Effects of Ammonium, Phosphate, and Salinity on Growth and Nutrient Contents of Seedlings of Mangrove *Rhizophora mangle L*.

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ABSTRACT

The purpose of this experimental study was to examine the growth responses and tissue nutrient contents of *Rhizophora mangle L*. to variations in salinity, nitrogen, and phosphate under greenhouse culture conditions. Salinity has long been recognized as an important factor regulating physiology, growth and zonation of all mangrove species. The extent to which a species can cope with fluctuations in salinity is an important determinant of species distribution patterns. Nutrient concentration is also considered to be an important factor in seedling establishment and distribution of species in mangrove communities. The availability of essential nutrients, especially nitrogen and phosphorous ions, in waterlogged mangrove soils is largely controlled by the redox (reduction-oxidation) potential of the sediment.

Besides nutrient concentration and salinity, many other factors, such as light, temperature, tidal variations, forest gaps, tree-height gradients that reflect complex spatial, within-stand differences in nutrient dynamics across narrow environmental gradients, soil fertility, soil redox potentials, pH, etc., in the fields also have great impacts on the growth and distribution of *Rhizophora mangle L*. The greenhouse culture conditions provide an environment to eliminate impacts on growth of *Rhizophora mangle L*. from these factors and isolate only the influences from salinity variations and nutrient concentration. Culture solutions of three NaCl concentrations (Om 5, or 20 ppt, or equivalent to 0, 85, or 342 mM) with five of

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NH₄*(N) and PO₄³⁻ (P) combinations (low N-P, Low N-high P, mid N-P, High N-Low P, and High N-P) are used throughout the experiment. Macronutrients consisted of KCl, CaCl₂, MgSO₄, and Fe were added in the basic culture solutions. The pH of the solution was adjusted to 6.3-6.5 with CaCO₃. The temperature in the greenhouse is set at 82°F at all times. As *Rhizophora mangle L*. needs an abundant of light, illumination is provided from 7 - 11 pm everyday in addition to the sunlight received in the greenhouse during daytime. Each of the propagules, later became seedlings, was planted in individual container with culture solutions so influences from tidal variations, forest gaps, environmental gradients were eliminated.

Towards the end of the experiment, Dry Ashing method was used by a private lab to derive the plant tissue nutrient contents. Inductively Coupled Plasma and Atomic Emission Spectrophotometer (ICP-AES) were used to analyze the Na, K, Ca, Mg, and total P in various parts of the plant tissues (leaves, stems, and roots). The total amount of nitrogen was determined by a CHN analyzer. PROBLEM

How do nitrogen, phosphorous, and salinity affect the growth and ionic contents of the seedlings of *Mangrove Rhizophora mangle L.* (Red Mangrove)?

HYPOTHESIS

A moderate salinity is an important factor regulating the growth and distribution of the *Rhizophora mangle L.* (Red Mangrove). Ammonium-nitrogen will have a positive contribution to the Red Mangrove's growth of shoots, leaves and roots, while phosphorus is a major nutrient limiting its growth. Both ammoniumnitrogen and phosphorous have significant effects on the nutrient contents in plant tissues. EXPERIMENTAL PROCEDURE

- Propagules of *Rhizophora mangle L.* (Red Mangrove) were bought from an aquarium/nursery located in Florida. Propagules ranging from 7 9 inches (17.8 23.0 cm) in length were used in this study.
- 2. Before the experiment, all propagules without leaves and roots were washed and rinsed with distilled water, blotted dry, and then weighed on an electronic scale of 0.001 precision. The exact lengths of the stems were measured.
- 3. While planting the propagules into the culture solutions, the bottom 2-in of each propagules were embedded in a plastic pot of sand/gravel. The pot is made of the bottom portion of a 2-liter coke bottle, approximately 3 3.5 in (8-9 cm) in height.
- 4. One propagule was placed in each plastic pot. A total of 15 propagules were planted and cultivated in water.
- 5. The propagules were kept in freshwater in greenhouse for 1 week before adding in any salt water.
- 6. The greenhouse temperature was adjusted to $82^{\circ}F(27.7^{\circ}C)$.
- 7. Illumination using 40-Watts lighting was placed in the greenhouse to supplement sunlight after day hours, from 7 pm 11 pm every evening.
- 8. A week later after the propagules were placed in the greenhouse, prior to ammonium-nitrogen (N) and phosphorous (P) nutrient treatments, salt water

of three different salinities (0, 85, or 342 mM, or equivalent to 0, 5, 20 ppt) were added into the plastic pots for a month to induce the development of cotyledons and roots.

9. Solutions of five $NH_4^+(N)$ and PO_4^{3-} (P) combinations were prepared and mixed with each of the three NaCl solutions to form the fifteen basic culture solutions for this experiment.

