

Short Communication

## Effect of Seawater on the Growth, Ion Content and Water Potential of *Rhizophora mucronata* Lam.

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**Growth and physiological responses at seedling (six months old) and sapling (12 months old) stages were studied under various seawater concentrations. The optimum growth of plants was obtained at 50% seawater and declined with further increases in salinity. Leaf water and osmotic potentials and xylem tension increased with an increase in media salinity both in seedlings and saplings, whereas stomatal conductance was decreased. Concentrations of sodium and chloride ions increased, while those of potassium and calcium decreased with an increase in salinity.**

**Key words:** Balochistan — Indus delta — Mangrove — *Rhizophora mucronata* — Seawater — Water potential

*Rhizophora mucronata* Lam. (Rhizophoraceae) is a much-branched large shrub or moderate sized tree, up to 10 meters tall, supported by adventitious prop roots (Ghafoor 1984). It is distributed along the coast of north Australia, Tropical Asia, the Indian and Pakistan subcontinent, and East and South Africa. In Pakistan, a few small populations are located in the Miani Hor estuary, Balochistan and in the Indus delta (Ghafoor 1984). The plant aids in coastal stabilization and provides nursery areas for economically important fishes and crustaceans in the tropics and sub-tropics (Tomlinson 1986). The forest stature of this species is regulated by residual effects of tidal and wave energy, soil salinity, nutrient availability and flooding frequency (Cintron *et al.* 1985, Feller 1995).

Like other halophytes, mangroves frequently face stress, in this case changes in salinity following shifts between flooding by ocean water and fresh water introduced by rain and run-off. Such spatial and temporal changes in salinity could affect the growth and physiology of plants (Naidoo 1985). Mangrove growth usually declines at high salinity, with optimal growth obtained at moderate salinity (Clough 1984). This pattern of growth is primarily a reflection of low external water potentials (Luttge 1997). The Leaf water potential tends to decrease when the plant is subjected to salinity stress (Clough 1984) as a function of tolerance. Other responses associated with salt stress in plants besides retarded growth and lowered water potentials include

changes in sap osmotic pressure, salt exclusion at root level and active salt excretion through leaves (Hutchings and Saengar 1987). Salt tolerant plants also maintain low osmotic potential and stomatal conductance (Clough 1984, Ball and Farquhar 1984, Naidoo 1985) through the accumulation of ions like sodium and chloride in their tissues. Such ions could have serious effects on plant metabolism. However, in salt tolerant plants, ions are usually sequestered in the vacuole and the lower osmotic potential is effectively countered by the synthesis of small organic solutes (Popp *et al.* 1984). These organic solutes serve as osmolytes and are also called compatible solutes.

The present study was designed to elucidate the morphological and physiological responses of *Rhizophora mucronata* when exposed to various concentrations of seawater. We investigated whether (1) *R. mucronata* growth would be maximal at 50% seawater, (2) *R. mucronata* would be more tolerant at the sapling stage and (3) *R. mucronata* would adopt the osmoconformer strategy with increasing salinity.

Mature propagules of *Rhizophora mucronata* were collected from Miani Hor, Balochistan during the summer and propagules ranging from 25 to 30 cm in size were used in this study. The bottom 5-cm of each propagule was embedded in a 36-cm diameter plastic bucket filled with acid-washed beach sand. Plants were grown in a green house under natural conditions and watered for two weeks through sub-irrigation. After two weeks, plants were progressively treated with seawater (0, 25, 50, 75 and 100%) fortified with nutrient solution (Popp and Polania 1989). The salinity level of the culture solution was maintained in the pots through daily sub irrigation with water and the solution replaced every seven days to avoid a build up in salinity. Dry weight, height, stem diameter at first internode and leaf area were measured twice at six-month intervals after the highest salinity was reached. Dry mass was determined after drying the plant samples in a fan-forced oven at 80 C.

