



Occurrence of annual growth rings in *Rhizophora mangle* in a region with low climate seasonality

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Manuscript received on January 5, 2015; accepted for publication on March 18, 2015

ABSTRACT

The formation of annual growth rings has been confirmed for several mangrove species in the last decade, among which is the *Rhizophora mangle*. However, the record of annual rings for this species was made in a region with high hydric seasonality, a widely recognized induction factor of annual rings in tropical species. In this sense, the present study aimed to verify the occurrence of annual growth rings in *R. mangle* in the mangroves of Guaratiba (Rio de Janeiro, Southeastern Brazil), a region with low hydric seasonality. For this purpose, the crossdating technique was applied in ten trees collected with known age (seven years). The growth rings are characterized by alternating layers of low vessel density (earlywood) and high vessel density (latewood). Multiple regression analysis indicated that growth rings width variation is driven by precipitation, water surplus, water deficit and water storage. Crossdating analysis confirmed the existence of annual growth rings in the *R. mangle* in Guaratiba. This discovery in a region with low hydric seasonality increases the dendrochronological potential of this species and suggests the importance of biological factors (eg. phenological behavior) as complementary inductors for the formation of growth rings in this species.

Key words: growth rings, dendrochronology, mangrove, dendroecology, proxy.

INTRODUCTION

The occurrence of annual growth rings in a woody plant is of great relevance, because it allows the use of the species in dendrochronological studies and their application in studies of climatology (Hughes 2002, Schongart et al. 2004), archeology (Kuniholm 2002, Towner 2002), and ecology (Fritts and Swetnam 1989, Rozendal and Zuidema 2011),

among others. In tropical regions, the formation of annual rings has been associated to environmental factors, such as the occurrence of a well-defined dry season or prolonged periods of inundation (Coster 1927, Worbes 1999, 2002, Dünisch et al. 2002, Schongart et al. 2002), and to biological factors, such as phenology and conservative rhythms (Alvim and Alvim 1978, Callado et al. 2001, Costa et al. 2013).

In the last fifteen years, the occurrence of annual growth rings has been confirmed in some

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mangrove species: *Rhizophora mangle* (Menezes et al. 2003), *R. mucronata* (Verheyden et al. 2004), *R. apiculata* (Yu et al. 2004), *Sonneratia apetala* and *Heritiera fomes* (Chowdhury et al. 2008), *Laguncularia racemosa* (Estrada et al. 2008) and *Lumnitzera racemosa* (Robert et al. 2011). In most of these studies, the formation of annual rings is related to some degree of climate seasonality. However, in low seasonality conditions, the distinctness of the growth rings may be lower, making it more difficult to identify their limits (Estrada et al. 2008), preventing them from being used in dendrochronological studies (Robert et al. 2011).

In the case of *R. mangle*, a species that presents a wide distribution along the biogeographic province of the Atlantic and East Pacific (Soares et al. 2012: from Bermuda Islands – 32°23'N – to Santa Catarina/Brazil – 28°28'S), the occurrence of distinct annual growth rings was demonstrated only for mangroves in the Northern coast of Brazil (Pará), where the water seasonality is quite pronounced (Menezes et al. 2003), which raises questions about the dendrochronological potential of this species in low seasonality regions. Thus, the present study aimed to fill in this gap by testing the

occurrence of annual growth rings in *R. mangle* in a region with low climate (hydric) seasonality.

MATERIALS AND METHODS

STUDY AREA

Sampling was conducted in a mangrove forest located in the Biological Reserve of Guaratiba (23°00'S; 43°34'W), Sepetiba Bay (Rio de Janeiro, Southeastern Brazil) (Fig. 1). According to the Köppen classification, the regional climate can be divided into Aw (tropical hot and wet with a dry season during the winter), which is typical of the lowlands, and Af (tropical hot and wet with no dry season), which is characteristic of the adjacent slopes. According to Estrada et al. (2008), the average annual temperature is 23.5 °C, and the average annual precipitation is 1,067 mm, with a weak tendency to wetter summers and drier winters. The same authors describe a high interannual variability in the precipitation pattern, with summer and winter alternating as the wetter period in the last 30 years.