<u>3 concentrations</u> <u>of NaCl</u> (salinity test)	<u>5 com</u> <u>NF</u> & I		
	<u>N</u>	<u>P</u>	
0 ppt	0.10 mM-N	0.05 mM-P	low N-P
or 0 mM	0.10 mM-N	0.50 mM-P	low N-high P
	0.50 mM-N	0.10 mM-P	mid N-P
	2.00 mM-N	0.05 mM-P	high N-low P
	2.00 mM-N	0.50 mM-P	high N-P
5 ppt	0.10 mM-N	0.05 mM-P	low N-P
or 85 mM	0.10 mM-N	0.50 mM-P	low N-high P
	0.50 mM-N	0.10 mM-P	mid N-P
	2.00 mM-N	0.05 mM-P	high N-low P
	2.00 mM-N	0.50 m M -P	high N-P
20 pp†	0.10 mM-N	0.05 m M -P	low N-P
or 342 mM	0.10 mM-N	0.50 mM-P	low N-high P
	0.50 mM-N	0.10 mM-P	mid N-P
	2.00 mM-N	0.05 mM-P	high N-low P
	2.00 mM-N	0.50 mM-P	high N-P

10. Of the total 15 combinations of culture solutions, each solution was

experimented with one propagule.

- The macronutrients in the basic solution also consisted of KCL, CaCl₂, MgSO₄, and Fe in elemental concentration (mM) of 6K, 4 Ca, 10 Mg, 10 SO₄, and 1 Fe.
- 12. Salinity, ammonium-N and Phosphate-P were adjusted as needed with NaCl, NH4Cl, and KH2PO4, respectively.
- 13. The pH of the solutions was adjusted to 6.3 6.5 with CaCO₃.
- 14. Plants growing in similar features were selected for further N and P nutrient treatments. The culture solutions were not aerated and were renewed every three weeks. Tap water was added as required to compensate for losses of solution through evapotranspiration.
- 15. The concentrations of N and P in the culture solutions were checked every 5-7 days and adjusted as needed to maintain initial culture concentrations.
- 16. Plants were kept in the greenhouse throughout the entire experiment (approximately 140 days) and were subject to salinity and nutrient treatments.
- 17. During the experimental period, plants were expected to grow, developing shoots, roots, leaves, into seedlings, at different rates subject to the various culture solutions treated.
- 18. 60 days after the plants were treated with N and P, seedlings were removed from the sand/gravel, cleaned in water, rinsed in distilled water, and blotted

dry. Total plant weight, stem length, leaf area, and root weight for each seedling were measured and recorded.

- 19. Total leaf area for each seedling was derived by tracing each leaf of a seedling on a graphing paper from which leaf areas were calculated.
- 20. A small section of a couple of root tips were clipped off from different seedlings as samples for deriving the total weight of roots for each seedling. Lengths and radii of the cutoff root sections were measured for root volume calculations. Weights were taken. Correlation between root volumes and root weights was established. Along with root length and volume information, root weight of each seedling was estimated and calculated.

21. Mean relative growth rates (RGR) over 100 days are calculated as follows:

- (1) $RGR_P = (W_2 W_1) / (t_2 t_1) \times 100$ for growth rate of plant weight
- (2) $RGR_s = (L_2 L_1) / (t_2 t_1) \times 100$ for growth rate in stem length (height)
- (3) RGR_L = $(A_2 A_1) / (t_2 t_1) \times 100$ for growth rate in leaf areas
- (4) $RRGR_R = (W_2 W_1) / (t_2 t_1) \times 100$ for growth rate in root weight

where W_2 and W_1 are tissue (plants and roots) weights, A_2 and A_1 are leaf areas, L_2 and L_1 are stem lengths (heights) at t_2 (the end of the first session of the experimental period) - t_1 (the beginning of the experimental period).

22. Since at t1, information on leaf areas and root weights were not available (propagules bought were without leaves and roots), only RGR's based on plant weights and stem heights were available at t2.

- 23. After measurements are taken, plants were placed back to gravel/sand in the pots and continued with nutrient treatments.
- 24. After 30 days, repeated procedure 18-21, but this time relative growth rates based on root weights and leaf areas were also available.
- 25. From the data derived, effects of how ammonium-nitrogen, phosphate and salinity of different concentrations have impacted the growth of the seedlings of mangrove *Rhizophora mangle L* were determined.
- 26.Leaves, stems, and roots were separated from the seedling. Plant tissues for each seedling were wrapped in paper towel, placed in envelopes, properly marked, and then sent to a private lab for further nutrient content analysis.
- 27. Private lab conducted the Dry Ashing Method to find out nutrient contents in various tissue parts for each seedling.
- 28. In the lab, individual plant tissues were dried at 80°C for 48 hours. Dry tissues were ground, then ashed at 485°C for 16 hours. The ash was further digested in concentrated HCl and HNO₃ solutions and dissolved in dilute acids to bring the minerals into solution. Sodium, potassium, calcium, magnesium, and total phosphorous were analyzed by Inductively Coupled Plasma and Atomic Emission Spectrophotometer (ICP-AES). The total amount of nitrogen was determined by a CHN analyzer.