Leaves were washed with distilled water prior to the determination of water and osmotic potential. Leaf discs, 5 mm in diameter, were punched from the middle of the laminae and placed in a C-52 sample chamber. After equilibration, water potential was determined with a Wescor HR 33 T Dew Point Micro-voltmeter. Osmotic potential was measured with a Dew Point Micro-voltmeter after freezing the disc in liquid nitrogen. Plant water status was measured

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Table 1. Means  $\pm$  SE ( $n=5$ ) of plant height, dry weight, stem diameter and leaf area after six months culture of *Rhizophora mucronata* seedlings in different seawater dilutions

Growth parameters	Seawater (%)				
	0	25	50	75	100
Plant height (cm)	82.0 <sup>b</sup> $\pm$ 1.2	84.3 <sup>b</sup> $\pm$ 2.2	104.9 <sup>c</sup> $\pm$ 3.1	80.3 <sup>b</sup> $\pm$ 2.8	69.0 <sup>a</sup> $\pm$ 1.9
Dry weight	6.1 <sup>b</sup> $\pm$ 0.8	6.2 <sup>b</sup> $\pm$ 1.2	8.6 <sup>c</sup> $\pm$ 1.2	6.2 <sup>b</sup> $\pm$ 1.1	4.4 <sup>a</sup> $\pm$ 0.8
Stem diameter (cm)	0.6 <sup>a</sup> $\pm$ 0.08	0.7 <sup>a</sup> $\pm$ 0.10	0.8 <sup>b</sup> $\pm$ 0.09	0.6 <sup>a</sup> $\pm$ 0.09	0.6 <sup>b</sup> $\pm$ 0.10
Leaf area (cm plant <sup>-2</sup> )	133.1 <sup>b</sup> $\pm$ 12.1	142.6 <sup>b</sup> $\pm$ 10.8	177.3 <sup>c</sup> $\pm$ 11.2	112.1 <sup>b</sup> $\pm$ 9.3	28.4 <sup>a</sup> $\pm$ 0.7

Mean values in rows for each parameter having different letters (a, b, c) are significantly different at  $P < 0.05$  by the Bonferroni test.

Table 2. Means  $\pm$  SE ( $n=5$ ) of plant height, dry weight, stem diameter and leaf area after 12 months culture of *Rhizophora mucronata* saplings in different seawater dilutions

Growth parameters	Seawater (%)				
	0	25	50	75	100
Plant height (cm)	85.1 <sup>b</sup> $\pm$ 2.1	95.7 <sup>b</sup> $\pm$ 3.1	120.5 <sup>c</sup> $\pm$ 3.5	91.3 <sup>b</sup> $\pm$ 3.2	74.1 <sup>a</sup> $\pm$ 1.9
Dry weight	6.3 <sup>b</sup> $\pm$ 1.1	7.2 <sup>b</sup> $\pm$ 1.3	10.9 <sup>c</sup> $\pm$ 1.1	7.1 <sup>b</sup> $\pm$ 0.9	5.0 <sup>a</sup> $\pm$ 0.8
Stem diameter	2.0 <sup>b</sup> $\pm$ 0.10	2.1 <sup>b</sup> $\pm$ 0.21	2.4 <sup>c</sup> $\pm$ 0.09	2.1 <sup>b</sup> $\pm$ 0.10	1.9 <sup>a</sup> $\pm$ 0.08
Leaf area (cm plant <sup>-2</sup> )	145.4 <sup>b</sup> $\pm$ 9.6	151.4 <sup>b</sup> $\pm$ 6.4	211.0 <sup>c</sup> $\pm$ 5.4	144.2 <sup>b</sup> $\pm$ 12.5	133.0 <sup>a</sup> $\pm$ 10.5

Mean values in rows for each parameter having different letters (a, b, c) are significantly different at  $P < 0.05$  by the Bonferroni test.

by stem xylem tension with a plant water status console (Arimad-2, Wagtech International Limited, UK) in five shoots for each treatment. Stomatal conductance was measured using an AP-4 porometer (Delta-T devices, UK) on the adaxial surface of fully expanded leaves at first node.

Half a gram of plant material was boiled in 10 ml of water for two hours at 100 C using a dry heat bath. This hot water extract was cooled and filtered using Whatman no. 42 filter paper. Chloride ion content was measured with a Beckman specific ion electrode whereas cations of plant organs ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ ) were analyzed using a Perkin Elmer model 360, atomic absorption spectrophotometer. For all growth parameters and water potential experiments, five replicates per salinity treatment were used. The results were analyzed using ANOVA methods and the Bonferroni test and the significance of individual treatments was measured using the Bonferroni test (SPSS 1996).

