at any  $T_{\text{leaf}}$ :

$$Q_{10} = e^{10 \times (b + 2cT_{\text{leaf}})} \quad (2)$$

The log-polynomial function (Equation 1) provided the strongest fit between observed (Supplementary Fig. S4) and predicted values of leaf  $R$  ( $R^2=0.994$  for both  $R_{\text{area}}$  and  $R_{\text{mass}}$ ), exhibiting a near perfect 1:1 relationship. Residuals were evenly distributed around zero across the full range of  $T_{\text{leaf}}$ .

Using the coefficients of the log-polynomial function, we calculated  $R_{\text{area}}$  and  $R_{\text{mass}}$  at 25 °C (herein,  $R_{\text{area}}^{25}$  and  $R_{\text{mass}}^{25}$ ), and the  $Q_{10}$  of  $R$  at 25 °C (herein  $Q_{10}^{25}$ ) for each seedling at each time point. Repeated estimates of  $R_{\text{area}}^{25}$ ,  $R_{\text{mass}}^{25}$ , and  $Q_{10}^{25}$  over time provide a direct way of determining the direction and magnitude of seasonal thermal acclimation. If seasonal thermal acclimation were occurring, we would expect a negative relationship between prevailing 5 d mean daily air temperature (herein  $T_5$  in °C) and  $R_{\text{area}}^{25}$ ,  $R_{\text{mass}}^{25}$ , or  $Q_{10}^{25}$ , with the slope of the relationship indicating the strength of acclimation (i.e. more negative slopes indicating stronger acclimation) (Aspinwall et al., 2021).

Finally, we quantified the degree of homeostasis of leaf  $R$  across seasons by calculating a thermal acclimation ratio ( $\text{Acclim}_{\text{Homeo}}$ , Slot and Kitajima, 2015). For each seedling and time point,  $\text{Acclim}_{\text{Homeo}}$  was calculated as *in situ*  $R$  at the time point where  $T_5$  was lowest (excluding the time point associated with the freezing event) divided by *in situ*  $R$  at each time point where  $T_5$  was higher (Sturchio et al., 2022). Ratios >1 indicated acclimation resulting in overcompensation, namely lower  $R$  at higher  $T_5$ . Ratios=1 indicated acclimation resulting in complete homeostasis of  $R$ , namely equivalent  $R$  at low and high  $T_5$ . Ratios <1 indicated acclimation resulting in partial homeostasis of  $R$  with increasing  $T_5$ , with lower ratios indicating less homeostasis. Ratios were plotted against the change in  $T_5$  across time points to assess changes in respiratory homeostasis with increasing seasonal temperatures, and the influence of species, population, and temperature treatment.

## Data analysis

All tests of statistical significance were conducted using RStudio (R v.3.6.1, RStudio v.1.2.1335; R Core Team, 2013) with  $\alpha=0.05$ . We used ANOVA to test the fixed effects of measurement date, temperature treatment, population, and their interactions on seedling stem volume, stem volume growth rate, total leaf count,  $R_{\text{area}}^{25}$ ,  $R_{\text{mass}}^{25}$ ,  $Q_{10}^{25}$ , and LMA. ANOVA was also used to test the effects of temperature treatment, population, and their interaction on total and component dry mass production. A preliminary ANOVA that included 'species' as a factor indicated that 'species' was the only factor affecting seedling growth. This was because *Avicennia* seedlings were ~2.5× larger than *Rhizophora* seedlings at the end of the experiment. Therefore, we analyzed and present data for each species separately.

We used analysis of covariance (ANCOVA) to test whether seasonal thermal acclimation was consistent across temperature treatments but different between tropical and subtropical populations. In this model,  $R_{\text{area}}^{25}$ ,  $R_{\text{mass}}^{25}$ , and  $Q_{10}^{25}$  were response variables,  $T_5$  was a covariate, and temperature treatment and population were factors. Interactions between  $T_5$  and treatment or  $T_5$  and population indicated that the slope of the relationship between  $R_{\text{area}}^{25}$ ,  $R_{\text{mass}}^{25}$ ,  $Q_{10}^{25}$ , and  $T_5$  differed between treatments or populations; that is, seasonal acclimation differed between treatments and populations. If interactions were not significant, but  $T_5$ , treatment, and population were significant, models were developed with different intercepts for each treatment and population, but a common slope. If only  $T_5$  was significant, this indicated that seasonal thermal acclimation was consistent across treatments and populations, and a single function (common slope and intercept) was used to describe the relationship between  $R_{\text{area}}^{25}$ ,  $R_{\text{mass}}^{25}$ , or  $Q_{10}^{25}$ , and  $T_5$ . ANCOVA was also used to test  $\text{Acclim}_{\text{Homeo}}$  (measure of respiratory homeostasis) as described for respiratory parameters. When factors were found to be significant, we used the package emmeans for post-hoc Tukey pairwise comparisons (Lenth et al., 2019).

