Title: The role of freezing in setting the latitudinal limits of mangrove forests

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Abbreviations:
EDX, Energy Dispersive X-ray Microanalysis
PLC, Percent Loss of Conductivity

Summary

- Mangrove trees dominate coastal vegetation in tropical regions, but are completely replaced by herbaceous salt marsh at latitudes above 32° N and 40° S. Because water deficit can increase damage due to freezing, we hypothesized that mangroves, which experience large deficits due to saline substrates, would suffer freeze-induced xylem failure.
- Vulnerability to freeze-induced xylem embolism was examined in the five most poleward mangrove species: *Avicennia germinans, Rhizophora mangle, Avicennia marina*, *Aegiceras corniculatum* and *Rhizophora stylosa*. Percent loss in hydraulic conductivity was measured following experimental manipulations of xylem tension; xylem sap ion concentration was determined using energy-dispersive X-ray microanalysis measurements on cryogenically frozen tissue.
- Species with wider vessels suffered 60-100% loss of hydraulic conductivity after freezing and thawing under tension, while species with narrower vessels lost as little as 13-40% of conductivity.
- These results indicate that freeze-induced embolism plays a fundamental role in setting the latitudinal limits of distribution in mangrove species, either through massive embolism following freezing, or through constraints on water transport due to vessel size.

Keywords: Mangrove, *Avicennia, Aegiceras, Rhizophora*, freeze-induced embolism, vessel diameter, xylem ion content

Introduction

Mangroves occur widely in the tropics but species diversity and stand complexity fall rapidly in temperate regions (Tomlinson, 1994; Spalding *et al.,* 1997; Duke *et al.,* 1998). Coastal ecosystems at latitudes above 32° N and 40° S lack woody vegetation, in contrast with the tropics, where an estimated 75% of coast can be classified as mangal (Spalding *et al.,* 1997). This world-wide transition from woody to herbaceous vegetation has long been attributed to temperature (McMillan, 1971; Lugo & Zucca, 1977; McMillan & Sherrod, 1986; Sherrod *et al.*, 1986; Tomlinson, 1994; Duke *et al.,* 1998), but the fundamental mechanisms excluding woody plants from tidal habitats at high latitudes remain unknown. Because mangroves are often considered tropical by definition (Tomlinson, 1994), few have questioned why they do not spread to temperate areas.

Mangroves are a large and diverse ecological assemblage. Trees of widely disparate families are found in the mangrove habitat, representing at least five major and many additional minor independent evolutions of salt-tolerant, anoxia-tolerant, woody plants (Tomlinson, 1994). Vivipary, breathing roots, and salt glands have evolved multiple times within this ecological classification (Ball, 1988; Duke *et al.,* 1998), suggesting strong, convergent selective pressures. Given the wide range of species filling this niche, and the opportunities for adaptation present in different lineages, it is surprising that natural selection has not produced woody plants that can tolerate the combined stresses of salinity and freezing.

Vulnerability to the disruption of water transport after freezing corresponds with range limits of many species (Sperry *et al.,* 1994; Pockman & Sperry, 1997; Langan *et al.,* 1997; Cavender-Bares & Holbrook 2001). Significant losses in the ability to supply water to stems and leaves can occur when air bubbles form in xylem sap during freezing. If the sap thaws under tension, these pockets of air can expand to block water transport (Tyree & Zimmerman 2002).

Whether a bubble will shrink or grow depends on the hydrostatic pressures within both the bubble and the surrounding fluid, as well as the force resulting from the gas:liquid interface. The bubble will expand when

$$P_B - P_x > \frac{2\gamma}{r} \qquad 1$$

where 2γ/r, the inward pressure due to surface tension γ divided by the radius of the bubble r, is less than the difference between the hydrostatic pressure of the xylem sap (P_x; typically <0) and the pressure of the gas within the bubble itself (P_B, >0). Thus, as pressure in the sap drops, smaller bubbles will cause xylem dysfunction (Davis *et al.,* 1999; Tyree & Zimmerman 2002). Mangroves experience comparatively large xylem tensions because of the high osmotic concentration in their substrates (Scholander *et al.,* 1962, Scholander, 1968). The xylem water potential of a tree growing in seawater will be ≤ -2.5 MPa even when stomata are closed. The magnitude of this tension increases during the day, as transpiration drives the water potential in the leaves below that of the soil. These substantial xylem tensions cannot be eliminated by shedding leaves, and appear to preclude the generation of positive root pressures as a means of refilling embolized conduits (Sperry *et al.,* 1988).