The growth of *Rhizophora mucronata* peaked at 50% seawater and decreased with increasing salinity. The Bonferroni test showed that dry weight, plant height, leaf area and stem diameter were significantly ( $P < 0.05$ ) increased in 50% seawater and decreased at high salinity i.e. 75 and 100% seawater (Tables 1 and 2). The growth and water potential are greatly affected by media salinity and plants adapt to such variations using different strategies

(Jefferies *et al.* 1979). Optimal growth of South African *R. mucronata* was observed in 25% seawater (Naidoo 1985) and similar responses were reported for other mangroves (Downton 1982, Clough 1984, Hwang and Chen 1995, Smith and Snedaker 1995, Ogrady *et al.* 1996). However, in *A. marina*, optimum growth was observed in 50% seawater (Karim and Karim 1993).

Study of the beneficial role of salts in regulating the growth of halophytes requires a physiological approach (Lee and Ignaciuk 1985, Murata *et al.* 1992). However, the decline in growth at higher salinity may reflect several factors including an inhibitory effect of high ionic content on photosynthetic efficiency, reduced stomatal conductance and diversion of resources to osmotica (Jefferies *et al.* 1979). A similar response in *Rhizophora stylosa* was reported above 25% seawater and it was suggested that a direct effect of salts is water stress at growing points of the plants (Clough 1984).

There was a significant ( $P < 0.001$ ) individual effect of leaf age, age of plants and seawater on tissue water and osmotic potential. Water potential in both young (second node from the apex) and old (lowest of node of the plant) leaves of seedlings and saplings gradually became more negative with the increase in media salinity (Fig. 1a). Osmotic potential in young and old leaves of seedlings and saplings became progressively more negative with the increase in salinity (Fig.

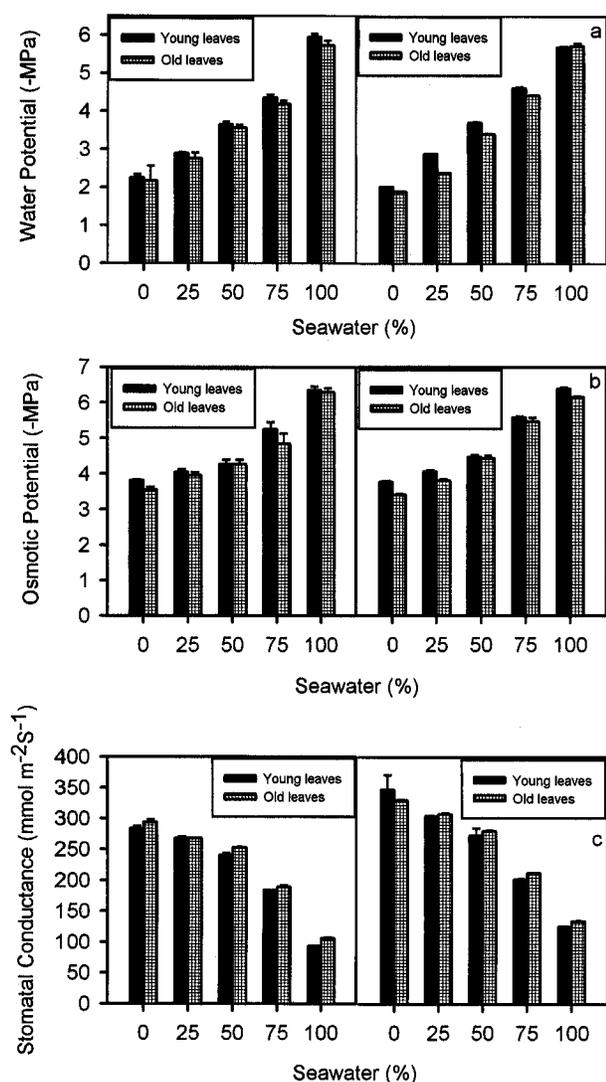


Fig. 1. Effect of NaCl (0, 25, 50, 75 and 100% seawater) on the water potential (a), osmotic potential (b) and stomatal conductance (c) of *Rhizophora mucronata* plants. Plants grown for 6 months after induction of salinity are referred to as seedlings (left) and those grown for 12 months as saplings (right). The leaves selected from the bottom node of the plant are designated as old and those collected from the second node from the apex as young. Bars represent means  $\pm$  standard errors.