The Guaratiba mangrove forests are characterized by a gradient of decreasing structural development from the fringe forests to the forests located

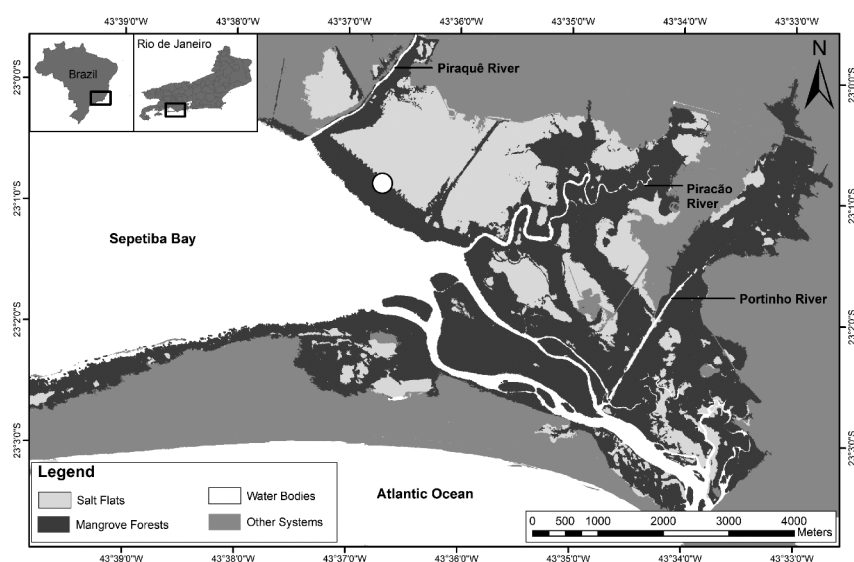


Figure 1 - Map of the study area. The sampling point is indicated with a white circle.

at the transition with adjacent salt flats (Estrada et al. 2013). This variation is determined by a gradual reduction in the tidal flooding frequency in the fringe-salt flat direction. Interstitial water salinity ranges from 35.3 ± 7.3 (in the fringe forests) to 42.2 ± 10.1 in the transition forests. *R. mangle* and *A. schaueriana* alternate as dominant or co-dominant in the fringe, basin and transitional forests, depending on the prevailing environmental conditions and the forest successional stage. The occurrence of *L. racemosa* is significantly lower.

METHODS

In July 2004, ten recently-recruited (five to six months before) saplings were tagged in transition forests (Fig. 1). Those saplings were part of a colonization process towards the salt flat. This colonization process was seen to initiate while the authors were in the field, measuring permanent plots located near the colonization area. In February 2011, the tagged trees were sampled (Table I). Each stem section, taken as close to the base as possible (avoiding stilt roots and deformations in the stem), was dried at room temperature, sanded with sandpaper of successively smaller particle sizes (80 to 600 grits/ cm²) and digitalized on a high-resolution scanner. Four rays (A, B, C and D) were plotted on the polished disks and, with the aid of a stereoscopic microscope, the rings were identified. The width of the rings was then measured in each transect using the Image Pro Plus 4.5 software. Growth rings identification and counting was verified through the crossdating method on the COFECHA software, which is based on the comparison of the chronologies of the sampled trees in order to facilitate the identification of false rings (Worbes 1995). With the aid of the Arstan software, the chronologies of each ray were obtained by indexation of the ring widths, which removes endogenous growth trends (Cook and Holmes 1986).

TABLE I
Diameters (measured at the base) of each sampled tree.