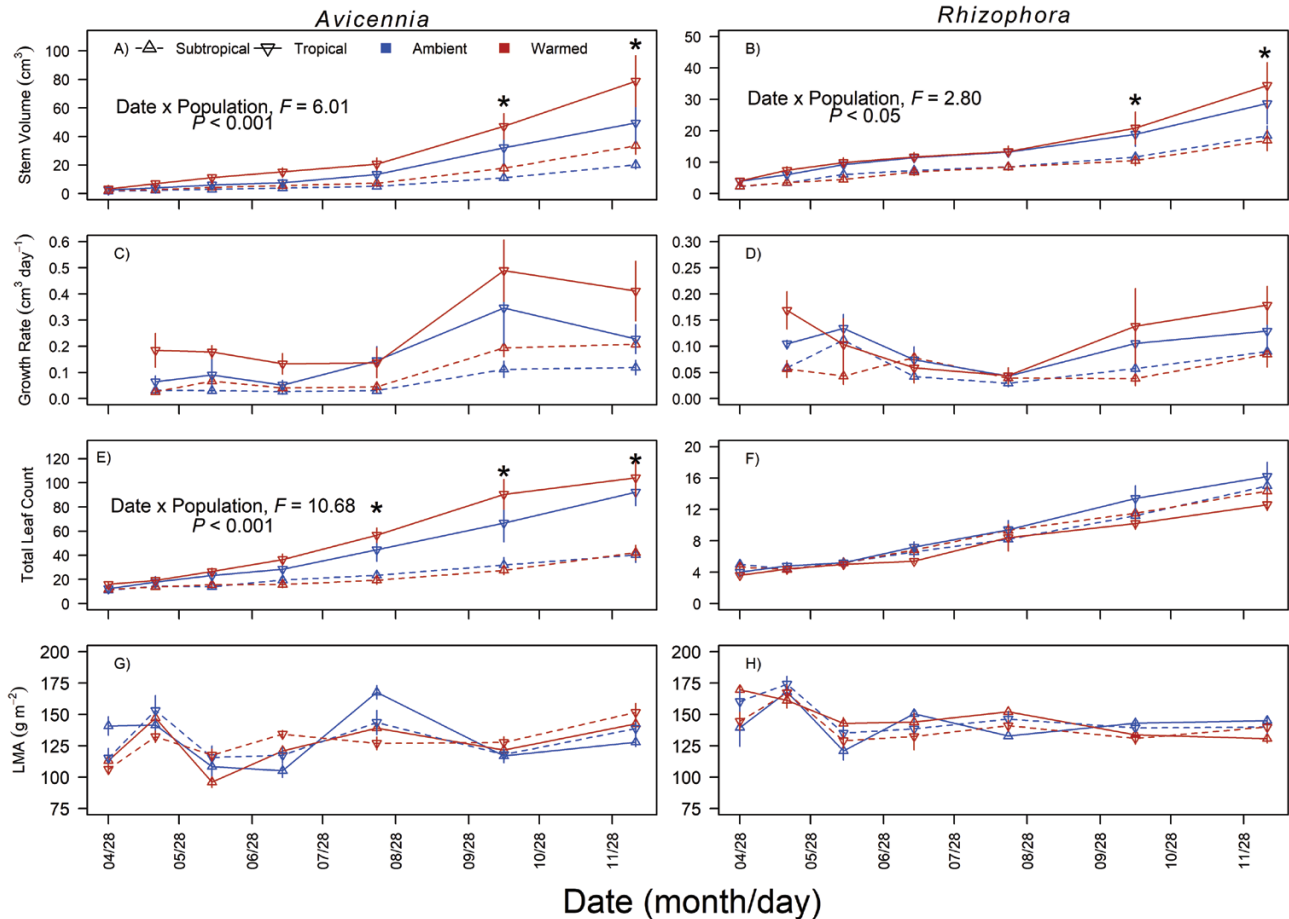
## Results

### Growth and biomass allocation

Stem volume was similar between populations during the first months, but tropical populations of both species showed higher stem volume than subtropical populations towards the end of the experiment (Fig. 2A, B; Supplementary Table S1). Averaged across dates, warming increased stem volume by 49.9% in *Avicennia* resulting from a 63.6% increase in growth rate ( $\text{cm}^3 \text{d}^{-1}$ ) compared with ambient conditions. Tropical *Avicennia* produced 22.3% more leaves under warming, while warming had no effect on leaf production in subtropical *Avicennia*. Generally, tropical *Avicennia* seedlings were larger than those from the subtropics. LMA was lower during warmer periods (Fig. 2G) but was unaffected by treatment or population (Supplementary Table S1).

In *Rhizophora*, warming had no significant effects on stem volume or growth rate (Supplementary Table S1). Yet, warming caused a modest (17.6%), albeit significant, decrease in leaf production in the tropical population with no effects of warming on leaf production in the subtropical population. For *Rhizophora*, LMA varied over time, with lower LMA during warmer periods (Fig. 2H).

In *Avicennia*, warming increased tropical seedling total dry mass by 51.5%, but did not affect subtropical seedling total dry



**Fig. 2.** Mean ( $\pm$ SE errors,  $n=6$ ) values for stem volume (A, B), growth rate (C, D), the number of leaves (E, F), and leaf mass per area (LMA; G, H) measured over seven time points for *Avicennia germinans* (left panels) and *Rhizophora mangle* (right panels). For each species, two populations (tropical from Belize, subtropical from Florida) were grown in a common garden under ambient (blue) and warmed (red) conditions. Asterisks denotes significant date $\times$ population effects within each panel.  $F$  and  $P$ -values for the model are included in each panel. Note: the y-axes are different for left- and right-hand panels.

mass (Fig. 3A), with root and leaf production being most responsive to warming (Supplementary Table S2). These results generally aligned with our hypothesis that warming would increase productivity in tropical populations more than in subtropical populations (Fig. 1A).