1b). In several studies, water potential became more negative in mangroves when subjected to increases in salinity (Naidoo 1985, Rada *et al.* 1989, Werner and von-Villert 1995, Medina *et al.* 1995, Hwang and Chen 1995). A negative osmotic potential in plants is caused by accumulation of inorganic solutes (Popp 1983) as well as low molecular weight organic solutes such as proline (Stewart and Lee 1974), cyclitols (Popp *et al.* 1993) and betaine (Storey and Wyn Jones 1975, Popp 1983). In addition, sequestration of most of the cellular NaCl within the vacuole is also a cause in some mangroves (Werner and Stelzer 1990). If a high vacuolar concentration of NaCl implies the need for an

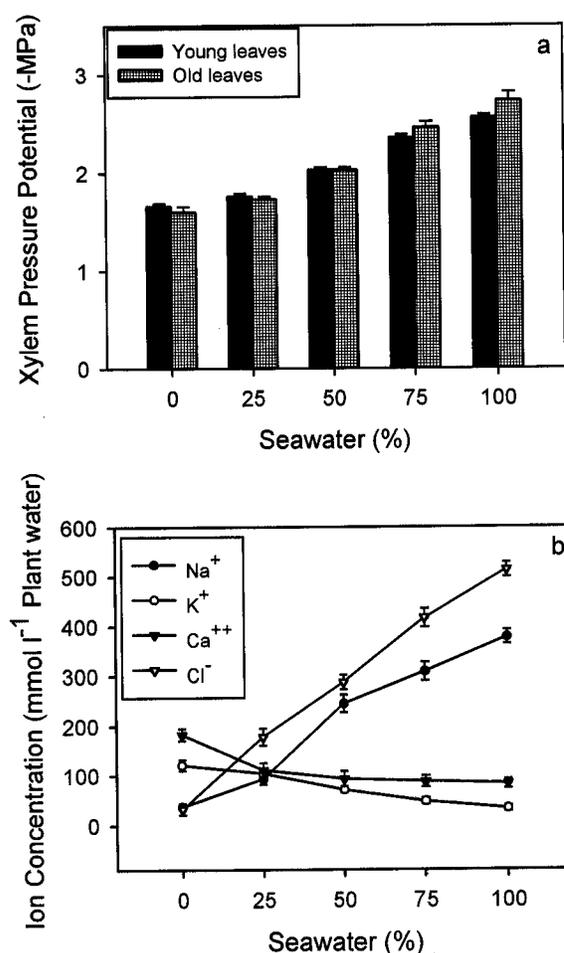


Fig. 2. Effect of NaCl (0, 25, 50, 75 and 100% seawater) on the xylem tension (a) and ion content (b) in *Rhizophora mucronata* saplings. Bars represent means  $\pm$  standard errors.

organic solute in the cytoplasm for intracellular osmotic adjustment (Jefferies *et al.* 1979), reallocation of nitrogenous organic resources for osmoregulation could severely limit plant growth (Naidoo 1985). The retarded growth of *R. mucronata* at higher salinity in the present study might be due to the presence of such organic solutes.

A three way ANOVA showed a significant individual effect ( $P < 0.05$ ) of leaf age, age of plants, and seawater ( $P < 0.001$ ) on stomatal conductance (Fig. 1c). The stomatal conductance of young and old leaves of seedlings and saplings decreased with increasing salinity (Fig. 1c). Xylem pressure potential also decreased (Fig. 2a). The lowering of osmotic potential in external medium results in decreased stomatal conductance (Naidoo 1985). It may reduce water absorption and translocation hence causing stomatal closures and increase the water-use efficiency of the plants (Werner and Stelzer 1990, Gordon 1993). The lowering of conductance decreases the rate of carbon dioxide accumulation and uptake (Apahalo and Jarvis 1993) and increases xylem tension (Ball and Farquhar 1984). Similar results were reported for other mangrove species (Werner and von-Villert 1995, Naidoo 1985). These results correspond to a lowering of

stomatal conductance indicating a limited water supply due to salt stress. This indicates that the water-use efficiency of plants increases with the increase in salinity (Ball and Farquhar 1984).