Plant life in saline habitats can be compatible with cold temperatures; well-developed salt marsh communities dominate tidal plains and estuaries where winter ocean isotherms fall below 10°C (Duke *et al.* 1998), and extend well into the arctic (Mitsch & Gosselink, 1986). The feature that distinguishes the salt marsh from the mangal is the lack of woody, arborescent plants. We hypothesize that the unique combination of freezing and tension due to salinity results in the exclusion of woody plants from coastal wetlands at latitudes where freezing is a regular occurrence.

We tested this hypothesis in three mangrove communities, sampling the five most poleward mangrove species on two continents at or near their absolute latitudinal limits, using *in vivo* and excised-branch techniques to examine how the interaction of freezing and tension influence hydraulic conductivity. Experimental manipulations of xylem tension were performed on excised branches to separate the effects of tension and freezing.

Materials and Methods

Plant material Five species of mangroves were collected from natural populations growing near their latitudinal limits. In Florida, USA, *Avicennia germinans* (L.) Stearn (Acanthaceae) and *Rhizophora mangle* L. (Rhizophoraceae) were collected at Marineland, (29°40' N, 81°12' W) and at Ponce Inlet (29°4' N, 80°55' W) respectively. In Australia, *Avicennia marina* (Forsk.) Vierh, var. *australasica* (Walp.) Moldenke (Acanthaceae) and *Aegiceras corniculatum* (L.) Blanco (Myrsinaceae) were collected in New South Wales, at Batemans Bay (35°42' S, 150°12' E), while *Rhizophora stylosa* Griff. (Rhizophoraceae) was collected in Queensland, near Jacobs Well (27°46' S, 153°22' E). It can be difficult to determine the age of mangrove wood in all species (Tomlinson, 1994) due to their generally aseasonal habitats (but see Verheyden *et al.*, 2005); however, all measurements were made on mature branches at least one year old and 2.5 to 7.7 mm in diameter; most of the variation in diameter was due to differences between species.

Occurrence of Freezing at Collection Sites In Florida, average winter minimums were high, 8.2°C in Jacksonville, near the Marineland site, and 9.5°C at Daytona Beach, near the Ponce Inlet site, but minimum recorded temperatures were -10°C and -9.4°C (21 and 30 year records respectively, NOAA-CIRES 2002). During the past century, severe freezes (< 8°C) occurred on average once every eight years (Henry *et al.,* 1994). In southern Australia, mild frosts are common: minimums of 0°C, -0.61°C and -3°C were recorded in mangrove communities during the study (data not shown) and thirteen-year record report minimums of -2.9°C (Commonwealth Bureau of Meteorology 2001).

Ion contents of xylem sap Ion contents of xylem vessels were measured using energy dispersive X-ray microanalysis (EDX) (McCully *et al.,* 2000) to determine an appropriate perfusing solution for use in hydraulic measurements. Physiologically relevant measurements of hydraulic conductivity are best made with a perfusing solution matched to the ion content of the xylem sap *in vivo*, as ion content can markedly affect measured conductivity (Zwieniecki, Melcher & Holbrook 2001, López-Portillo, Ewers & Angeles 2005).

One stem cross-section was taken from each of three individuals growing at an estuarine site in Nelligen, NSW, Australia, and three individuals from a marine site in Batemans Bay, NSW, Australia (n=3). Replicates were rapidly frozen by submersion of intact, attached branches in liquid nitrogen (-196°C). After removal from the tree, segments were cut from each branch and transported to the lab in a cryo-shipper at -170°C.

Samples were planed with a diamond knife at -80°C (McCully *et al.,* 1998; McCully *et al.,* 2000), coated with aluminum, and transferred to the cryo-stage of a scanning electron microscope (Oxford CT 1500, Oxford Instruments, Oxford, UK) where they were viewed through a beryllium window. Ion concentrations in 6-12 filled xylem conduits per sample were measured using EDX (Link eXL, Oxford Instruments, Oxford, UK).