- 29. From the data received from the private lab, nutrient contents in each of the plant's tissues were derived.
- 30. Charts/graphs were plotted to help further analysis of the growth rates and tissue nutrient contents *Rhizophora mangle L*. in a greenhouse environment.

MATERIALS

- ✓ *Rhizophora mangle L.* (Red Mangrove) X 15 propagules
- ✓ Plastic containers/pots bottom portion of the 2-liter coke bottle, approximately 3 - 3.5 in (8-9 cm) in height X 15 counts
- ✓ Gravel X 15 pounds
- ✓ Sand X 20 pounds
- ✓ Chemicals to be dissolved in tap water for culture solution preparations:

To prepare for three saline solutions:

• Salt (NaCl) X 2 kilograms

0 ppt (0 mM) 5 ppt (85 mM) 20 ppt (342 mM)

To prepare for five combinations of $NH_4^+(N)$ and $PO_4^{3-}(P)$:

- Ammonium Chloride (NH₄Cl) X 50 grams
- Potassium Phosphate manobasic (KH₂PO₄) X 35 grams

 Low N-P:
 0.10 mM-N + 0.05 mM-P

 Low N-High P:
 0.10 mM-N + 0.50 mM-P

 Mid N-P:
 0.50 mM-N + 0.10 mM-P

 High N-Low P:
 2.00 mM-N + 0.05 mM-P

 High N-P:
 2.00 mM-N + 0.50 mM-P

To prepare for the basic solution of macronutrients

- Potassium Chloride (KCl) X 40 grams
- Calcium Chloride (CaCl) X 30 grams
- Epson Salt (MgSO₄) X 50 grams
- Iron (Fe) X 5 grams

To help maintain pH of the solution at 6.3 - 6.5

• Calcium Carbonate (CaCO₃) X 50 grams

EXPERIMENTAL FACILITY & APPARATUS

✓ Greenhouse facility - temperature/humidity controlled with sun roofing



- ✓ Electronic scale 0.001-gram precision
- ✓ Soil pH test kit
- ✓ pH Meter





✓ Desktop lamp with 40-Watt light bulb for evening lighting supplement





- ✓ Lab Apparatus for dry ashing plant tissues:
 - Inductively Coupled Plasma Vista Simultaneous ICP-AES
 - Nitrogen Analyzer Perkin-Elmer CHN/SO Series II
 - Muffle Furnace Thermolyne 6000







Nitrogen Analyzer



Muffle Furnace

GRAPHS

Average RGR's of Plant Tissues

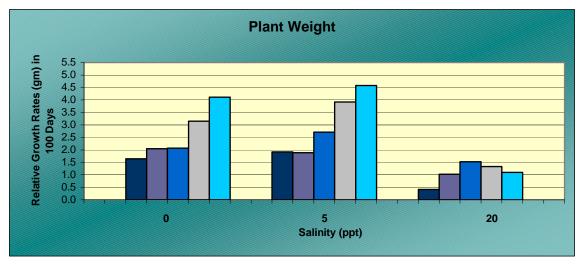
Average Relative Growth Rates for Plant Weights & Stem Lengths for Seedlings in the Same Culture Solutions for 100 Days RGR = Relative Growth Rate

 t_1 = beginning of the experimental period (September 22, 2003) t_2 = end of the first session of the experimental period (January 13, 2004)

t₂ -t₁ = 113 days

 RGR_P = Growth rate of plant weights in 100 days = $(W_2 - W_1)/(t_2 - t_1) \times 100$ RGR_S = Growth rate of stem lengths in 100 days = $(L_2 - L_1)/(t_2 - t_1) \times 100$

Low N-P Low N-High P Mid N-P High N-Low P High N-P



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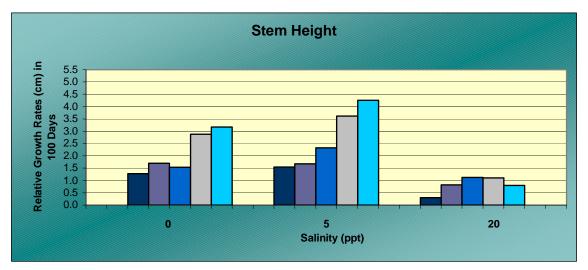
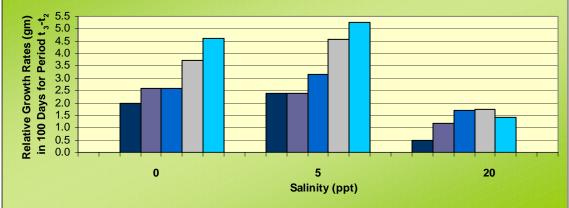


Fig. 2

Average Relative Growth Rates for Plant Weights & Stem Lengths for Seedlings in the Same Culture Solutions for 100 Days