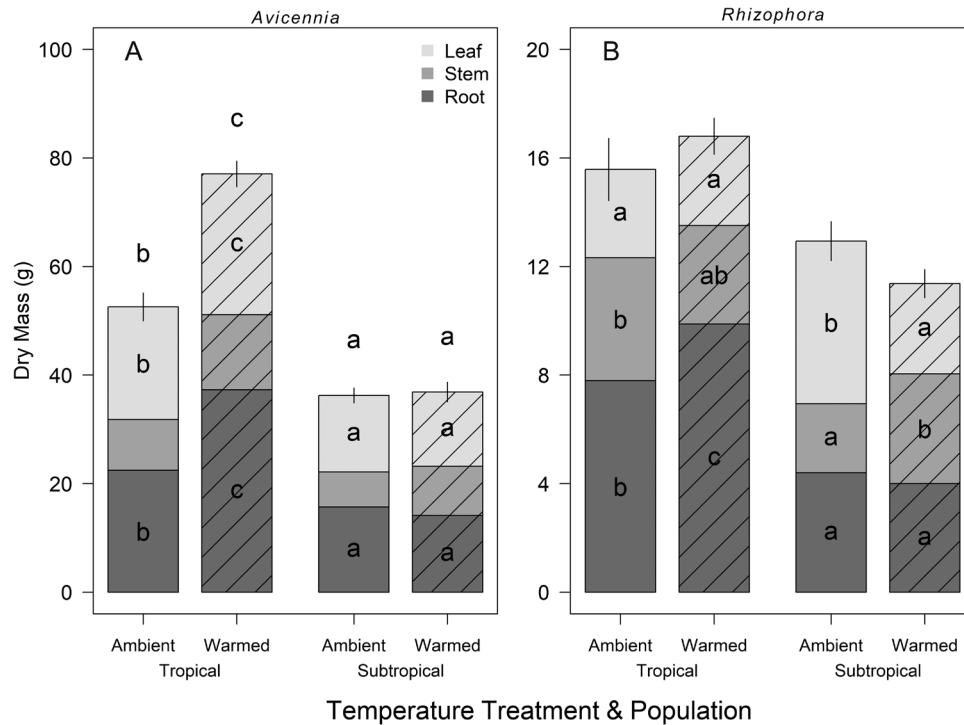
In *Rhizophora*, warming effects on total dry mass were similar between populations (i.e. no treatment $\times$ population effect, Supplementary Table S2), although total dry mass was 34.4% higher in tropical seedlings than in subtropical seedlings. However, warming effects on leaf, stem, and root dry mass differed between populations. In subtropical seedlings, warming decreased leaf dry mass by 44.4% but increased stem dry mass by 59.1% (Fig. 3B). Warming increased root dry mass by 26.7%. These warming effects were also reflected through changes in biomass (Fig. 3B).

#### Seasonal acclimation of leaf R

In both *Avicennia* and *Rhizophora*, temporal variation in leaf respiratory physiology was observed (Supplementary Fig. S4), with few differences across populations or warming treat-

ments (Supplementary Table S3; Supplementary Fig. S5). In both species, seasonal changes in  $R_{\text{area}}^{25}$ ,  $R_{\text{mass}}^{25}$ , and  $Q_{10}^{25}$  were largely explained by changes in mean daily air temperature (i.e.  $T_5$ ). In *Avicennia* and *Rhizophora*, we found a negative relationship between  $R_{\text{area}}^{25}$  and  $T_5$  that was consistent across warming treatments and populations (Fig. 4A). However,  $R_{\text{area}}^{25}$  declined more steeply with increasing  $T_5$  using data collected prior to the freeze event compared with using data from all time points (Table 1). We also found a negative relationship between  $R_{\text{mass}}^{25}$  and  $T_5$ , which was consistent across treatments and populations; however, this trend was restricted to pre-freeze data in *Avicennia* (Fig. 4C) and only apparent when using all time points in *Rhizophora* (Fig. 4D). In contrast to our expectations, seasonal acclimation of  $R_{\text{area}}^{25}$  and  $R_{\text{mass}}^{25}$  was consistent across tropical and subtropical populations.

In both species, seasonal changes in  $Q_{10}^{25}$  were dependent upon population (significant  $T_5 \times$  population effect, Supplementary Table S3). In subtropical *Avicennia*, we found a positive relationship between  $T_5$  and  $Q_{10}^{25}$ , indicating higher



**Fig. 3.** Dry mass of *Avicennia germinans* (A) and *Rhizophora mangle* (B). For each species, populations (tropical from Belize, subtropical from Florida) were grown in a common garden under ambient (clear boxes) and warmed conditions (hatched boxes). Data are presented as components of dry mass ( $\pm$ SE): leaves (lightest gray), stems, and roots (darkest gray). Letters represent significant differences within that dry mass component, where letters that are shared represent no significant differences. Letters above bars are for total biomass. No letters indicate no significant temperature  $\times$  treatment population effects.

sensitivity of leaf  $R$  to temperature during summer (Fig. 4E). However,  $Q_{10}^{25}$  was rather consistent across seasons in tropical *Avicennia*, with these trends restricted to data collected prior to the freeze event. In *Rhizophora*, the subtropical population showed consistent  $Q_{10}^{25}$  across seasons while the tropical population increased  $Q_{10}^{25}$  as  $T_5$  increased (Fig. 4F). In both species, these results indicate that seasonal adjustments in  $Q_{10}^{25}$  differed between tropical and subtropical populations. Still, experimental warming did not affect the relationship between  $Q_{10}^{25}$  and  $T_5$ .