Interaction of freezing and tension in excised branches Terminal, leaf bearing branches ~1 m in length were collected during periods of minimal transpiration and xylem tension between

20:00 and 22:00 for all species except *Rhizophora stylosa*, which was harvested between 5:00 and 7:00. For each species, 23 to 15 individuals were sampled; this was reduced to 10 in *R. mangle*, where only one experiment was conducted. Branches were cut in pairs, with one branch was assigned to the freezing treatment and the other to the cold-storage control. "Tension" and "Tension Relieved" samples were gathered on successive days. In "Tension" treatments, branches were cut from each tree in air. In "Tension Relieved" treatments branches from the same tree were cut under a solution containing 25 mM NaCl and allowed to hydrate until leaf water potentials (Ψ_L) measured with a pressure chamber (Scholander *et al.,* 1964) were ≥ 0.5 MPa. All branches were enclosed in plastic bags immediately following excision to prevent water loss and thus preserve xylem tensions at either their native ("Tension") or hydrated ("Tension Relieved") values.

Prior to beginning the freezing treatments, the water potential of one leaf from each branch was measured using a pressure chamber. Branches were placed in a temperature-controlled chamber and cooled from 0°C to -10°C at a rate of 2°C h^{-1}, held at -10°C for one hour, and thawed at 2°C min^{-1}. Moist towels were placed in the freezing chamber to prevent further water loss. Freezing and thawing rates reflected rates of temperature change experienced during freezing in nature (Sperry & Sullivan, 1992, Sperry *et al*., 1994, Pockman & Sperry, 1997 and Cordero & Nilsen 2002). Control branches were stored in sealed plastic bags at 4°C during the time the experimental branches were in the freezing chamber.

Thermocouples were attached to branches during freezing and branch temperatures were logged using a datalogger (Campbell Scientific, Logan, Utah USA). Freezing exotherms were observed and recorded for each branch. Because freezing is a stochastic process, freezing was observed to occur at a variety of temperatures between -2 and -10°C. A sample thermocouple trace for a freezing branch appears in Figure 1.

The effect of freezing on xylem hydraulic conductivity was quantified by comparing percent loss of hydraulic conductivity (PLC) in paired frozen and unfrozen (control) branches. A 3-to-7 cm segment, located >1× maximum vessel length from the cut end, was excised from each branch. Maximum vessel lengths were determined using the air injection method of Zimmerman & Jeje (1981). All cuts were made under 25 mM NaCl solution to prevent entry of air into the test segment; this solution, passed through a 0.2 μm syringe filter, was also used for perfusion. The flow rate through each segment was then measured with a steady-state flow meter (Brodribb & Feild, 2000) at an initial hydraulic head of ~2.3×10^{-2} MPa (US) or ~1.9×10^{-3} MPa (Australia). These pressures, which occurred before the first resistor, are insufficient to flush emboli from open vessels in all five species, based on measurements of maximum vessel diameter. The actual delivery pressures experienced by the branches were on the order of half that supplied by the initial pressure head.

Each segment was then flushed with perfusing solution using a syringe to remove air emboli from vessels. Segments were flushed at ~100 kPa for five minutes, or until no more bubbles emerged from the distal end of the segment, and then re-measured to estimate maximum conductivity. Measurements are reported as PLC:

$$PLC = (1-(K_i / K_m)) \times 100 \qquad 2$$

where K_i indicates initial conductivity after treatment and K_m indicates maximum conductivity after flushing. The short length of the segments ensured that a majority of vessels were cut open at both ends. This approach has been used by Cochard *et al.* (2002) and Brodribb *et al.* (2003) to determine PLC, and was chosen because tests showed conductivity decreased in *Avicennia* *marina* during the longer flushing times required to dissolve emboli, possibly due to wounding effects (data not shown). However, if the majority of vessels are open, emboli can be pushed out the end of the segment using only the pressure necessary to move a meniscus along an open capillary. Because vessel endings often contribute the majority of hydraulic resistance in the stem (Wheeler *et al.,* 2005, Choat *et al.,* 2006), a measurement excluding them would be misleading in comparisons; therefore, we do not report sapwood-specific conductivity.

