A new method to assess herbivory levels on *Rhizophora mangle* L. fruits in mangroves with different degrees of forest complexity

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Abstract
Consumption levels on mangrove leaves and propagules are well known worldwide, but information on fruit herbivory is still missing. We assess herein herbivory levels on attached fruits of *Rhizophora mangle* in three mangroves with different degrees of structural complexity in Paranaguá Bay (South Brazil). Consumption levels were estimated through a new quantitative methodology using volumetric differences between intact and damaged fruits. Besides deeper tissue damage, fruits also presented surface lesions, which were visually estimated. Consumption levels on fruits were usually very low and did not relate to local mangrove structural complexity. However, our results still suggest that herbivory processes on fruits must be quantitatively investigated to avoid underestimations of herbivory as an important path for organic matter flux in mangroves. Fruits are locally consumed not only by tree-dwelling crabs and insects, as previously recognized, but also by birds, which may play a more significant role in mangrove herbivory.

Key words: consumption levels; fruit predation; frugivory; water displacement method; Brazil

Resumo: Um novo método para avaliar níveis de herbivoria sobre frutos de *Rhizophora mangle* L. em manguezais de diferentes graus de complexidade estrutural. Níveis de consumo sobre folhas e propágulos de manguezal são mundialmente conhecidos, mas informações de herbivoria sobre frutos são ausentes. Este trabalho analisa níveis de herbivoria sobre frutos ainda presos às árvores de *Rhizophora mangle* de três manguezais com diferentes graus de complexidade estrutural na Baía de Paranaguá (Sul do Brasil). Os níveis de consumo foram estimados por uma nova metodologia quantitativa utilizando diferenças volumétricas entre frutos intactos e predados. Além de lesões profundas, os frutos também apresentaram lesões superficiais, que foram estimadas visualmente. Os níveis de consumo sobre frutos foram geralmente muito baixos e não relacionados à complexidade estrutural dos manguezais locais. No entanto, os resultados sugerem que processos de herbivoria sobre frutos devem ser quantitativamente investigados para evitar subestimativas de herbivoria como uma importante via de fluxo de matéria orgânica em manguezais. Os frutos não são consumidos localmente apenas por caranguejos arborícolas e insetos, como reconhecido previamente, mas também por aves, que desempenham um papel mais significativo na herbivoria em manguezais.

Palavras chave: níveis de consumo; predação sobre frutos; frugivoria; método de deslocamento de água; Brasil

Introduction

Herbivory not only affects carbon microbial transformations in mangroves, but also the amount of organic carbon available for exportation (Kristensen *et al.* 2008). Leaf herbivory levels in both global and regional scales are well studied, but exact values may vary according to the species and sites considered (Cannicci *et al.* 2008). However, it is likely that total herbivory levels in mangroves are even

higher, since many studies have revealed a consistent consumption on twigs, large branches, stems, roots, flowers, fruits, and propagules (Farnsworth & Ellison 1991, Robertson 1991, Feller & Mathis 1997, Feller 2002). Despite an extensive literature regarding herbivory in mangroves, sound quantitative information on fruit predation is completely missing.

Fruit and propagule predation play an important role in determining seedling distribution patterns in mangrove stands. Crabs and insects are the primary propagule consumers, though their preferences vary with respect to different species, ages, and sizes of trees (Michinton & Dalby-Ball 2001, Sousa *et al.* 2003). Values from a worldwide survey of pre-dispersal predation rates among 42 sites and 10 mangrove species ranged from 0 to 93 % within stands, with a global total predation rate of 23.3 % and a mean level of 28.3 % across sites (Farnsworth & Ellison 1997). Pre-dispersal propagule predation must be accounted for in estimates of reproductive output, stand health and availability for restoration efforts, while post-dispersal frugivory is viewed as an important factor in structuring many mangrove communities (Farnsworth & Ellison 1997, Steele *et al.* 1999). Injury in fruit and propagules may affect their viability before and after fall and dispersion, therefore limiting seedlings´ survival and affecting the establishment, recolonization and zonation patterns in mangroves (Robertson 1991, Michinton & Dalby-Ball 2001, Krauss & Allen 2003).