#### In situ respiration and homeostasis

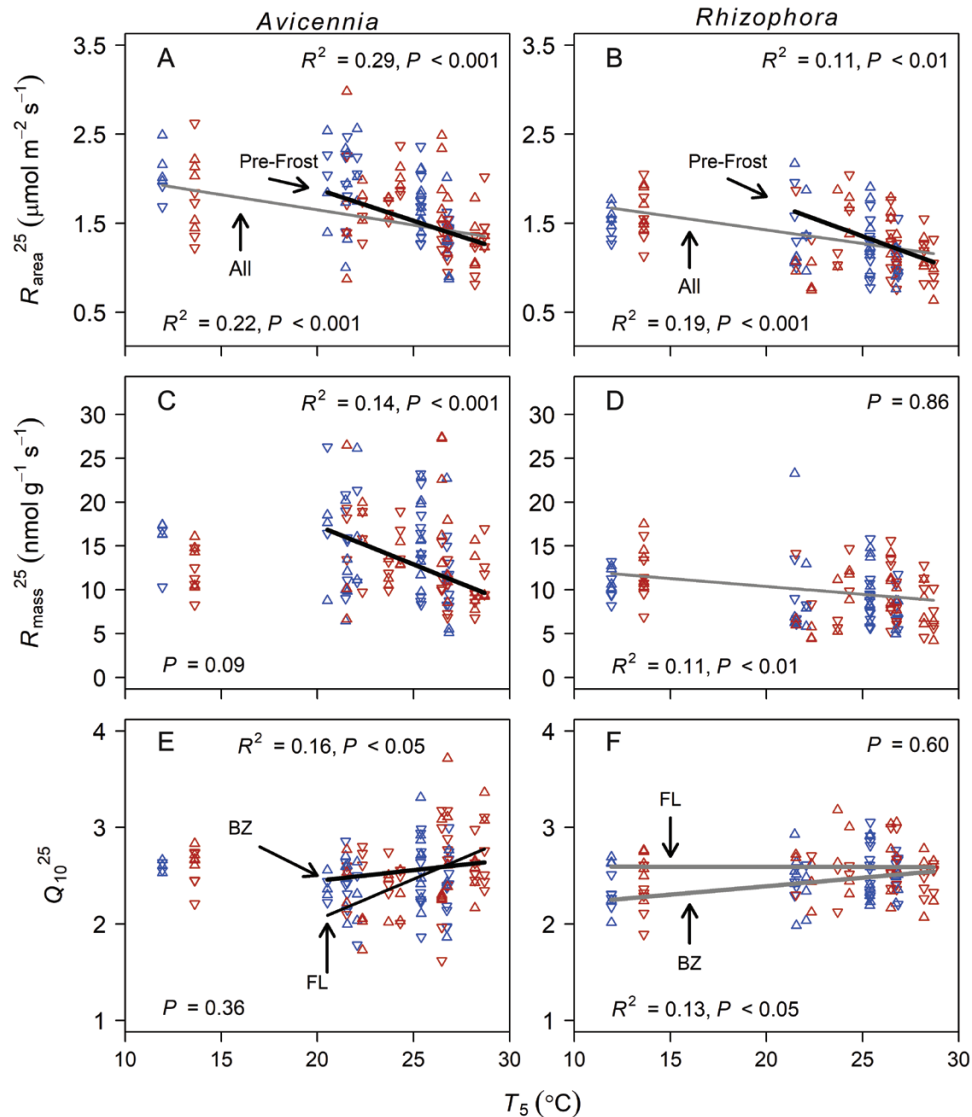
In *Avicennia*, we observed no relationship between  $\text{Acclim}_{\text{Homeo}}$  (measure of respiratory homeostasis) and seasonal warming (Fig. 5A, C), with no significant population effect. However, across seasons, ambient-grown plants exhibited greater homeostasis of  $R_{\text{area}}$  ( $\text{Acclim}_{\text{Homeo}} = 0.88 \pm 0.06$ ) than warm-grown plants ( $\text{Acclim}_{\text{Homeo}} = 0.69 \pm 0.06$ ,  $F = 6.22$ ,  $P = 0.02$ ). Across populations and treatments,  $\text{Acclim}_{\text{Homeo}}$  of  $R_{\text{mass}}$  was  $0.93 \pm 0.05$ , indicating near-complete homeostasis across seasons.