RGR = Relative Growth Rate t₂ = beginning of the 2nd session of the experimental period (January 13, 2004) t₃ = end of the experimental period (February 12, 2004) t₃ -t₂ = 30 days RGR_P = Growth rate of plant weights in 100 days = (W₃ - W₂)/ (t₃ - t₂) ×100 RGR₅ = Growth rate of stem lengths in 100 days = (L₃ - L₂)/ (t₃ - t₂) ×100 Low N-P Low N-High P Mid N-P High N-Low P High N-P Plant Weight



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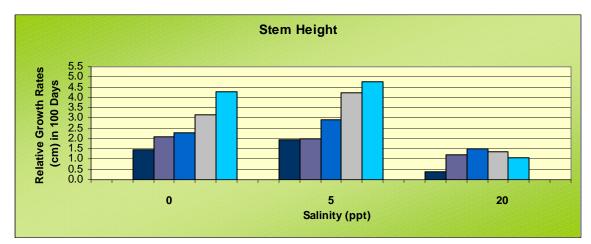


Fig. 4

Average Relative Growth Rates for Leaf Areas & Root Weights for Seedlings in the Same Culture Solutions for 100 Days RGR = Relative Growth Rate

 t_2 = beginning of the 2nd session of the experimental period (January 13, 2004) t_3 = end of the experimental period (February 12, 2004)

$t_3 - t_2 = 30 \text{ days}$

 RGR_P = Growth rate of leaf areas in 100 days = $(A_3 - A_2)/(t_3 - t_2) \times 100$ RGR₅ = Growth rate of stem root weights in 100 days = $(W_3 - W_2)/(t_3 - t_2) \times 100$

Low N-P Low N-High P Mid N-P High N-Low P High N-P

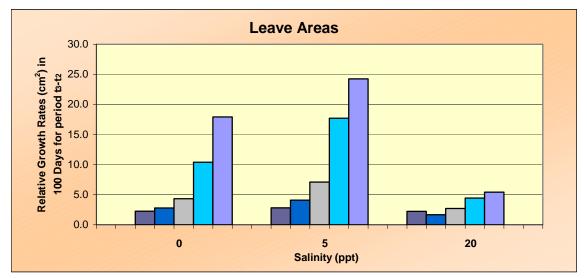


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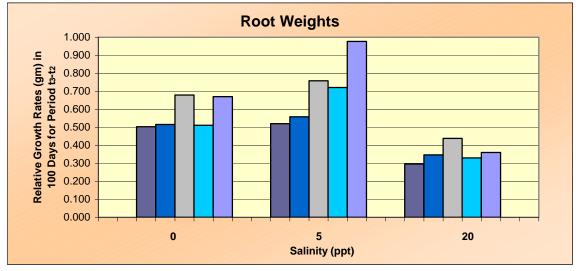
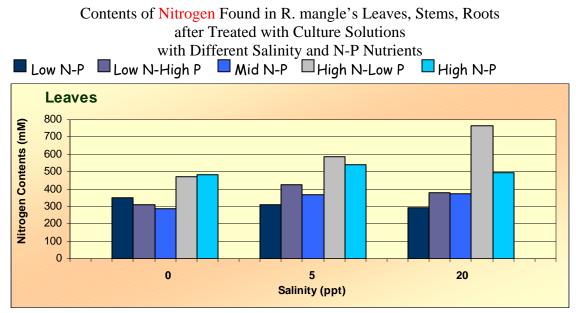
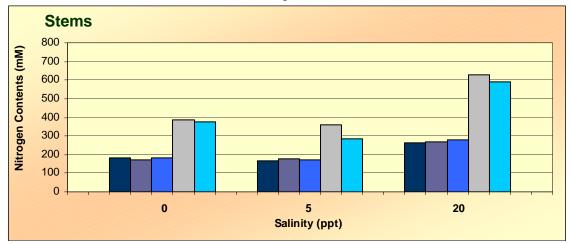


Fig. 6

Nutrient Contents Found in Plant Tissues









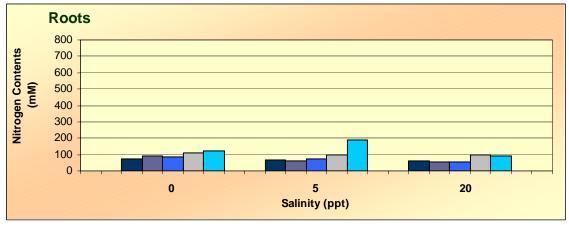
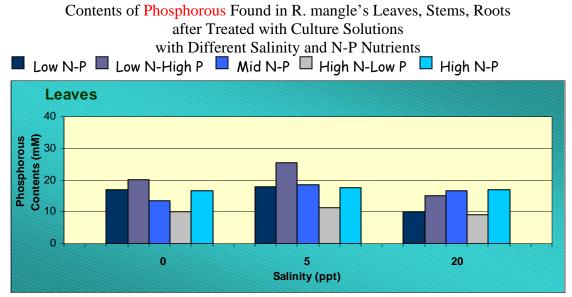