Higher levels of herbivory are associated with enhanced nutritional quality of plants growing in benign rather than nutrient-stressed environments. Onuf *et al.* (1977) found that nutrient enrichment in *R. mangle* was responsible for increased primary productivity, higher percent nitrogen of leaves (= higher nutritive value for consumers) and increased losses to herbivores. Nutrient ratios should determine plant nutritive quality and palatability as an adaptive response to herbivory that evolved under constraints of availability of resources in the environment. It has been demonstrated that both herbivory rates and growth responses in red mangrove trees increase with artificial fertilization (Feller 1995), suggesting a preference of these herbivores for well developed trees with higher availability of nutrients.

We use herein a new method to assess consumption levels on attached fruits of *Rhizophora mangle* in mangrove stands with known different degrees of forest complexity in a large subtropical estuarine system (Paranaguá

Bay, Brazil). A previous study in this subtropical estuary (Faraco & Lana 2004) found that a dwarf mangrove with lower amounts of nitrogen and phosphorus in soils and leaves experienced less leaf-herbivory than structurally well developed mangroves. We hypothesized that fruit consumption will also be lower in this dwarf mangrove compared to others of higher forest complexity in the same region. We presumed that an increase in forest structural complexity might be responsible for greater losses to herbivory.

Materials and methods
Study area
Our study was carried out in Paranaguá Bay, southern Brazil (25° 30' S, 48° 25' W). Climate is warm-humid subtropical with rainy summers and cold dry winters. Mean annual rainfall is about 2 500 mm (maximum of 5 300 mm), and mean precipitation during the rainy season is three times higher than during the dry season (Lana *et al.* 2001).

Local mangroves are composed of *Rhizophora mangle*, *Avicennia schaueriana* Stapf & Leechman ex Moldenke, and *Laguncularia racemosa* Gaerth, generally without well defined zonation patterns (Faraco & Lana 2004). *R. mangle* flowers locally between April and June, and the fructification begins in May (personal observations). The tree-dwelling crab *Aratus pisonii* is recognized as the main herbivore at canopy levels (Brogim & Lana 1997). We sampled three marginal riverine stands with known distinct levels of structural development, previously classified by Couto (1996), Sessegolo (1997) and Silva (2007), according to varying criteria such as species richness, density, tree height and diameter. All the studied mangroves were located in the high energy, euhaline (average salinity ~ 25-30 ppt) outer sector of the bay (Fig. 1).

Site 1 is located in Maciel tidal creek (25° 33' 41" S and 48° 25' 07" W), which drains 8 km² of a mangrove area. It holds the most developed forest with the highest complexity structure. The three mangrove species are present, with 6 to 9 m high *R. mangle* and *A. schaueriana* being predominant. *L. racemosa* also occurs, but is not as common nor as tall as the other two species (Faraco & Lana 2004). The samplings were done in *R. mangle* fringes adjacent to the waterline.