Sample identification	Diameter (cm)
Rh08	2.4
Rh09	3.5
Rh10	4.0
Rh11	2.4
Rh14	3.0
Rh15	2.3
Rh17	2.3
Rh18	1.9
Rh19	3.0
Rh20	3.0

To assess climate variability in the Guaratiba region, average monthly temperature and monthly cumulative rainfall from 1985 to 2010 were obtained from the meteorological station of Marambaia, operated by INMET ('National Institute of Meteorology'). Based on this data, the sequential (monthly) water balance was also calculated, following the method described by Thornthwaite and Mather (1955). To verify the influence of climate variables on the widths of the growth rings, simple and multiple regression analyses ($p < 0.05$) were tested between the master chronology (average between each of the rays from all the sampled trees) and the climate parameters: precipitation, mean temperature, water storage in the soil, water deficit and water surplus. Climate parameters were calculated for the period between October and September of the following year (12 months). This procedure was adopted based on both the results and the literature, which indicate that the late wood is formed in the winter (Verheyden et al. 2004).

RESULTS

The macroscopic examination revealed distinct growth rings (Fig. 2a), but they were scarcely distinct or indistinct microscopically (Fig. 2b). The growth rings are composed by a sequence of a darker and thicker layer, characterized by low density

vessels, followed by a clear and narrower layer, where the vessel density is higher (Fig. 2). The first layer formed from the pith is dark and probably began to be formed in the summer of 2003/2004, since the marked plants were recruited about six months before the tagging, as mentioned above. The last layer observed in all trees at the sampling time (February / 2011) is rather narrow, but it is also dark, characterizing an incomplete growth ring. From the pith to the bark, seven complete growth rings were found in all samples, corresponding to the period of seven years between 2004 and 2011. Crossdating analysis (Fig. 3) showed a significant ($p < 0.01$) intercorrelation coefficient of 0.94 among all sampled trees, confirming that the growth rings are formed with annual periodicity.

The climate seasonality in Guaratiba is not well-pronounced, as indicated by the analysis of the monthly water balance of the last 25 years (Fig. 4). Although there is a trend of increased water storage and surplus in the summer months and lower storages and larger deficits in the winter

months, in several years this pattern was reversed or no pattern was observed. The graphical analysis indicates some level of coincidence between the master chronology and the climate parameters (Fig. 5). None of the simple correlation tests as shown to be significant. However, multiple correlation tests indicated significant results, with high coefficients of determination (R^2), for the following combinations of variables (Table II): “precipitation + water deficit” ($R^2 = 0.86$), “water surplus + water deficit” ($R^2 = 0.96$), and “water surplus + water storage” ($R^2 = 0.79$). All the other combinations of variables resulted in not-significant regressions.

DISCUSSION

The same pattern of vessel density variation in the growth rings of *R. mangle* in Guaratiba is also observed for *R. mangle* in North Brazil (Menezes et al. 2003), *R. mucronata* in Kenya (Verheyden et al. 2004) and *R. apiculata* in China (Yu et al. 2004). Verheyden et al. (2004), using cambial marking and monitoring techniques, observed that the dark