Fig. 9





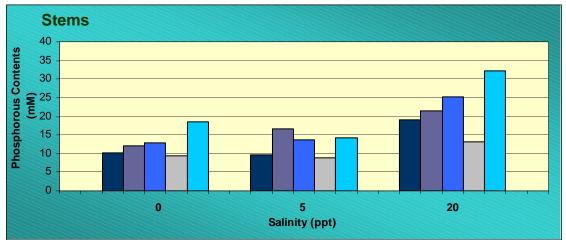


Fig. 11

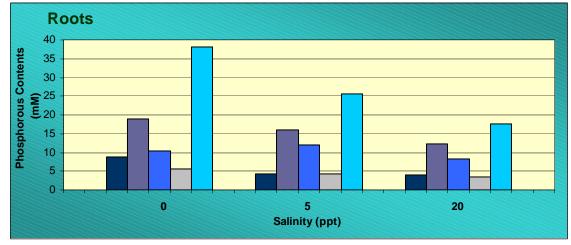
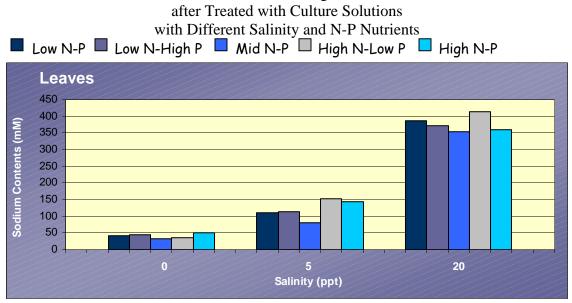
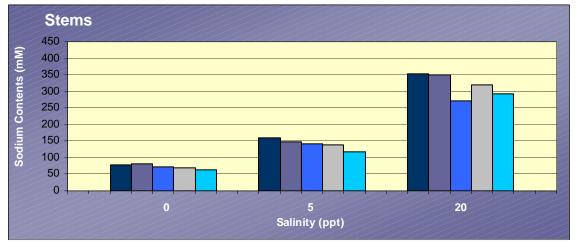


Fig. 12



Contents of Sodium Found in R. mangle's Leaves, Stems, Roots







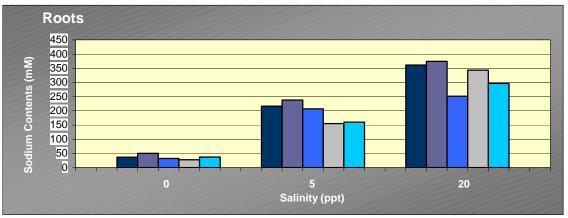
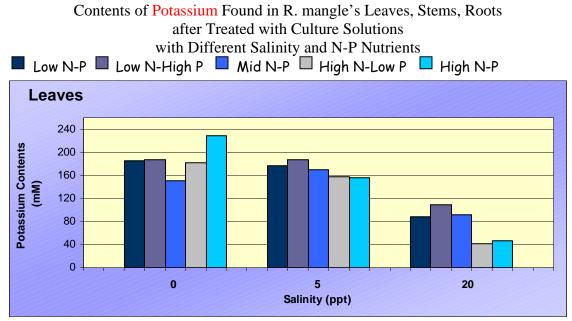
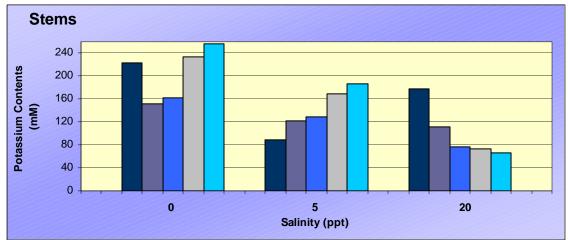


Fig. 15









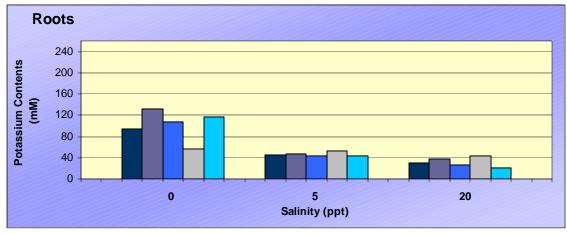
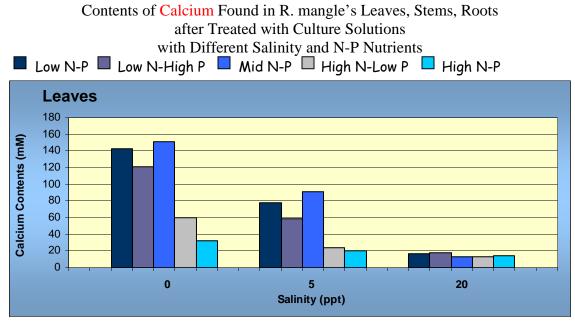
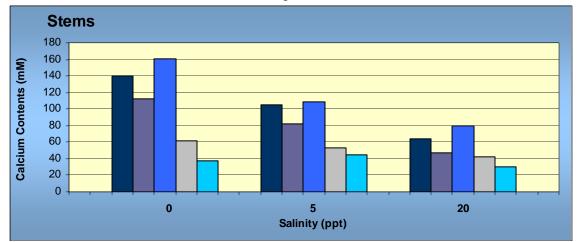