Site 2 is located in Perequê tidal creek (25° 34' S and 48° 21' 30" W), composed of the three mangrove species, but largely dominated by *L. racemosa*. The forest is formed by a mosaic of contrasting patches with distinct structural
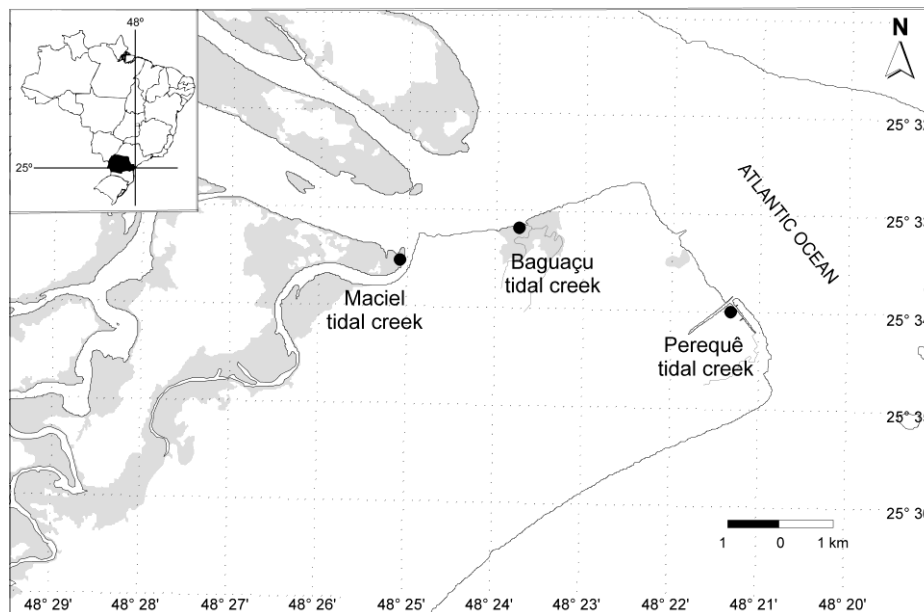
Figure 1. Location of study sites in Paranaguá Bay, south coast of Brazil, indicated by black dots. Grey areas correspond to regional mangrove cover.

complexity (Silva 2007). We sampled at the riverine fringe with 2.6 km², occupied only by *R. mangle*, restricted to a few layers of trees along the border of the creek.

Site 3 is located in Baguaçu tidal creek (25° 33' 15" S and 48° 23' 25" W), composed mainly by *L. racemosa*, with some basins occupied by *A. schaueriana* and *R. mangle* trees restricted to fringes in the margins of the creek and the Bay (Faraco & Lana 2004). We surveyed a 1.0 km² area covered by a dwarfed stand of trees (< 2 m height), considered as the less structurally developed site and functionally classified by Lugo & Snedaker (1974) as scrub mangrove, typical of nutrient-poor soils.

Sampling methods

Firstly, *R. mangle* fruits still attached to the trees were randomly obtained in a pilot survey to establish the relationships between volume *versus* length and volume *versus* diameter (n = 88). Then, subsequent surveys were carried out between August and October 2004 at each of three mangrove sites (Total n = 1530) (fig. 2) to test for differences among mangroves. In *Rhizophora*, the fruit is the unit that remain together with the cotyledons on the tree after the seedling or propagule is abscised from the cotyledons (Tomlinson, 1986). At each site, a 50 m transect was established parallel to the creek. Fruit were collected from the tree that stood closer to each of six randomly selected points along the transect. Five branches holding a variable number of fruits were randomly sampled

from each entire tree, with a pruning hook and scissors.

Fruits were processed immediately after sampling at the lab to avoid water loss. Mean diameter, length, and volume were measured for all damaged and non-damaged fruits. Diameters and lengths were measured with a calliper and a rule, and the volumes by a simple water displacement technique, based on the principle that the volume of a solid tissue submerged in a graduated cylinder is equal to the volume of the displaced water. We chose a cylinder that was large enough to fit the fruit being measured into, and filled it enough so that the fruit was fully submerged. The meniscus was read to determine the initial water volume, and was recorded again after placing a fruit in the cylinder. The final volume was calculated by subtracting the volume of the water and fruit from the volume of the water alone. A few drops of domestic detergent were added to lower surface tension and to facilitate the adherence of liquid to the injuries.

Data analysis

The relationships between volume *versus* length and volume *versus* diameter were assessed through a linear regression model using intact fruits obtained in the pilot survey (n = 88). The best coefficient of determination ($r^2 = 0.917$) was found for volume *versus* length, and this equation was used to estimate the predicted volumes of damaged fruits in the subsequent samplings. To predict the volume of injured fruits before damage, the observed measures of injured fruits

were applied to the volume *versus* length regression model, generating the expected volume of the fruits as if they were intact. The difference between the predicted and the observed volume of damaged fruits is, in turn, an estimate of the volume ingested by herbivores, which was converted to a percentage. When damage altered the fruit length, the data were not used to run regressions.