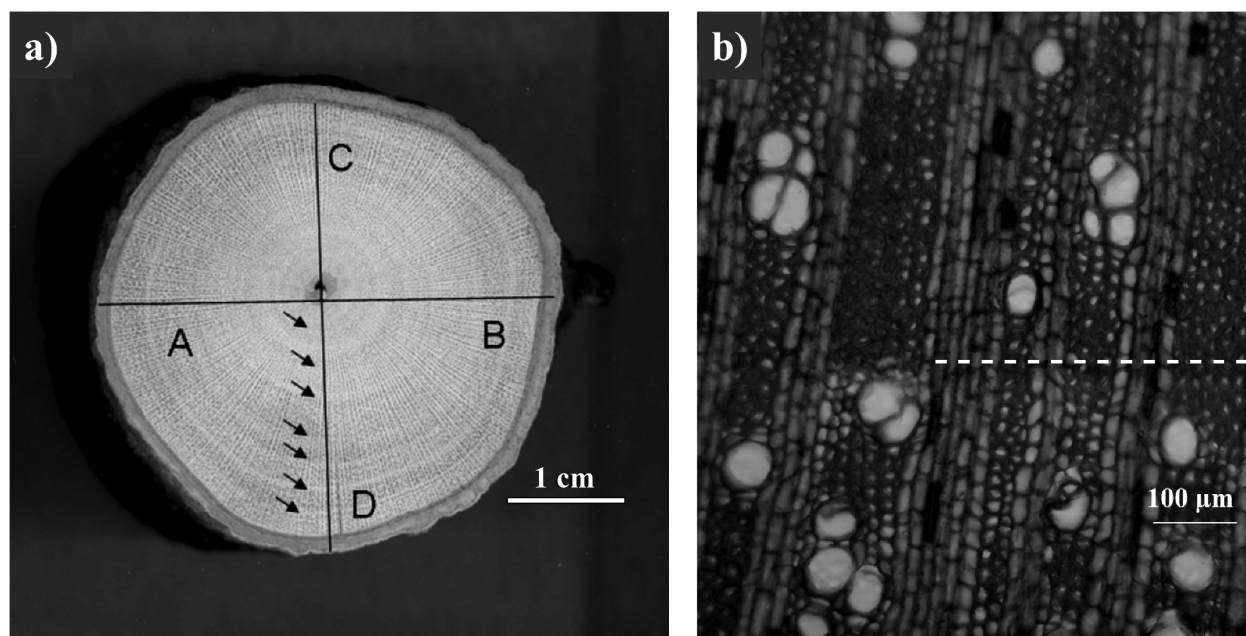


Figure 2 – Macro - (2a) and microscopic (2b) aspects of the growth rings observed in *R. mangle*. In a), the black lines represent the transects (A, B, C, and D) and the arrows indicate the growth rings boundaries. In b), the dashed line indicates the limit between a latewood (below) and an earlywood (above).

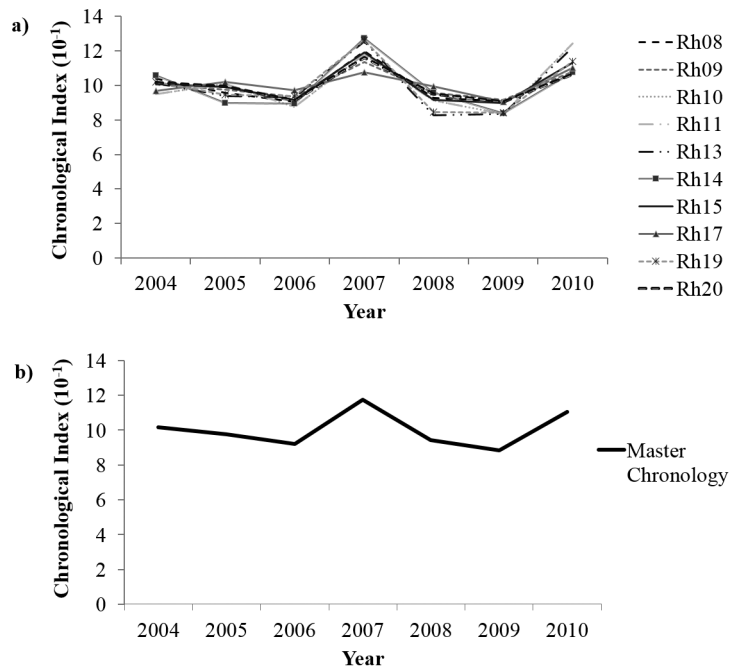


Figure 3 - Indexed chronologies of each tree (a) and the indexed master chronology (b).

TABLE II
Simple and multiple regression equations between growth ring widths and climate variables.

Regression Equation	R ²	t	F	p	SEE
RW = 1.03839 - 0.00003 (PREC)	0.01	-0.19	-	0.86	0.1136
RW = - 0.12028 + 0.04857 (TEMP)	0.06	0.54	-	0.61	0.1109
RW = 0.99065 + 0.00014 (WSUR)	0.17	0.31	-	0.77	0.1130
RW = 0.93126 - 0.00049 (WDEF)	0.42	-1.92	-	0.11	0.0866
RW = 1.09560 - 0.00015 (WSTO)	0.26	-1.31	-	0.25	0.0985
RW = 0.40651 + 0.00037 (PREC) - 0.00111 (WDEF)	0.86	-	12.69	<0.05	0.0471
RW = 0.77725 + 0.00099 (WSUR) - 0.00097 (WDEF)	0.96	-	51.36	<0.05	0.0257
RW = 1.14338 + 0.00110 (WSUR) - 0.00039 (WSTO)	0.79	-	7.62	<0.05	0.0581