Fig. 18









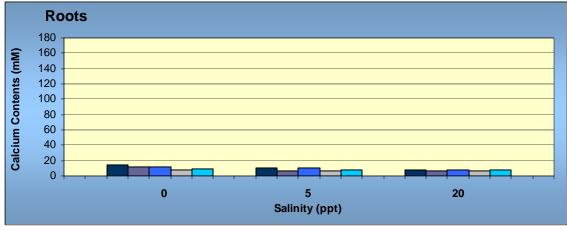
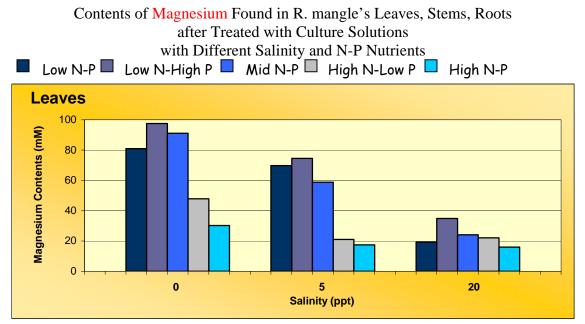
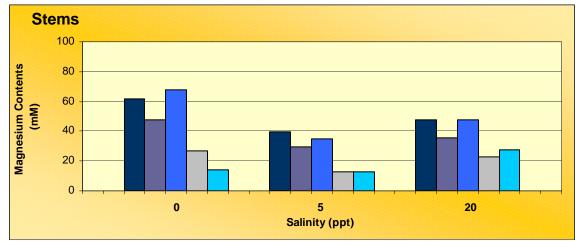


Fig. 21









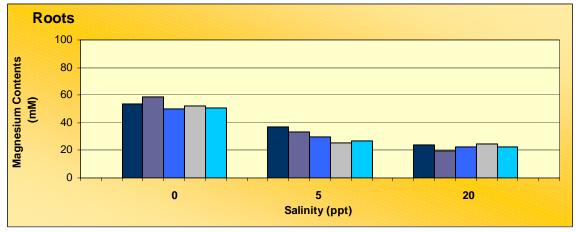
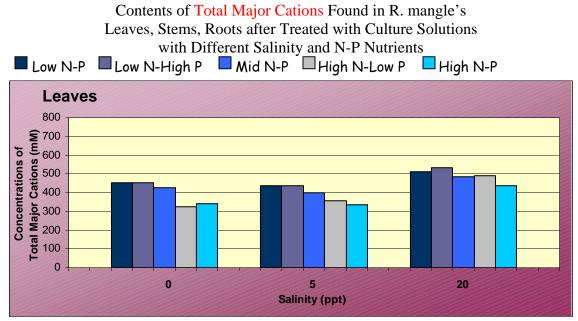
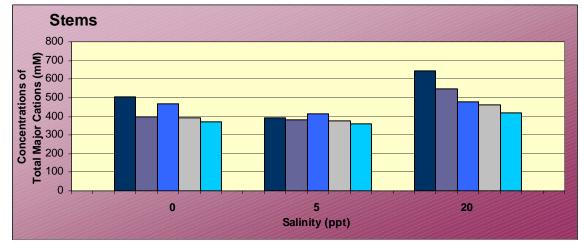


Fig. 24









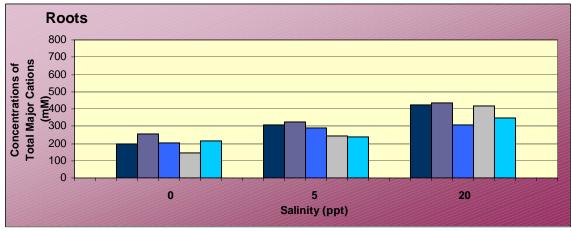
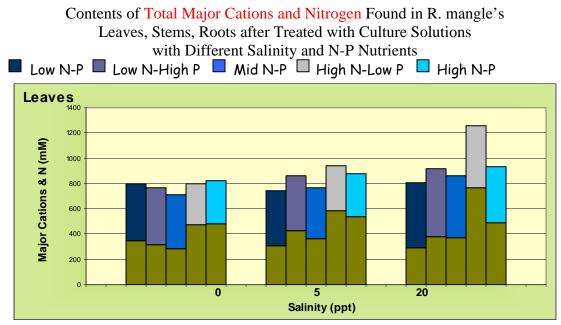
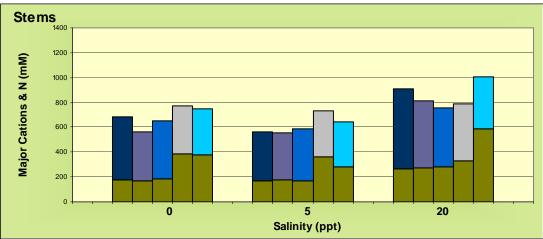