Volume losses were calculated only for fruits with larger injuries, hereafter called "deep lesions". "Superficial lesions", too small to be measured by the water displacement method,

were visually estimated as the percentage of injured surface area in relation to the total fruit surface. A one way ANOVA was adopted to estimate univariate differences among sites. Homogeneity of variance was examined using Levene's test ($p < 0.05$). No transformation was necessary to stabilize the variances or normalized the data.

Results

Based on linear regression analysis, the volume varied consistently with the length and diameter of fruits (Fig. 2).
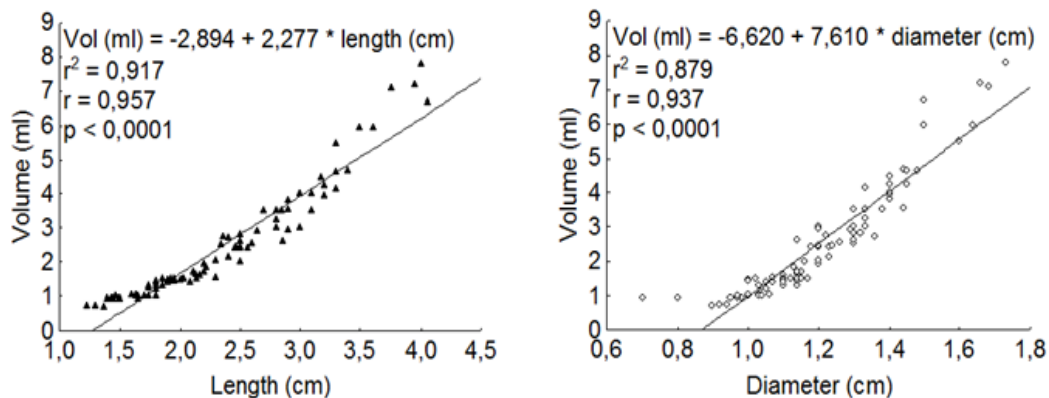
Figure 2. Volume (ml) *vs*. length (cm) and Volume (ml) *vs*. diameter of *R. mangle* fruit (n = 88)

The total frequency of fruit deep lesions was extremely low, corresponding to only 3.6 % of the 1 530 observed fruits. Superficial lesions were more frequent, reaching 30 % of the fruits. Overall consumption levels on fruits with deep lesions, expressed as volumetric percent losses per fruit, ranged from 0 % to 0.15 % at Maciel;

from 0.2 % to 0.7 % at Perequê; and from 0 % to 1.4 % at Baguaçu. For superficial lesions, mean consumption levels were represented as the proportion of fruit area lost to herbivores, which varied from 0.3 % to 2.8 % of total fruit surface at Maciel; from 0.6 % to 3.2 % at Baguaçu; and from 1.1 % to 3.3 % at Perequê (Table I).

Table I. Percent of consumption per fruit for deep and superficial lesions found at Maciel, Perequê and Baguaçu tidal creeks (*n* = number of fruits sampled in each mangrove).

Sites	Type of Consumption	Mean consumption per fruit (%)	Minimum consumption per fruit (%)	Maximum consumption per fruit (%)
Maciel	Deep lesions	0.08	0.0	0.15
(*n* = 629)	Superficial lesions	1.49	0.3	2.8
Perequê	Deep lesions	0.33	0.2	0.7
(*n* = 562)	Superficial lesions	2.28	1.1	3.3
Baguaçu	Deep lesions	0.53	0.0	1.4
(*n* = 339)	Superficial lesions	2.02	0.6	3.2

Consumption levels did not vary greatly with mangroves, and ANOVA indicated no significant differences in both deep and superficial herbivory amongst sites (Table II, Fig. 3, Fig. 4), regardless of differences in structural complexity.

Conversely, fruits growing on Maciel tidal creek, the most developed and structurally complex site, tended to exhibit the lowest consumption levels both for superficial and deep herbivory (Fig. 3, Fig. 4).

Table II. ANOVA results ($p < 0.05$) of consumption levels on *R. mangle* fruits with deep and superficial lesions for significant differences among the three sites (Maciel, Perequê and Baguaçu). The percentage of all fruits in the sample that were damaged by herbivores and the number of fruits sampled (= to the number of fruit used in the analysis) are both given in parenthesis.