In *Rhizophora*,  $\text{Acclim}_{\text{Homeo}}$  for  $R_{\text{area}}$  and  $R_{\text{mass}}$  declined with increasing seasonal temperatures. The decline in  $\text{Acclim}_{\text{Homeo}}$  for  $R_{\text{area}}$  was similar between populations and treatments (popu-

lation slope =  $-0.067$ , treatment slope =  $-0.046$ ), indicating a similar reduction in respiratory homeostasis with seasonal warming, but the intercept differed between populations and temperature treatments (Fig. 5B). On an area basis, the intercept of the  $\text{Acclim}_{\text{Homeo}}$ –seasonal warming relationship was lower in the subtropical population (intercept = 1.016) than the tropical population (intercept = 1.166), and lower in the warmed treatment (intercept = 0.814) than the ambient treatment (intercept = 1.063, Fig. 5B). Thus, across seasons, respiratory homeostasis was generally lower in the subtropical population and warmed treatment. On a mass basis, the intercept of the  $\text{Acclim}_{\text{Homeo}}$ –seasonal warming relationship was lower in the warmed treatment (intercept = 1.071) than the ambient treatment (intercept = 1.336), indicating that warming generally reduced the degree of respiratory homeostasis across seasons (Fig. 5D).

#### Population and warming effects on cold susceptibility

In *Avicennia*, subtropical seedlings ( $n = 12$ ) exhibited no freeze injury. In tropical seedlings, eight out of 12 exhibited some freeze injury, with two seedlings exhibiting severe injury (>50% of the canopy affected). Among the tropical seedlings that exhibited freeze injury, half were grown under ambient conditions and half were grown under warmed conditions,



**Fig. 4.** Relationships between prevailing 5 d mean air temperatures ( $T_5$ ) and area-based leaf respiration at 25 °C ( $R_{area}^{25}$ ), mass-based leaf respiration at 25 °C ( $R_{mass}^{25}$ ), and the  $Q_{10}$  of leaf respiration at 25 °C ( $Q_{10}^{25}$ ) for subtropical (Florida, open triangles) and tropical (Belize, open circles) populations of *Avicennia germinans* and *Rhizophora mangle*. Seedlings were grown in a common garden under ambient (blue) and warmed conditions (red). Data were analyzed using all data ('All' seven sampling dates) and data collected prior to a freeze event ('Pre-freeze' six sampling dates). Gray trend lines denote significance using all data, while black lines denote significance restricted to pre-freeze data. In (E) and (F) we observed a significant temperature × population effect, so relationships for each seedling population are denoted using arrows. The  $R^2$  (when significant) and  $P$ -values for analyses using all data are reported in the bottom left of each panel; model fit parameters using data pre-freeze are reported in the top right of each panel.

indicating no effects of growth temperature on freeze susceptibility.

We observed no visible freeze injury in *Rhizophora* seedlings. However, during the biomass harvests all leaves were easily detached (compared with previous samplings where leaves were detached for leaf  $R$  measurements). While we had no method for quantifying premature abscission, it appeared that all seedlings were in the process of dropping leaves. This indicated susceptibility of *Rhizophora* to freeze events, with no effects of temperature treatment or population.

## Discussion

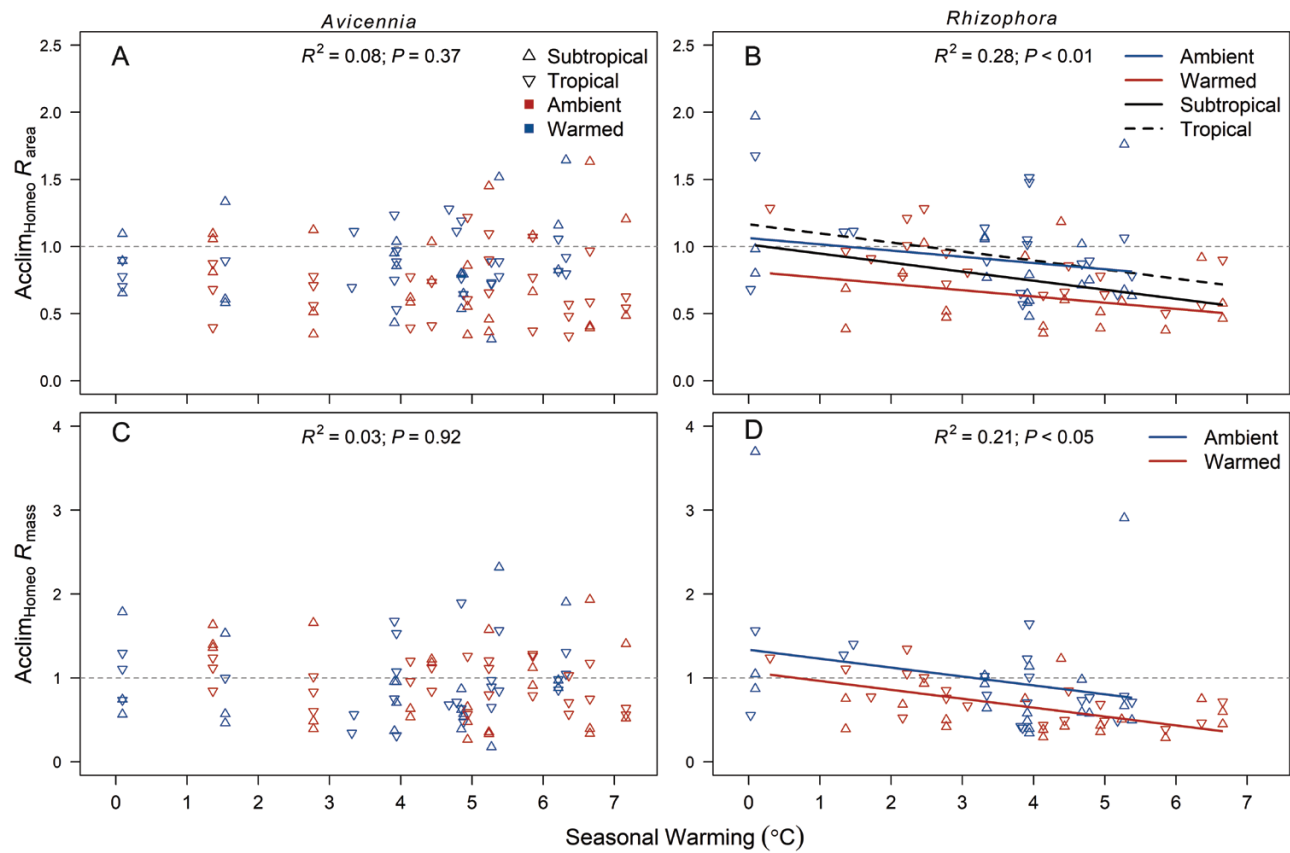
We found general support for our hypotheses that tropical and subtropical mangrove populations would show different growth responses to warming and freezing. Tropical *Avicennia* grown at the species northern range limit increased total dry mass in response to warming, while subtropical *Avicennia* seedlings showed no productivity response to warming. Tropical *Avicennia* was also more susceptible to freezing than subtropical *Avicennia*, while both *Rhizophora* populations appeared