The concentration of sodium and chloride ions significantly ( $P < 0.001$ ) increased as the salinity of the medium increased, whereas potassium and calcium concentrations decreased (Fig. 2b). *Rhizophora mucronata* accumulated high concentrations of sodium and chloride ions when cultured at a non-zero NaCl salinity (i.e. in 25% seawater and above). Potassium and calcium levels were higher in 0% seawater. The decline in potassium and calcium in the tissues with the increase in salinity indicates that these ions were replaced by sodium at higher salinity levels. Trends in the distribution of ions in our results were similar to those of several salt marsh halophytes and mangroves (Jefferies *et al.* 1979, Naidoo 1985, Slim *et al.* 1996). High concentrations of ions in the tissues of *R. mucronata* at high salinities (75 and 100%) may have caused a reduction in growth by inhibiting biochemical processes such as enzyme activities and protein synthesis (Gibson *et al.* 1984).

Our results suggest that *Rhizophora mucronata* is a highly salt tolerant species, which maintains its salt balance like a true halophyte though its growth is retarded at very high salinity. This species could be re-introduced into the Indus delta because of its high salinity tolerance and growth stimulation in 50% seawater. Along the coast of Pakistan, the diversion of river water has caused an overall increase in media salinity, which might favor the growth of this particular species.

### References

- Apahalo, P.J. and Jarvis P.G.** 1993. Separation of direct and indirect responses of stomata to light: Results from a leaf inversion experiment at constant intercellular CO<sub>2</sub> molar fraction. *J. Exp. Bot.* **44**: 791-800.
- Ball, M.C. and Farquhar, G.D.** 1984. Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina* to long term salinity and humidity conditions. *Plant Physiol.* **74**: 1-6.
- Cintron, G., Lugo, A.E. and Martinez, R.** 1985. Structural and functional properties of mangrove forests. In W.G. D'Arcy and M.D. Correa, eds., *The Botany and Natural History of Panama*. Monographs in Systematic Botany. Missouri Botanical Garden, St. Louis, Missouri, USA. pp. 53-66.
- Clough, B.F.** 1984. Growth and salt balance of mangroves *Avicennia marina* (Forssk.) Vierh. and *Rhizophora stylosa* Griff. in relation to salinity. *J. Plant Physiol.* **11**: 419-430.
- Downton, W.J.S.** 1982. Growth and osmotic relation of the mangrove *Avicennia marina*, as influenced by salinity. *Austral. J. Plant Physiol.* **9**: 519-528.
- Feller, I.C.** 1995. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove *Rhizophora manglæ*. *Ecol. Monogr.* **65**: 477-505.
- Ghafoor, A.** 1984. Rhizophoraceae. In *Flora of Pakistan*. Edited by Nasir, E. and Ali, S.I., Karachi, Pakistan.
- Gibson, T.S., Spiers, J. and Brady, C.J.** 1994. Salt tolerance in plants. II. *In vitro* translation of mRNAs from salt tolerant and salt sensitive plants on wheat germ ribosomes. Responses to ions and compatible organic solutes. *Plant Cell Environm.* **7**: 579-587.
- Gordon, D.M.** 1993. Diurnal water relations and salt content of two contrasting mangroves growing in hypersaline soils in tropical arid Australia. In H. Lieth and A. Al-Masoom, eds. *Towards the Rational Use of High Salinity Tolerant Plants*. The Netherlands, **1**: 193-216.
- Hutchings, P.A. and Saenger, P.** 1987. *Ecology of mangroves*. University of Queensland Press, St. Lucia, Australia.
- Hwang, Y.H. and Chen, S.C.** 1995. Salt tolerance in seedlings of the mangrove *Kandelia kandel* (L.) Druce, Rhizophoraceae. *Bot. Bull. Acad. Sin.* **36**: 25-31.
- Jefferies, R.L., Rudmik, T. and Dillon, E.M.** 1979. Responses of halophytes to high salinities and low water potentials. *Plant Physiol.* **64**: 989-994.
- Karim, J. and Karim, A.** 1993. Effect of salinity on the growth of some mangrove plants in Bangladesh. In Lieth, H and Al Masoom, A. eds., *Towards the Rational Use of High Salinity Tolerant Plants*. The Netherlands, **1**: 193-216.
- Lee, J.A. and Ignaciuk, R.** 1985. The physiological ecology of strand line plants. *Vegetatio* **62**: 319-326.
- Luttge, U.** 1997. *Physiological Ecology of Tropical Plants*. Springer-Verlag, Berlin.
- Medina, E., Lugo, A.E. and Novelo, A.** 1995. Contenido Mineral del Tejido Foliar de Especies de Manglar de la Laguna de Sontecomapan (Veracruz, Mexico) y su. *Biotropica* **27**: 317-23.
- Murata, S., Kobayashi, M. and Sekiya, J.** 1992. Sodium stimulates regeneration of phosphoenol pyruvate in mesophyll chloroplast of *Amaranthus tricolor*. *Plant Cell Physiol.* **33**: 1247-1250.
- Naidoo, G.** 1985. Responses of the mangrove *Rhizophora mucronata* to high salinities and low osmotic potentials. *S. African J. Bot.* **52**: 124-128.
- Ogrady, A.P., McGuinness, K.A. and Eamus, D.** 1996. The abundance and growth of *Avicennia marina* and *Rhizophora stylosa* in the low shore zone of Darwin Harbour, Northern Territory. *Austral. J. Ecol.* **21**: 272-279.
- Popp, M.** 1983. Chemical composition of Australian mangroves. I. Inorganic ions and organic acids. *Z. Pflanzenphysiol.* **113**: 395-409.
- Popp, M. and Polania, J.** 1989. Compatible solutes in different organs of mangrove trees. *Ann. Soc. Forst.* **46**: 842-844.
- Popp, M., Larher, F. and Weigel, P.** 1984. Chemical composition of Australian mangroves. III. Free amino acids, total methylated onium compounds and total nitrogen. *Z. Pflanzenphysiol.* **114**: 15-25.
- Popp, M., Polania, J. and Weiper, M.** 1993. Physiological adaptations to different salinity levels in mangrove. In Lieth, H and Al Masoom, A. eds., *Towards the Rational Use of High Salinity Tolerant Plants*. The Netherlands, **1**: 217-224.
- Rada, F., Goldstein, G., Orozco, A., Montilla, M., Zabala, M. and Azocar, A.** 1989. Osmotic and turgor relations of