	DF	MS	F	p
Deep lesions (3.6, n = 1530)	2	0.199	1.333	0.310 (n.s.)
Superficial lesions (30.1, n = 1530)	2	97.29	0.734	0.586 (n.s.)

Consumption levels on fruits with surface injuries tended again to be higher and very similar between Perequê and Baguaçu tidal creeks, and lower at Maciel (Fig. 4), though not statistically significant.

Discussion

Conventional methods to assess consumption levels on propagules are limited to visual recognition and categorization of different damage types (Allen *et al.* 2003, Krauss & Allen 2003, Michinton & Dalby-Ball 2001, Sousa *et al.* 2003). As such, they are not suitable for reliably assessing fruit consumption, and for properly evaluating mangrove carbon flow. Our novel method allowed for a reliable quantification of fruit herbivory, as evidenced by the very significant regressions. The displacement technique was also a non destructive, cheap, accessible, and quick volume estimator. Curran *et al.* (1996) compared a number of volumetric methods (pycnometry, object upthrust after immersion, and water volume displacement) and showed no systematic under- or overestimation of the actual volume by any of them, thus settling on the displacement method because of its greater simplicity. However, consumption levels can be underestimated as negative values and may be recorded in the case of fruits with very small herbivore marks. Our technique is adequate for partially consumed fruits, but obviously disregards completely consumed ones, or internal tissue loss by insect miners.

The patterns of consumption levels on fruits suggest that variations in mangrove structural complexity may not be a sufficient explanation of variation in herbivory, which is probably related to the low frequency of occurrence of consumed fruits.

Most deep lesions on fruits presented sharp edges with a "V" shape, and field observations of birds feeding on a *R. mangle* fruit suggest that frugivorous birds may be the main agents of such damage. Local birds as the sayaca tanager (*Thraupis sayaca*) and the brazilian tanager (*Ramphocelus bresilius*) have strong beaks, easy access to the canopy, and enough strength to overcome the mechanic barrier of the extremely hard fruits. Mangrove plants are known to produce an excess of tannins and other phenols, depending on the tissue or senescence level of the plant, as defence strategies against herbivore attack (Mooney & Gulmon 1981, Robertson 1988). However, many birds are tolerant to low palatability substances as tannins (Bairlein 1996), and even higher concentrations of tannin in *R. mangle* hypocotyls (Lacerda *et al.* 1998) would not keep them away from the fruits. Urban growth along the Perequê tidal creek may concentrate more opportunistic birds in the adjacent mangrove fragments (Mestre *et al.* 2007), thereby increasing local consumption levels on fruits.
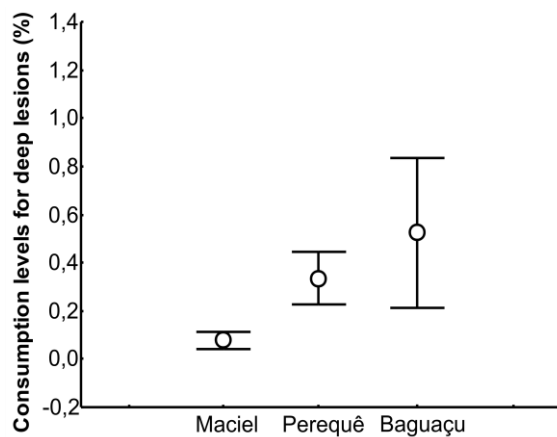
Figure 3. Mean consumption levels (± standard error) on fruits with deep tissue damage found at Maciel, Perequê and Baguaçu tidal creeks.
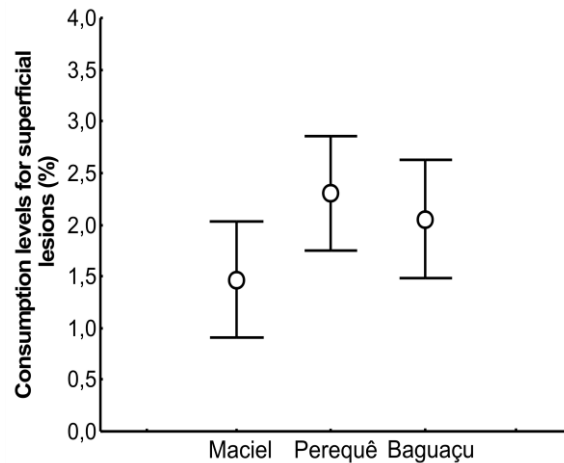
Figure 4. Mean consumption levels (± standard error) of fruits with superficial tissue damage found at Maciel, Perequê and Baguaçu tidal creeks.