**Table 1.** Slopes and intercepts from significant models examining the relationship between leaf respiratory parameters and 5 d mean prevailing temperature

Species	Data	Population	Response	Slope	Intercept	R <sup>2</sup>
<i>Avicennia</i>	All	Both	$R_{area}^{25}$	−0.034	2.334	0.22
	Pre-freeze	Both	$R_{area}^{25}$	−0.071	3.304	0.29
		Both	$R_{mass}^{25}$	−0.881	34.930	0.14
		Subtropical	$Q_{10}^{25}$	0.084	0.358	0.16
		Tropical	$Q_{10}^{25}$	0.022	2.007	–
<i>Rhizophora</i>	All	Both	$R_{area}^{25}$	−0.031	2.038	0.19
		Both	$R_{mass}^{25}$	−0.181	14.017	0.11
		Subtropical	$Q_{10}^{25}$	−3.009xe-04	2.597	0.13
		Tropical	$Q_{10}^{25}$	0.017	2.043	–
	Pre-freeze	Both	$R_{area}^{25}$	−0.079	3.323	0.11

Data for *Avicennia germinans* and *Rhizophora mangle* were analyzed using the entire dataset (Data=All) or data obtained prior to a freeze event, which occurred prior to the final sampling (Data=Pre-freeze).



**Fig. 5.** The degree of acclimation from homeostasis ( $Acclim_{Homeo} = in situ R$  at the coolest temperature divided by  $in situ R$  at all other points during the experiment) of area- and mass-based leaf respiration ( $R_{area}$  and  $R_{mass}$ , respectively) by seasonal warming (i.e. the difference in the 5 d mean air temperature from the coolest time point from all other time points during the experiment). Solid blue and red lines indicate significant relationships between  $Acclim_{Homeo}$  and the degree of warming for ambient- and warm-grown seedlings, respectively. The solid and dashed black lines indicate significant relationships between  $Acclim_{Homeo}$  and the degree of warming for subtropical and tropical populations, respectively.

equally frost susceptible. *Rhizophora* populations showed similar productivity responses to warming, although the tropical population increased root mass with warming. Importantly, we found little evidence that populations differed in temperature

acclimation of leaf  $R$ . These results provide evidence for temperature adaptation at the whole-plant scale but little evidence for population differences in thermal acclimation of leaf physiology.

### Genetic differentiation in growth responses to warming and freezing susceptibility

We expected that tropical populations of both mangrove species would exhibit a positive growth response to warming at the species' northern range limit, as warming would push  $T_{\text{air}}$  closer to that of their home environment (Feeley *et al.*, 2007; Way and Oren 2010; Drake *et al.*, 2015). Indeed, we found that tropical populations generally increased biomass production with warming, particularly in leaves and roots (Fig. 3). Warming did influence subtropical seedlings, although changes in productivity were small and primarily related to biomass allocation. A few studies have also observed population differences in growth responses to warming among tropical and subtropical populations of widely distributed species, assumed to be reflective of differences in temperature adaptation (Drake *et al.*, 2015).

As expected, tropical *Avicennia* were more susceptible to freezing temperatures than subtropical *Avicennia*; however, both populations of *Rhizophora* were highly sensitive to freezing temperatures. These results provide mixed support for our original hypothesis, but generally align with previous observations. Markley *et al.* (1982) found that susceptibility to freezing temperatures increased as latitude of origin decreased in seed sources of *A. germinans* and *R. mangle* (seeds sourced from 8°N to 27°N). Cook-Patton *et al.* (2015) also observed phenotypic variation in freeze tolerance across a 200 km latitude gradient in Florida, indicating strong potential for genetic differentiation in freeze tolerance among populations. *Avicennia* is considered the most freeze tolerant of North American mangroves, with −4 °C identified as the threshold for leaf damage (Osland *et al.*, 2020). In comparison, *Rhizophora* has been shown to exhibit damage at 0–3 °C in south Florida (Ross *et al.*, 2009; Zhang *et al.*, 2016), and Devaney *et al.* (2021) found that *Rhizophora* was generally less tolerant of freezing than *Avicennia*. In our study, temperatures during the freeze event were −2.1 °C to −1.0 °C which may have been too cold to elucidate any population differences in freeze tolerance in *Rhizophora* (i.e. freezing temperatures were below the injury threshold for both populations). Although we cannot rule out the potential for genetic differentiation in *Rhizophora* freeze tolerance, our results indicate differences in freeze tolerance between tropical and subtropical *Avicennia* populations, and no genetic differentiation in freeze tolerance in *Rhizophora*.