Fig. 27









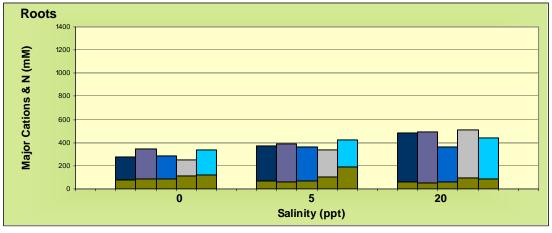


Fig. 30

EXPERIMENTAL RESULTS

- Salinity has a significant impact on the growth of *Rhizophora mangle L*. The red mangrove seedlings show the fastest growth rates in all plant tissues (i.e. leaves, stems, and roots) at the salinity of 5 ppt under all circumstances. Growth rates at 0 ppt salinity come into second. High salinity (20 ppt) severely limits the growth of *R. mangle L.* (Fig. 1-6).
- There was a noticeable interaction between ammonium-nitrogen (N) concentrations and the growth rates of *R. mangle's* seedlings when the salinity is low. At 0 and 5 ppt salinity, high N concentration at 2mM greatly increased the growth of the aboveground tissues, i.e. leaf area and stem height. This same tendency is not so obvious when the salinity is at 20 ppt (Fig. 1-6).
- At 0 and 5 ppt salinity, high concentration of P *alone* does not show much impact on the growth rates of the seedlings' overall plant weights, stem heights, as well as leaf areas (Fig. 1-5). However, high P concentration accompanied with the phenomenal impacts from high N concentration pushes the seedlings to further spur dramatically. This high growth rate is more noticeable at 5 ppt than at 0 ppt salinity.
- Root growth does not show too much of a response to the P or N concentration variations. Based on the experiment, roots show a common preference in a medium concentration of both N and P under all 3 salinity conditions (Fig. 6). The growth of roots, however, is mainly affected by

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salinity. Roots grow faster under low salinity conditions, with 5 ppt salinity still the most preferable condition.

∔ Tissue Nutrient Contents:

🕷 🛛 Nitrogen (N):

Ammonium-Nitrogen in the culture solutions has a direct effect on the N contents in plant tissues. This is particularly significant in the leaves and the stems (Fig. 7-8) under all salinity conditions. Roots show much less impact from the N treatment (Fig 9).

Phosphorous (P):

High P concentration in culture solutions shows a significant impact on root growth when N concentration is high (Fig. 12). The same effect, however, is not too noticeable in leaves or stems.

Salinity, in general, does not show too much of an interaction with P treatment, though the seedlings' stems do show higher P contents at 20 ppt salinity (Fig. 11).

Tissue Ionic Contents: Salinity greatly affected the concentration of cations in the tissues of *R. mangle.*

Sodium (Na):

Na⁺ contents in seedlings' tissues have a position relation with the salinity in culture solution (Fig. 13-15). The higher the salinity level,

the higher the Na⁺ contents found in all plant tissues, i.e. leaves, stems, and roots.

There is not much interaction between the N-P concentrations in the culture solutions and Na⁺ contents in tissues; though at 20 ppt, plant tissues show a litter higher Na⁺ contents in general when P concentration is high (Fig. 13-15).

🍀 🛛 Potassium (K):

At low salinity conditions (0 and 5 ppt), K^{+} is the dominant inorganic cation in the seedlings' leaves and stems (Fig. 16-17). As the salinity increases in the culture solutions, Na⁺ gradually replaces the K⁺ and becomes the dominant cation in plant tissues.

At 5 ppt salinity, K⁺ contents in leaves shows literally no difference under any N-P concentrations; whereas at 20 ppt salinity, K⁺ contents in leaves decrease quite significantly when N-concentration increases (Fig. 16).

🗮 🛛 Calcium (Ca):

 Ca^{2+} contents are significantly higher at low salinity than at high salinity in both leaves and stems (Fig 19-20). Ca^{2+} contents in leaves and stems show an inverse relation with N concentrations in culture solutions, i.e higher Ca^{2+} contents when N-concentration is low; though Ca^{2+} is found to be at the highest under almost all salinity conditions when N-P concentrations are set at the medium level (i.e. 0.50 mM-N + 0.010 mM-P).

Ca²⁺ contents are found to be extremely low in the seedlings' roots compared to leaves and stems in all culture solutions (Fig. 21).

🍀 🛛 Magnesium (Mg):

There is an inverse relation between Mg²⁺ contents with salinity in leaves and roots. At 0 and 5 ppt salinity, Mg²⁺ contents in leaves are significantly higher than that at 20 ppt salinity (Fig. 22 & 24). This trend, however, is not evident in stems (Fig. 23).