The method developed to assess frugivory levels did not allow us to reliably distinguish among the different species/guilds of invertebrates responsible for the fruit damage. However, observations made during our field work and by other studies show that other local herbivores with access to tree canopy may be potential fruit consumers, such as the crabs *Aratus pisonii*, *Sesarma rectum, Goniopsis cruentata*, *Chasmagnathus granulata*, and insects as grasshoppers and ants (Brogim & Lana 1997, Faraco & Lana 2004). Despite the key role played by crabs in the maintenance of mangroves structure and function, other invertebrates can not be underestimated (Cannicci *et al.* 2008). Tree-living predator ants may also indirectly influence consumption rates of fruits still attached to the mother plant, since they protect the plants from herbivorous insects (Offenberg *et al.* 2004). The active removal of such insects by the ants decreases leaf damage, and could also discourage crab and other invertebrates attacks to the fruits growing in the trees.

We investigated mangrove herbivore levels on *R. mangle* fruits using a successful quantitative approach, first developed and applied in this study. The lack of relationships between herbivory and different structural complexity shown by our results suggests that other factors are influencing consumption levels in Paranaguá Bay mangroves. Nevertheless, our study strongly suggests that herbivory processes upon fruits must be quantitatively investigated to avoid underestimations of herbivory as an important path for organic matter flux in mangroves. Consumption on *R. mangle* fruits is not only practiced by invertebrates, as previously recognized, but also by frugivorous birds.

References
Allen, J. A., Krauss, K. W. & Hauff, R. D. 2003. Factors limiting the intertidal distribution of the mangrove species *Xylocarpus granatum*. **Oecologia**, 135: 110-121.

Bairlein, F. 1996. Fruit-eating in birds and its nutritional consequences. **Comparative Biochemistry and Physiology**, 113: 215-224.

Brogim, R. A. & Lana, P. C. 1997. Espectro alimentar de *Aratus pisonii*, *Chasmagnathus granulata* e *Sesarma rectum* (Decapoda, Grapsidae) em um manguezal da Baía de Paranaguá, Paraná. **Iheringia Série Zoológica**, 83: 35-43.

Cannicci. S., Burrows. D., Fratini, S., Smith, III T. J., Offenberg, J. & Dahdouh-Guebas, F. 2008. Faunal impacts on vegetation structure and ecosystem function in mangrove forests: A review. **Aquatic Botany**, 89: 186-200.

Couto, E. C. G. 1996. Caracterização estrutural do manguezal da Foz da Gamboa do

Maciel (Paranagua - PR). **Arquivos de Biologia e Tecnologia**, 39: 497-507.

Curran, M., James, P. & Allaway, W. G. 1996. The measurement of gas spaces in the roots of aquatic plants: Archimedes revisited. **Aquatic Botany,** 54: 255-261.

Faraco, L. F. D. & Lana, P. C. 2004. Leaf-consumption levels in subtropical mangroves of Paranaguá Bay (SE Brazil). **Wetlands Ecology and Managament**, 12: 115-122.

Farnsworth, E. J. & Ellison, A. M. 1991. Patterns of herbivory in Belizean mangrove swamps. **Biotropica**, 23: 555-567.

Farnsworth, E. J. & Ellison, A. M. 1997. Global patterns of pre-dispersal propagule predation in mangrove forests. **Biotropica**, 29: 318-330.