RW = Ring width (mm); PREC = Annual precipitation (mm); TEMP = Mean annual temperature (°C); WSUR = Annual water surplus (mm); WDEF = Annual water deficit (negative values) (mm); WSTO = Annual water storage (mm); R² = Coefficient of determination; t = t-test critical value of the simple regression; F = ANOVA critical value of the multiple regression; p = significance of t- or F-test; SEE = Standard error of estimation. In the multiple regression equations all the partial regression coefficients are significant (p < 0.05).

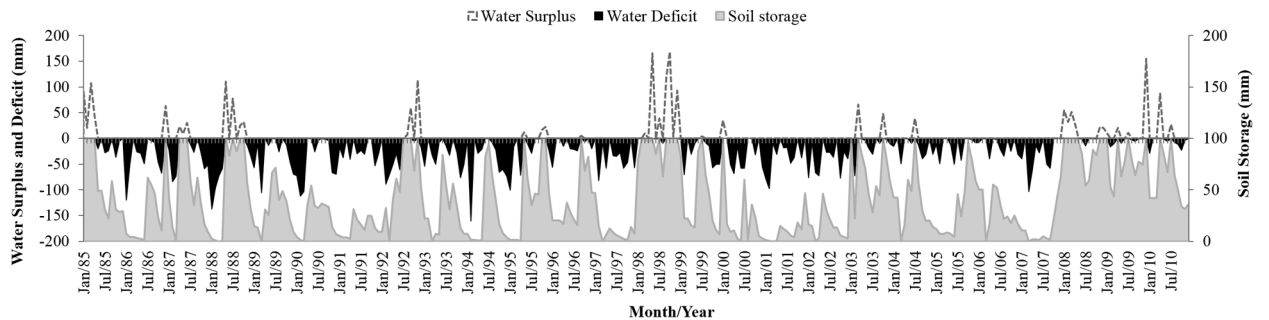


Figure 4 - Monthly water balance from 1985 to 2010 in the Guaratiba region.