We also tested whether seedlings of both species and populations would show increased freeze damage when grown under experimental warming. Following observations in some temperate and boreal species, we assumed that warmer growth temperatures would limit cold acclimation and increase freeze susceptibility (Pagter and Arora, 2013; Bokhorst *et al.*, 2018). We found no evidence that experimental warming increased mangrove seedling susceptibility to freezing. These results are similar to those of Song *et al.* (2020) who found that experimental warming had no impact on cold tolerance of two

Indo-Pacific mangroves (*Avicennia marina* and *Bruguiera gymnorhiza*). We conclude that climate warming may not alter mangrove responses to freezing. Instead, exposure to prior chilling events or other abiotic factors such as humidity or salinity could be more important determinants of freezing tolerance and recovery (Devaney *et al.*, 2021).

### Consistent acclimation to seasonal temperatures across populations

We found that tropical and subtropical populations of both species showed similar thermal acclimation of respiratory capacity to seasonal temperature changes and experimental warming (Fig. 4). This finding contradicts our expectation that home-thermal environment would influence thermal acclimation of leaf  $R$  in both *Avicennia* and *Rhizophora*. However, this result is consistent with a few studies with different species that found little evidence of within-species (population-level or genotypic) variation in thermal acclimation (Bolstad *et al.*, 2003; Atkin *et al.*, 2005; Lee *et al.*, 2005). The lack of population-level differences in thermal acclimation could be due to the environmental conditions under which the sporophyte embryo develops (Bossdorf *et al.*, 2008). For instance, Kvaalen and Johnsen (2008) found seeds of *Picea abies* collected from latitudinal and elevation gradients to exhibit several traits (growth rates, temperature tolerance, and timing of bud break) linked to the thermal conditions of the seed's home environment; however, when germinated under common conditions, these traits converged, highlighting the importance of environment during development in trait expression. Nonetheless, others have found some population-level differentiation in acclimation capacity relating to home-thermal environment. For instance, Tjoelker *et al.*, (2009) found that warm-origin provenances of *Pinus banksiana* (a widespread boreal conifer) acclimated more strongly to prevailing temperatures than those from cooler origins. Yet, few studies have specifically examined species which span tropical to temperate ecosystems. One study using multiple provenances of two tree species with distributions spanning warm-temperate to tropical regions found stronger acclimation to elevated growth temperatures in temperate provenances of *Eucalyptus tereticornis* compared with tropical provenances, with no provenance differences in thermal acclimation of respiratory capacity in temperate and tropical *Eucalyptus grandis* (Drake *et al.*, 2015).

While our findings were consistent with many studies that found minimal population-based differences in acclimation capacity, we did observe population-based differences in adjustments of  $Q_{10}^{25}$  to prevailing conditions (Fig. 4). Subtropical *Avicennia* and tropical *Rhizophora* increased  $Q_{10}^{25}$  as prevailing  $T_{\text{air}}$  increased, which is the opposite of what would be expected if Type I thermal acclimation occurred (Slot and Kitajima, 2015). Interestingly, tropical *Avicennia* and subtropical *Rhizophora* exhibited consistent  $Q_{10}^{25}$  values across a  $T_{\text{air}}$  range >10 °C. While the proximate cause of increased  $Q_{10}^{25}$

with increasing  $T_{\text{air}}$  in subtropical *Avicennia* and tropical *Rhizophora* remains unclear, there are a few possible explanations. First, some studies have found that  $Q_{10}$  values increase with soluble sugars (Azcón-Bieto, 1983; Atkin *et al.*, 2005; Ow *et al.*, 2010). This positive relationship could reflect substrate limitation of the respiratory enzyme activity (Atkin and Tjoelker, 2003). A second explanation for population differences in seasonal changes in  $Q_{10}^{25}$  could be population differences in leaf nitrogen (N) or phosphorus (P) concentrations over seasons. Studies including many species at many sites, and some studies with individual species at individual sites, have found that leaf N and P tend to scale positively with  $R_{\text{area}}^{25}$  or  $R_{\text{mass}}^{25}$  (Meir *et al.*, 2001; Tjoelker *et al.*, 2008; Crous *et al.*, 2011; Atkin *et al.*, 2015; Sturchio *et al.*, 2022). A third explanation for population differences in seasonal changes in  $Q_{10}^{25}$  could be population differences in the low temperature threshold required to increase  $Q_{10}^{25}$ . In *Eucalyptus pauciflora*, Atkin *et al.* (2000) found that the  $Q_{10}$  of  $R$  was constant ( $\sim 2.0$ ) when prevailing mean daily  $T_{\text{air}}$  varied between 6 °C and 16 °C. When mean daily  $T_{\text{air}}$  dropped below  $\sim 6$  °C, the  $Q_{10}$  of  $R$  increased to 3.0–4.0. Although seedlings in our study experienced brief periods of freezing, mean daily  $T_{\text{air}}$  was generally above 10 °C. Thus, conditions during the experiment may not have been cool enough to trigger increased  $Q_{10}^{25}$  in some populations.