Vessel diameter measurements Vessel diameters were measured on sections taken from segments used for hydraulic measurements in both the US and Australia. Three images were taken per cross-section in each stem, accounting for ~3/4 of total area of the section. For each species two stems each from five individuals were used. In the US, segments were shaved smooth with a razor blade, oven-dried and sputter coated, and viewed on a calibrated Quanta 200 ESEM (FEI Co., Hillsboro, Oregon, USA). In Australia, segments preserved in ethanol were hand-sectioned, stained with toluidine blue, and photographed at 100× magnification on an Axioskop light microscope (Carl Zeiss, Oberkochen, Germany) with a Spot Camera (Diagnostic Instruments, Sterling Heights, Michigan, USA). All images were analyzed using thresholding and particle analysis utilities in analySIS 3.2 (Soft Imaging System, Gulfview Heights, South Australia, Australia).

Results

Ion contents of xylem sap EDX analysis of both *Avicennia marina* and *Aegiceras corniculatum* revealed relatively low concentrations of ions in the xylem sap (Table 1). In both species, concentrations of K^+ were frequently below detectable limits, while Mg^{2+}, P^+, S^{2-}, and Ca^{2+} were only rarely above the limits of detection in xylem conduits. There was no significant difference between ion concentrations in xylem conduits of plants growing at the higher salinity coastal site and the estuarine site, allowing measurements to be pooled. *Avicennia marina* had higher average ion concentrations than *Aegiceras corniculatum*, but again there was no significant difference between species.

Based on these measurements, a perfusing solution containing 25 mM NaCl was selected for use in all five species. This agrees with previous reports of mangrove xylem sap content (Tomlinson, 1994, Ball, 1988) and represents 92-94% exclusion of substrate salt. Although both *Avicennia marina* and *Aegiceras corniculatum* have previously been thought to have higher sap salinities due to the fact that they have salt excretion glands, our results do not support this. These results are also consistent with reports of 24.4-36.6 mM NaCl by Melcher *et al.* (2001) in *Rhizophora mangle*, (a non-excreting species) however, they are less than the 56-316 mM reported by López-Portillo *et al.* (2005) *Avicennia germinans* and *Conocarpus erectus*.

Interaction of freezing and tension in excised branches There was severe xylem impairment in three of the five species after freezing at native tension; in two species, there was no significant effect of freezing at native xylem tensions (Fig. 2). A separate one-way ANOVA was performed for each species, showing that branches of *Avicennia germinans*, *Rhizophora mangle* and *Rhizophora stylosa* frozen under tension showed significantly ($P < 0.05$ for all tests, Tukey HSD) greater loss in conductivity than in control branches. Freezing under tension did not significantly impair conductivity in either *Aegiceras corniculatum* or *Avicennia marina* as compared to unfrozen branches Fig. 2).

These tests also showed that, among species susceptible to freeze-induced damage, *Avicennia germinans* and *Rhizophora stylosa* showed significantly greater dysfunction after freezing under tension than after freezing with tension relieved. Measurements on tension-relieved stems of *Rhizophora mangle* were not made due to time limitations and inaccessibility of the sample site.

In *Aegiceras corniculatum,* there was no significant effect of freezing, with or without tension. In *Avicennia marina*, branches frozen after tension was relieved showed significantly greater impairment than hydrated, unfrozen branches or branches frozen under tension. However, the overall loss of conductivity in this species was low (< 40%) and comparable to, or less than, the measurements for control branches in other species. Although the treatments were statistically distinguishable in *Avicennia marina*, the magnitude of impairment in each case was so low that it is unlikely to have a distinguishable effect on the survival of the plant. Losses in conductivity of this magnitude are not likely to lead to death of the plant; while this difference is statistically significant, it is small enough in scale that it is unlikely to be physiologically important.