Feller, I. C. 1995. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). **Ecological Monographs,** 65: 477-505.

Feller, I. C. & Mathis, W. N. 1997. Primary herbivory by wood-boring insects along an architectural gradient of *Rhizophora mangle*. **Biotropica**, 29: 440-451.

Feller, I. C. 2002. The role of herbivory by wood-boring insects in mangrove ecosystems in Belize. **Oikos**, 97: 167-176.

Krauss, K. W. & Allen, J. A. 2003. Factors influencing the regeneration of the mangrove *Bruguiera gymnorriza* (L.) Lamk. on a tropical Pacific island. **Forest Ecology and Management,** 176: 49-60.

Kristensen, E., Bouillon, S., Dittmar, T. & Marchand, C. 2008. Organic carbon dynamics in mangrove ecosystems: a review. **Aquatic Botany,** 89: 201-219.

Lacerda, L. D., Jose, D. M. V. & Francisco, M. C. F. 1998. Nutritional status and chemical composition of mangrove seedlings during development. **Brazilian Journal of Biology**, 48: 401-405.

Lana, P. C., Marone, R. M. L. & Machado, E. C. 2001. The subtropical estuarine complex of Paranaguá Bay, Brazil. *In*: Seeliger, U., Kjerfve, B. (Eds.). **Coastal Marine Ecosystems of Latin America**. Springer-Verlag, Berlin, 360 p.

Lugo, A. E. & Snedaker, S. C. 1974. The ecology of mangroves. **Annual Review of Ecology and Systematics**, 5: 39-64.

Mestre, L. A., Krul, R. & Moraes, V. S. 2007. Mangrove bird community of Paranaguá Bay – Paraná, Brazil. **Brazilian Archives of Biology and Technology**, 50: 75-83.

Minchinton, T. E. & Dalby-Ball, M. 2001. Frugivory by insects on mangrove propagules: effects on the early life history of *Avicennia marina*. **Oecologia,** 129: 243-252.

Mooney, H. A. & Gulmon, S. L. 1981. Constraints on leaf structure and function in reference to herbivory. **BioScience,** 32: 198-206.

Offenberg, J., Havanon, S., Aksornkoae, S., Macintosh, D. J. & Nielsen, M. G. 2004. Observations on the ecology of weaver ants (*Oecophylla smaragdina* Fabricius) in a Thai mangrove ecosystem and their effect on herbivory of *Rhizophora mucronata* Lam. **Biotropica,** 36: 344-351.

Onuf, C. P., Teal, J. M. & Valiela, I. 1997. Interactions of nutrients, plant growth and herbivory in a mangrove ecosystem. **Ecology,** 58: 514-526.

Robertson, A. I. 1988. Decomposition of mangrove leaf litter in tropical Australia. **Journal of Experimental Marine Biology and Ecology,** 116: 235-247.

Robertson, A. I. 1991. Plant-animal interactions and the structure and function of mangrove forest ecosystems. **Australian Journal of Ecology**, 16: 433-443.

Sessegolo, G. C. 1997. Estrutura e produção de serapilheira do manguezal do rio Baguaçu, Baía de Paranagua – PR. **PhD. Thesis**. Universidade Federal do Parana, Parana, 110 p.

Silva, L. G. M. 2007. Estrutura especial e variabilidade temporal do manguezal do Rio Perequê (Pontal do Sul – Parana). **Monograph**. Universidade Federal do Parana, Parana, 60 p.

Sousa, W. P., Quek, S. P. & Mitchell, B. J. 2003. Regeneration of *Rhizophora mangle* in a Caribbean mangrove forest: interacting effects of canopy disturbance and a stem-boring beetle. **Oecologia,** 137: 436-445.

Steele, O. C., Ewel, K. C. & Goldstein, G. 1999. The importance of propagule predation in a forest of non-indigenous mangrove trees. **Wetlands,** 19: 705-708.

Tomlinson, P. B. 1986. Seedlings and seeds. *In*: Tomlinson, P. B. (Ed.). **The botany of mangroves**. Cambridge University Press, Cambridge, 436 p.