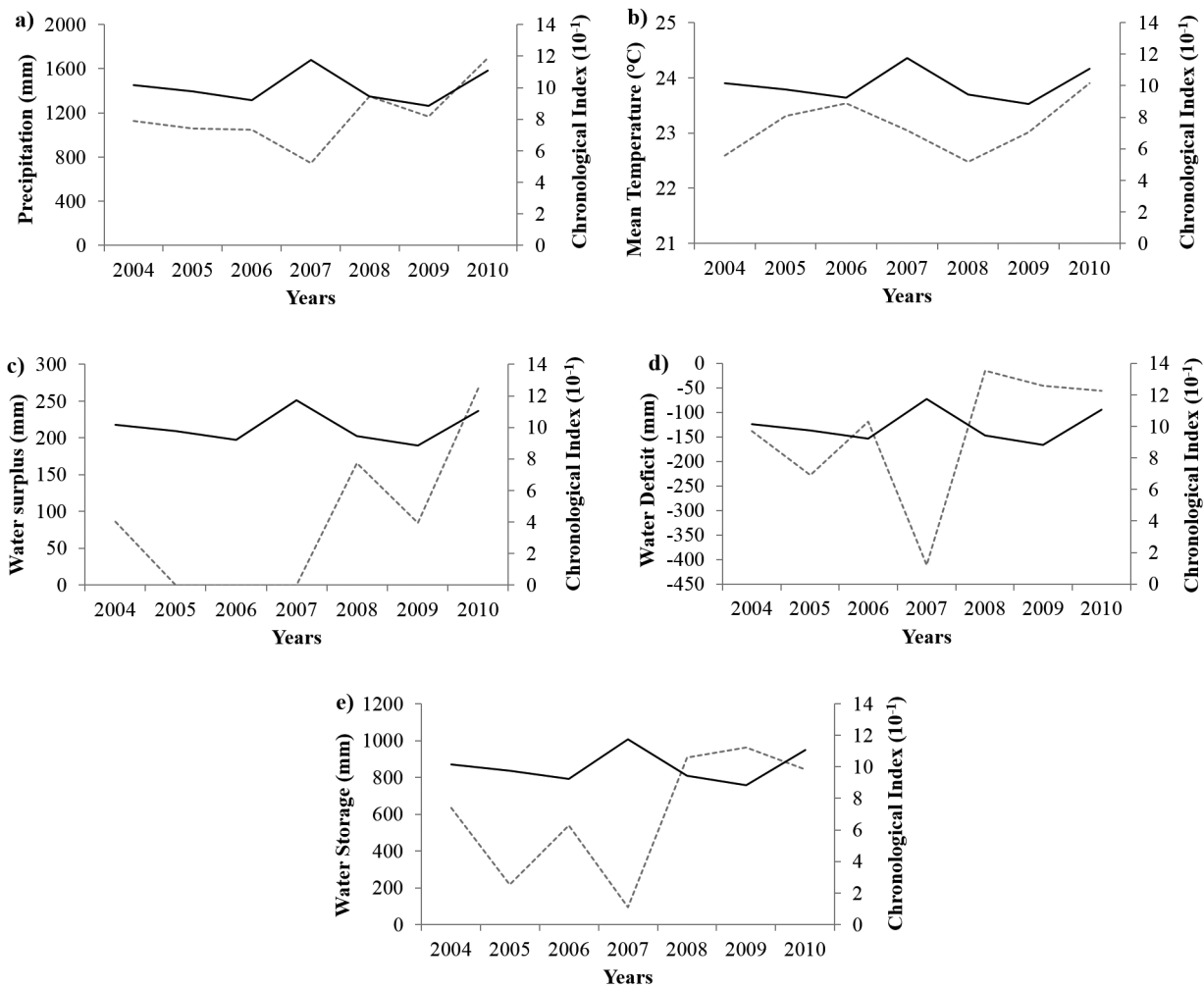


Figure 5 - Line plots showing the indexed master chronology (black lines) against climate parameters (grey dashed lines): (a) precipitation; (b) mean temperature; (c) water surplus; (d) water deficit; and (e) water storage. No significant correlation ($p < 0.05$) between the master chronology and the climate parameters was found.

layers (with lower vessel density) are formed in the rainy season, being classified as earlywood, and that the clear layers (with higher vessel density) are formed in the drier season, when the water and salt stress increase, being classified as latewood. The increase of vessel density as a strategy to resist or tolerate drought in the drier season is a common trend in growth rings of many species, as stated by Carlquist (2001), for example. This author explains that an increase in vessel density leads to a higher safety of water conduction and, thus, to a decreased risk of death by embolism.

Considering that the trees were sampled in February 2011, when a layer of low vessel density was being formed, it can be inferred that the earlywood is formed in part during the summer in Guaratiba. Despite the high interannual variability, summer is the season with the largest number of occurrences of peaks of water storage and /or surplus (Fig. 5), suggesting that although weak, there is a relationship between the early formation and water availability. Following this logic, and what was described by Verheyden et al. (2004), it is possible to suggest that the latewood in Guaratiba is

formed over the winter months, a season with lower historical incidence of water storage and surplus peaks in the region. Although the relationship with climate seasonality is weak, this hypothesis of latewood formation in the winter is consistent with what was observed in the samples: seven complete rings, plus one incomplete earlywood, formed in the period between the summer of 2004 and the summer of 2011, during which seven winters occurred.

The formation of annual growth rings in conditions of low climate seasonality suggests the interaction between climatic variables (eg. rainfall, water balance, photoperiod) and the phenological behavior of the species in the induction of latewood, as demonstrated earlier in Southeastern Brazil (Callado et al. 2001, 2004, Costa et al. 2013) for some tropical species. It is known that *R. mangle* has a marked seasonal cycle of both vegetative and reproductive phenology (Fernandes 1999, Mehlig 2006). However, as there is no available data on the phenology of *R. mangle* for the Guaratiba region the possible effect of phenology on the induction of latewood cannot be assessed in the present study.