Vessel diameter measurements Mean and hydraulically weighted average vessel diameter in all five species corresponded with both latitudinal limits and observed loss of conductivity after freezing at native xylem tensions (Table 2). The percentage loss in hydraulic conductivity of excised branches frozen under tension increased with the hydraulically weighted average vessel diameter (r^2=0.72) (Fig. 3). These data show that when the native tension was retained, interspecific differences in vessel diameter accounted for as much as 72% of the variation in freeze-induced embolism. Thus, for species inhabiting saline environments, vulnerability to freeze-induced embolism can be reduced by a decrease in the diameter of xylem vessels.

Discussion

We tested the hypothesis that tension generated by osmotic pressure makes mangroves more vulnerable to freeze-induced cavitation. In three of five species, a combination of freezing at environmental temperatures and *in vivo* tensions caused severe embolism. Freezing under tension caused also more severe loss of conductivity than did freezing with tension relieved in the two species for which this was examined. However, our results also indicate that *in vivo* tensions were not sufficient to cause significant xylem failure in *Aegiceras corniculatum* or *Avicennia marina*, species with mean vessel diameters of 17.05 and 19.09 µm (Table 2). We believe that these species were protected from embolism by their narrow vessels. Differences in hydraulically weighted average vessel diameter corresponded directly with loss of conductivity due to freezing (Fig. 3). In turn, loss of conductivity in response to freezing to -10°C corresponded with the latitudinal limits of the species in this study.

Previous work has correlated conduit diameter with hydraulic impairment due to freezing (Sperry *et al.,* 1994, Davis *et al.,* 1999), showing that primarily for reasons of volume (Tyree & Zimmermann 2002, Pittermann & Sperry, 2003) 30 µm is the threshold at which severe (>50%) loss of conductivity occurs (Davis *et al.,* 1999). In our results, all species above 22 µm showed significant loss in conductivity after freeze/thaw treatment; for species below this threshold, there was no major impairment after freezing.

Vessel Diameter and Latitudinal Range The average vessel diameters for *Avicennia germinans* and *Rhizophora mangle* collected from sites in Florida at 29°N were 54% greater than those of *Avicennia marina* and *Aegiceras corniculatum* collected in Australia at 35°S. The latter two species have the most poleward range of all mangroves. The mean vessel diameters of 19 and 17µm in *Avicennia marina* and *Aegiceras corniculatum*, respectively, are among the smallest observed in angiosperms (Tomlinson, 1994). Future work might be designed to address whether the smaller vessels diameters observed in these two populations are a result of plasticity or an innate trait. A comparative study of vessel diameter in *Avicennia marina* and *Aegiceras corniculatum* across latitude could address this question.

The climatic profiles of the collecting sites in Florida and New South Wales may contribute to the differences observed. In Florida, average winter minimums are warm, ranging from 8.2 to 9.5°C, but frosts as severe as -10°C are regular events, occurring, on average, once every 8 years over the past century (Henry *et al.,* 1994). For a mangrove growing under these conditions, there is no benefit to resisting freezing in an average year, and producing narrow vessels represents a significant fitness cost in years without frost. This suggests that where mangroves are able to achieve reproductive success, either directly or through dispersal, they may face little or no pressure to adapt to intermittent frosts.

By contrast, climate in southern New South Wales provides conditions that may select for frost resistance. Climate records indicate that frosts occur at least once a year in Bateman's Bay, with an average of 5.6 days with minimums ≤0°C per year, but frosts are less severe, with no temperatures below -3°C (Commonwealth Bureau of Meteorology 2006). Our experiments showed that when leaves are attached, xylem freezing may occur at or near this temperature (Fig. 1). We suggest that freezing is a pressure mangrove species in this area face before reproductive age. Our results indicate that the smaller vessel diameters and higher vessel densities found at this site successfully limit freeze-induced embolism even under tension. Yet, these narrow vessels contribute to keeping *Avicennia marina* and *Aegiceras corniculatum* from exploiting more seasonal habitats, as carbon gain is typically limited by high resistance in stems. The extremely long periods of time between flowering and fruiting (>1 year, Duke, 1990) observed in the southernmost populations of *Avicennia marina* suggest that these populations have difficulty acquiring the photosynthate needed for reproduction. With shorter growing seasons at high latitudes, this could explain why these seemingly well-adapted species do not reach colder climates.