Both leaves and stems show an Mg^{2+} contents that are inversely related to N-concentration in culture solution (Fig. 22-23).

Mg²⁺ contents are found to be at higher levels compared to Ca²⁺ in the seedlings' roots (Fig. 24). There is, however, not much interaction between the Mg²⁺ contents and N-P concentrations in roots.

Total Major Cations (Na⁺, K⁺, Ca²⁺, and Mg²⁺):

Total major cations maintain at a level of around 400-650 mM in the seedlings' leaves and stems (Fig. 25-26). Roots show lower total cation contents, but still maintain at levels of around 200-400 mM (Fig 27).

Total cation concentrations are the highest at 20 ppt salinity for all leaves, stems, and roots due to high Na^+ content at 20 ppt salinity (Fig. 25-27).

Total Major Cations (Na⁺, K⁺, Ca²⁺, and Mg²⁺) + Nitrogen (N):

Rhizophora mangle L. is able to regulate and accumulate high cation contents in tissues at various N-P treatments, even when it is cultured without NaCl (Fig. 28-30).

CONCLUSION

This research supports the hypothesis that a moderate salinity is an important factor regulating the growth of *Rhizophora mangle L*. It also demonstrates that various salinity conditions do have different impacts on the growth rates of a mangrove seedling. In this experiment, salinity at 5 ppt gives the mangrove seedlings the fastest relative growth rates (Fig. 1-6). High salinity at 20 ppt significantly limits the growth of the mangrove seedlings, and the effects are not relieved by the addition of high concentrations of either nitrogen or phosphate.

In general, a high N concentration alone in the culture solution has a positive contribution to the growth of *R. mangle* seedlings, whereas a high P concentration alone does not. However, deviating from the hypothesis, phosphorous does not *always* limit the growth of the mangroves. This experiment shows that increasing P concentration accompanied by a high N-concentration can actually spur the seedlings' growth, particularly at low salinity level.

The hypothesis is also supported by the experimental results that both ammonium-nitrogen and phosphorous do have significant effects on the nutrient contents in plant tissues, though to different extents in leaves, stems, and roots (Fig. 1-6). *R. mangle* seedlings can accumulate a high concentration of cations in tissues under all salinity conditions, including at 0 ppt salinity. Potassium is the major cation in the seedlings tissues at low salinities, but is replaced by sodium as the concentration of NaCl in the culture solution, and consequently in plant tissues, increases (Fig. 13-18). Results of this greenhouse research show that the Na⁺

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contents in the *R. mangle* seedlings' leaves are closely related to the Na concentration of the culture solution.

Though the optimal salinity for *R. mangle* in the greenhouse environment is found to be at 5 ppt NaCl level in this experiment, and seedlings growth are inhibited at high salinity of 20 ppt, the red mangrove, however, exhibits exuberant growth even at porewater salinities ranging from 0 to 27 ppt in Florida coastlines (Twilley, 1996). Availability of freshwater supply in the field is thought to be responsible for this apparent discrepancy in optimal growth salinity. *R. mangle L.* preferentially utilized rain-derived freshwater during the wet season (Lin and Sternberg, 1992). While greenhouse seedlings were continuously exposed to constant salinity conditions, plants in the fields are routinely inundated with relatively freshwater as well as higher salinity porewater. FUTURE APPLICATIONS

"Mangroves may be declining faster than rain forests.....less than half of the mangrove forests that once covered the world's tropical coastlines are now gone....." ~An alert from Ecologists and Environmentalists~

Mangroves are one of the world's most species-rich ecosystems and help to protect coasts from storms and floods. They offer significant and unique habitat to birds, mammals, crustaceans, and fish populations through a complex marine food chain, and create breeding habitat and establish restrictive areas for the protection of maturing offspring. Mangroves also contribute to improved water quality by filtering and assimilating pollutants, stabilizing bottom sediments, protecting shorelines from erosion, and are a valuable source of protection for coral reefs.

As increases in population, waterfront development, agriculture, aquaculture, boating, recreational activities, as well as land reclamation are severely damaging the worldwide mangrove forests, the importance and necessity of implementing mangrove replenishment and replanting plans have become a more and more urgent issue.

It is hoped that this greenhouse experiment can help establish a foreground based on the data collected for future mangrove replanting projects. The specific nature as to how ammonium, phosphate, and salinity impact the growth rates and ionic nutrient contents of the mangrove seedlings is expected to help in developing an ecological model for mangrove replenishment. Continued studies and researches

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are encouraged to examine the feasibility of converting the greenhouse environment into the replanting fields in order to compensate the loss of mangroves habitats due to the human activities.

> "IF there are no mangrove forests, the sea will have no meaning. It is like having a tree with no roots, for the mangroves are the roots of the sea." ~A fisherman on the coast of the Andaman Sea, Trang Province, Thailand~