The trees, regardless of the absolute growth rate, showed a very similar pattern of growth rings width variation (Fig. 3). This similarity indicates the presence of the same external factor influencing the growth of trees, a basic principle of crossdating (Worbes 1995). As indicated by the significant multiple regression analysis (Table II), climate variables related to water availability may be considered external factors driving growth rings width variation. However, although these regressions are significant, the coefficients of partial regression present unexpected signs for water deficit and water storage. Therefore, the equations indicate that ring width increases as precipitation and water surplus increase. Considering that salinity in the soil of mangroves decreases with increased rainfall (Semeniuk 1983, Cohen et al. 1999), the observed dependence of ring width on precipitation

and water surplus is sustained and is comparable with other studies (Stahle 1999, Worbes 1999, Schongart et al. 2006). In contrast, the equations also indicate that ring width increases as water deficit increases (water deficit data are represented with a negative sign) and as water storage decreases. A similar trend has previously been observed for juvenile tropical trees by Soliz-Gamboa et al. (2011), who associated it to a positive correlation between rainfall and cloudiness (Clark and Clark 1994, Lovejoy and Schertzer 2006). Considering that juvenile trees growing under a dense canopy (which is the case of the studied trees) are subjected to light limitation, a possible explanation for this unexpected trend is that when precipitation is lower (and thus water deficit is higher or water storage is lower) cloudiness is probably lower, decreasing light limitation. It should not be discarded, however, the possibility that this unexpected trend, as well as the non-significance of the simple regressions, are a result of the low sample size. The low age (seven years) of the trees means few rings and thus a small sample size ($n = 7$) for the regression test. As this analysis is highly sensitive to the sample size (Zar 1996), it is possible that these results do not capture the true relationship between ring widths and climate variables. These trends should be re-assessed with longer chronologies in the future.

CONCLUSION

It was shown that *R. mangle* forms annual growth rings not only in regions with high climate seasonality, as demonstrated earlier by other authors, but also in low climate seasonality conditions, as observed in the Guaratiba region (Southeastern Brazil). This discovery increases the dendrocronological potential of this species and suggests the importance of biological factors (eg. phenological behavior) as complementary inductors for the formation of growth rings in this species.

ACKNOWLEDGMENTS

The authors are thankful to Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support.

RESUMO

A formação de anéis de crescimento anuais tem sido confirmada para diversas espécies de mangue na última década, dentre as quais *Rhizophora mangle*. Contudo, o registro de anéis anuais para esta espécie foi feito em região com alta sazonalidade hídrica, um fator de indução de anéis anuais em espécies tropicais amplamente reconhecido. Nesse sentido, o presente estudo teve como objetivo verificar a ocorrência de anéis de crescimento anuais em *R. mangle* nos manguezais de Guaratiba (Rio de Janeiro, sudeste do Brasil), região com baixa sazonalidade hídrica. Para isso, a técnica de datação cruzada foi aplicada em dez árvores coletadas com idade conhecida (sete anos). Os anéis de crescimento são caracterizados pela alternância entre uma camada com baixa densidade de vasos (lenho inicial) e outra com alta densidade de vasos (lenho tardio). A análise de regressão múltipla indicou que o padrão de variação de largura dos anéis de crescimento é determinado por precipitação, excedente hídrico, déficit hídrico e armazenamento hídrico. A análise de datação cruzada confirmou a existência de anéis de crescimento anuais no lenho de *R. mangle* em Guaratiba. Essa descoberta em região com sazonalidade hídrica pouco pronunciada aumenta o potencial dendrocronológico desta espécie e sugere a influência de outros fatores (ex. comportamento fenológico) como indutores complementares para a formação dos seus anéis de crescimento desta espécie.

Palavras-chave: anéis de crescimento, dendrocronologia, manguezal, dendroecologia, proxy.

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