Freezing as a Disturbance Cold temperatures alone do not exclude vegetation from saline habitats, and salt marshes dominated by low-growing grasses (e.g., *Spartina,*) reeds (e.g., *Juncus*,) and succulents (e.g., *Salicornia*) are widespread. Although the mangrove to salt marsh transition has not been extensively considered, forest to grassland boundaries occur widely and are well-studied (Schultz *et al.,* 1955, Longman & Jeník, 1992). Successional theory suggests that where nutrient availability permits, trees are the long-term climax vegetation (Clements, 1916). However, grass- or herb-dominated ecosystems persist where disturbances, such as fire, grazing or drought, occur regularly. Freezing has occasionally been considered in this context, although not as widely as fire or grazing (Longman & Jeník, 1992).

Our results suggest that at least two separate mechanisms are responsible for limiting the range of the mangroves species within the climates included in our study. For *Avicennia germinans*, *Rhizophora mangle*, and *Rhizophora stylosa*, rare but severe freezes may act as disturbances which favor the herbaceous salt marsh vegetation. For *Avicennia marina* and *Aegiceras corniculatum*, severe freezes are apparently not lethal, and mechanisms not tested here must be suggested to account for their distribution.

Many different factors can cause the death of a mangrove tree, and may be locally responsible for limiting the expansion of a particular stand. Cold ocean temperatures and drier land (Duke *et al.,* 1998), a lack of continuity in suitable habitat, and cold damage to living membranes (McMillan, 1975, Markley *et al.,* 1982) may all have helped establish the present geographic range of mangrove taxa. Our data add to these previous explanations, suggesting that freezing in a saline environment can severely limit the ability to supply water to leaves. This may be one more vital clue in understanding why cold-weather mangroves have never evolved in any group.

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Table 1. Xylem sap ion concentrations for Na, Cl and K ions.

Species	Na (µM)[1]	Cl (µM)[1]	K (µM)[1]
Aegiceras corniculatum	30.00±19.97	31.80±18.57	5.19±5.43
Avicennia marina	27.26±22.29	24.68±17.02	2.93±5.48

[1] Measurements were made using energy dispersive X-ray microanalysis (EDX) on branch tissue visualized by cryo-scanning electron microscopy. Data for each ion are given as means ± standard error (*n* = 3).

Table 2. Vessel diameters for five mangrove species from the northern and southern limits of distribution.

Species	D (μm)[1]	D_h (μm)[2]	Latitude[3]
Aegiceras corniculatum	17.1±0.68	24.2±1.22	35°42'30"S
Avicennia marina	19.1±0.35	29.0±0.66	35°42'30"S
Avicennia germinans	31.0±1.01	40.9±1.41	29°40'08"N
Rhizophora mangle	30.8±2.52	38.3±2.94	29°4'35"N
Rhizophora stylosa	22.2±1.17	35.2±0.90	27°46'41"S

[1] Mean vessel diameter (D)
[2] Hydraulically weighted mean vessel diameters, calculated as $D_h = \Sigma D^5/\Sigma D^4$
[3] Collection latitude