Interestingly, the slope of the relationship between  $R_{\text{area}}^{25}$  and  $T_5$  prior to the freeze event ( $-0.071$  for *Avicennia* and  $-0.079$  for *Rhizophora*) was similar between species. These slope estimates are similar to what others have observed in temperate-subtropical *Eucalyptus tereticornis* (slope  $-0.070$ , Aspinwall *et al.*, 2016) and temperate *Fagus sylvatica* L. ( $-0.070$ , Rodríguez-Calcerrada *et al.*, 2010). However, the area-based slope for *A. germinans* in our study was 85% higher than the slope of the relationship between  $R_{\text{area}}^{25}$  and  $T_5$  found in potted *A. germinans* seedlings in Aspinwall *et al.* (2021). Our slope estimate for the  $R_{\text{mass}}^{25}$  versus  $T_5$  relationship ( $-0.881$ ) was more than double the slope estimate from Aspinwall *et al.* (2021). Importantly, that study used a different *A. germinans* population; one from northeast Florida that is presumably adapted to the coolest temperatures in the species range. Although the strength of acclimation differed between studies, both results indicate consistent acclimation responses in *Avicennia* across different abiotic conditions (i.e. salinity and experimental warming). However, a recent study with *Avicennia* trees growing under natural conditions in northeast Florida found little evidence of thermal acclimation of respiratory capacity; only reductions in  $Q_{10}^{25}$  with increasing temperature (Sturchio *et al.*, 2022). The generality of thermal acclimation responses in *Avicennia*, and mangroves in general, is unclear, and further work is required to determine how genetic and environmental variation affects temperature controls of respiratory  $\text{CO}_2$  fluxes in mangrove ecosystems.

Signatures of temperature adaptation were apparent at the whole-plant scale, but thermal acclimation of leaf  $R$  was rather consistent between tropical and subtropical populations of both

species. Why might thermal acclimation of leaf physiology be consistent between populations adapted to different thermal environments? Thermal acclimation of  $R$  is thought to optimize the supply of ATP and carbon skeletons for plant growth, cell maintenance processes (e.g. protein turnover), and active transport under fluctuating temperature conditions (Atkin and Tjoelker, 2003; Atkin *et al.*, 2005). Stronger or weaker acclimation results in greater or less respiratory homeostasis (Slot and Kitajima, 2015), with potential consequences for plant performance. In some cases, greater homeostasis of  $R$  is associated with higher uniformity in plant growth rates across growth temperatures (Kurimoto *et al.*, 2004). However, at the leaf scale, thermal acclimation of leaf  $R$  may come at a cost if it does not optimize processes that support maintenance of photosynthetic capacity under natural conditions (Dewar *et al.*, 1999; Aspinwall *et al.*, 2016; Smith *et al.*, 2020; Wang *et al.*, 2020). Thus, similarities in thermal acclimation of leaf  $R$  between populations from different thermal environments might arise due to similarities in maintenance of photosynthetic processes across changing growth temperatures. New studies that examine coordination of thermal acclimation of leaf  $R$  and photosynthetic capacity among populations within species, as well as potential costs and benefits of acclimation (Wilson and Franklin, 2002; Arnold *et al.*, 2019), and epigenetic effects, may provide new insights into the evolution of thermal acclimation processes.

## Supplementary data

The following supplementary data are available at [JXB online](https://onlinelibrary.wiley.com/doi/10.1111/jxb.15111).

Fig. S1. Daily minimum and maximum temperatures and monthly mean precipitation from tropical Twin Cayes, Belize, and subtropical Fort Myers, Florida.

Fig. S2. Daily values of air temperature, relative humidity, vapor pressure deficit, and differences between ambient and warmed values of daily temperature during the experiment.

Fig. S3. Density of readings from environment sensors for the course of the experiment.

Fig. S4. Natural log-transformed area-based and mass-based respiration by leaf temperature.

Fig. S5. Mean values for area- and mass-based leaf respiration at 25 °C and the  $Q_{10}$  of leaf respiration.

Table S1.  $F$ -values from the three-way ANOVA of sample date, population, warming, and their interactive effects on stem volume, growth rate, and total leaf count.

Table S2.  $F$ -values from the two-way ANOVA of warming and population on plant dry matter, dry matter allocation, and whole-plant traits.

Table S3.  $F$ -values from the three-way ANOVA of sampling date, population, warming treatment, and their interactions on area- and mass-based leaf respiration at 25 °C and the  $Q_{10}$  of leaf respiration.

Table S4. ANCOVA of 5 d mean temperature, population, warming treatment, and their interactions on

area- and mass-based leaf respiration at 25 °C and the  $Q_{10}$  of leaf respiration.

## Author contributions

JC, KH, and MJA: conceptualization and design; JC, KH, MS, SD, and EG: data collection; JC and MJA: data analysis; JC and MJA: writing the manuscript with input from all authors.

## Conflict of interest

The authors declare no conflicts of interest.

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## Data availability

All data used in this manuscript are publicly available and can be accessed at <https://github.com/jjchieppa/Mangroves>.

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