- three mangrove ecosystem species. Austral. J. Plant Physiol. **16**: 477-486.
- Slim, F.J., Gwada, P.M., Kodjo, M. and Heminga, M.A.** 1996. Biomass and litterfall of *Ceriops tagal* and *Rhizophora mucronata* in the mangrove forest of Gazi Bay, Kenya. Mar. & Fr. Wat. Res. **47**: 999-1007.
- Smith, S.M. and Snedaker, S.C.** 1995. Salinity responses in two populations of viviparous *Rhizophora mangle* L. seedlings. Biotropica **27**: 435-440.
- Stewart, G.R. and Lee, J.A.** 1974. The role of proline accumulation in halophytes. Planta **120**: 279-289.
- Smith, S.M., Yang, Y.Y., Kamiya, Y. and Snedaker, S.C.** 1996. Effect of environment and gibberellins on the early growth and development of the red mangrove, *Rhizophora mangle* L. Plant Growth Regulator Bull **20**: 215-223.
- SPSS Inc** 1996. SPSS: SPSS 7.0 for Windows 95. SPSS Inc. USA.
- Storey, R. and Wyn Jones, R.G.** 1975. Betaine and choline levels in plants and their relationship to NaCl stress. Plant Sci. Lett. **4**: 161-168.
- Tomlinson, P.B.** 1986. The Botany of Mangroves. Cambridge University Press, London.
- Werner, A. and Stelzer, R.** 1990. Physiological responses of the mangrove *Rhizophora mangle* grown in the absence and presence of NaCl. Plant Cell Environm. **13**: 243-255.
- Werner, B.H. and von Villert, D.J.** 1995. Dynamic changes in bulk water relations during stomatal oscillations in mangrove species. Continuous analysis using a dew point hygrometer. Physiol. Plant, **94**: 479-485.

(Received June 28, 2000; accepted March 15, 2001)