Stuart et al., Figure 1

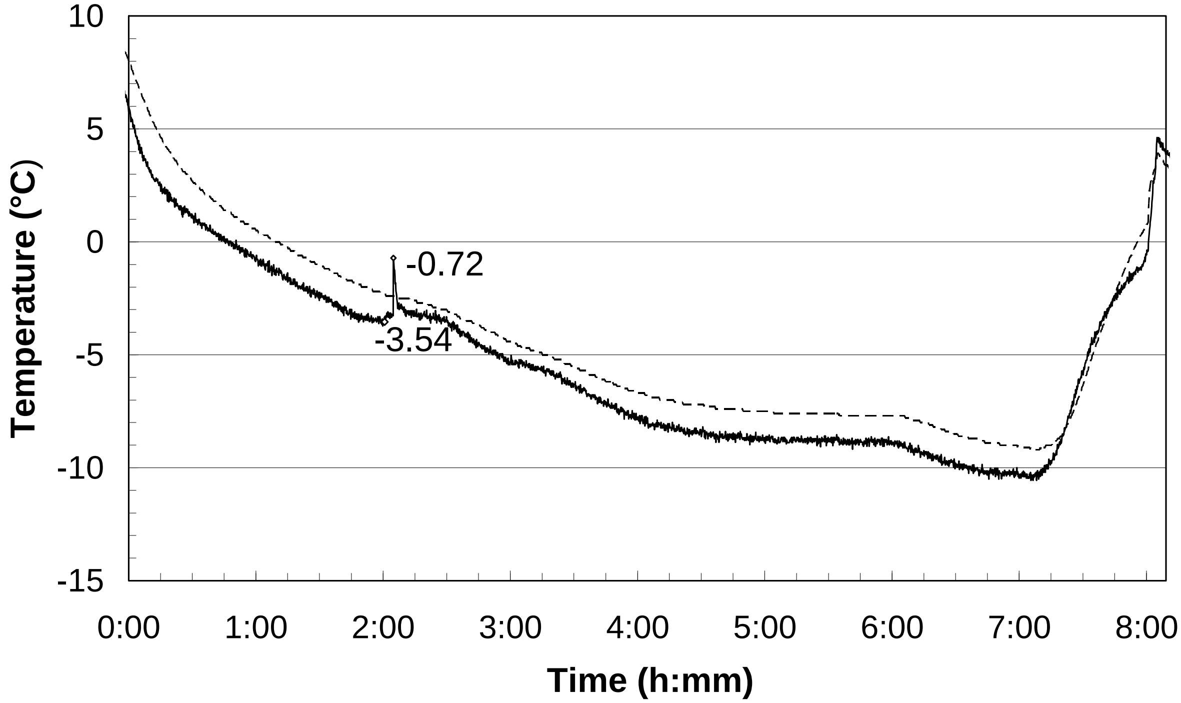

Figure 1 Graph of temperature versus time for one branch frozen on January 7th, 2003. The dark trace shows branch temperature; the dotted line shows the temperature of a reference thermocouple placed distal to the chilling unit. The temperature at which freezing occurred (-3.54°C) and the highest temperature of the freezing exotherm (-0.72°C) are labelled. Temperatures at which freezing occurred varied between -2 and -10°C.

Stuart et al., Figure 2

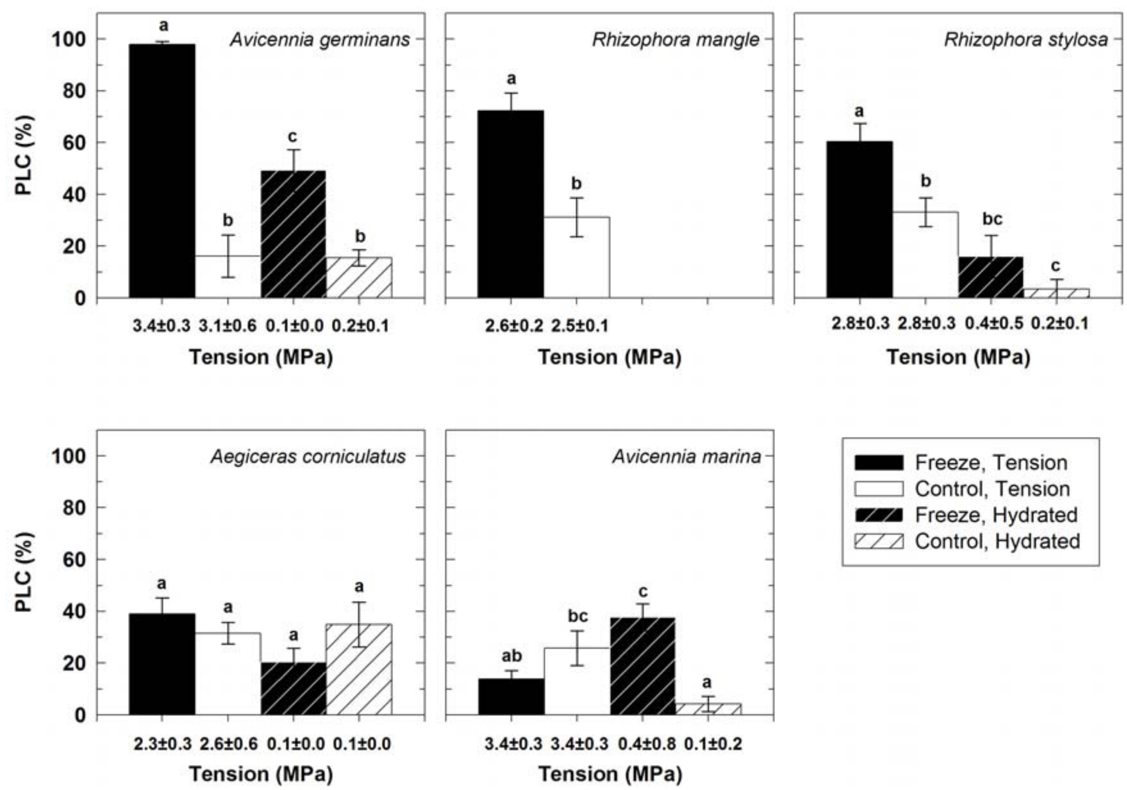

Figure 2 Interactive effects of freezing and tension on percent loss in stem hydraulic conductivity (PLC) in five mangrove species. The four treatments, indicated by pattern, were: branches frozen or held at 4°C at native xylem tension, and branches cut under sap solution and hydrated to relieve xylem tension. Mean water potentials for frozen + control treatments are reported below each group. Error bars represent standard error calculated for each mean, $n = 5$ to 13, with an average size of 9; unevenness of group sizes was due to loss of samples because of high rates of clogging in some species. A separate ANOVA test was performed for each species; letters indicate groups which were significantly different at $p \leq 0.05$ using Tukey HSD. For each tension treatment, ‘frozen’ and ‘control’ samples were paired branches from a single individual, for a total of 10-20 individuals per species.

Figure 3

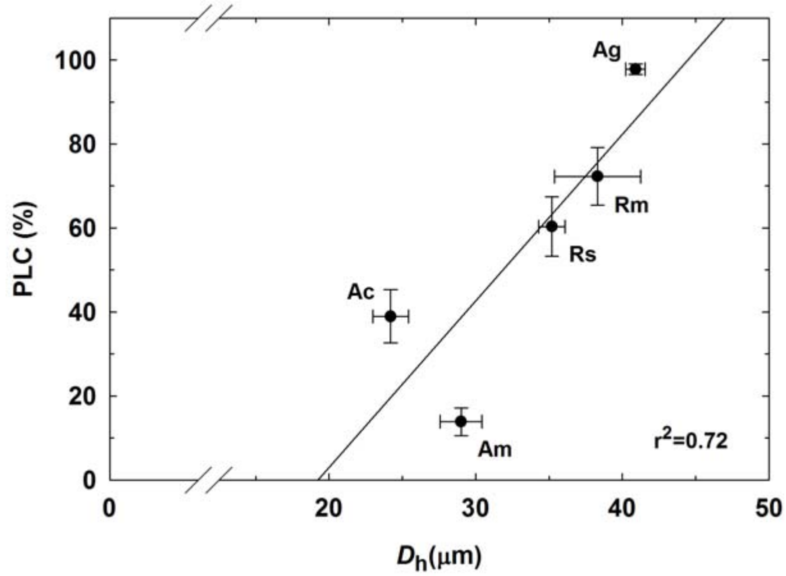

Figure 3 Freeze-induced percent loss in stem hydraulic conductivity (PLC) at native xylem tensions as a function of hydraulically weighted vessel diameter (D_h) in µm. Hydraulically weighted vessel diameters ($D_h = \Sigma D^5/\Sigma D^4$) account for the disproportionate contribution of larger vessels to conductivity. Regression line is y = 3.961x-76.08 with $r^2 = 0.72$. Bars denote standard error of the mean, with n = 5-13 stems for PLC and n = 5 stems for vessel diameters. Two letter abbreviations indicate genus and species: *Aegiceras corniculatum* (Ac), *Avicennia germinans* (Ag), *Avicennia marina* (Am), *Rhizophora mangle* (Rm), and *Rhizophora stylosa* (Rs).