

## **The Effects of Salinity on Water Hyacinth (*Eichhonia crassipes*)**

**Jack Cheng**

**Abstract** The water hyacinth's ability to cope with various ecosystems has been the primary reason why it has been so successful in infesting fresh and brackish waterways. The high productivity and absorption capacity of the hyacinth causes damage by obstructing navigation, impeding drainage, destroying wildlife resources, and reducing outdoor recreation. This study examines the productivity of water hyacinth at different salinity. The experiment was conducted in a greenhouse with eighteen 15L tanks (one control and five treatments replicated three times) over seven days. Each tank contained water, nutrients, and salt. Salinity concentration for the control was 0 ppt while the treatments had concentrations of 0.5 ppt, 2.25 ppt, 4.5 ppt, 9 ppt, and 18 ppt. Leaf number, length, width, and plant color were measurements were taken three times a week as a measure of growth and health. The results from this study suggest that water hyacinth cannot survive past a salinity concentration of 9 ppt. Any salinity level above the oligosaline range (0.5 ppt-5 ppt) appears to weaken and damage the hyacinth. Information from this study would be useful in assessing the risk of water hyacinth infestation and possible methods of control. Future research can look at a longer study period as well as a finer gradient of salinity concentrations.

## Introduction

The South American floating aquatic plant water hyacinth (*Eichhonia crassipes*) has a history of being a worldwide invasive plant. Water hyacinth was first introduced to the Sacramento River in California by ornamental enthusiasts in 1904 (Toft, 2003). Water hyacinth's ability to flourish in various ecosystems and environmental ranges has been one of the main reasons why water hyacinth has been so successful in infesting new habitat (Tag El Seed, 1975). Its ability to cope with varying temperatures, lighting conditions, pH levels, drought resistance, and salinity has made water hyacinth ideal for almost any environment (Tag El Seed, 1975).

In addition to its ability to tolerate a wide range of environmental conditions, water hyacinth is also capable of rapid reproduction. Water hyacinth can reproduce vegetatively and sexually. A study in Louisiana in 1948 showed that ten plants were able to vegetatively reproduce 1610 plants in three months (Penfound, 1948). In the study conducted by Tag El Seed in 1973, hyacinth managed to cover an area of one thousand kilometers in two years. The ability for vegetative propagation is probably due to the plant's rate of photosynthesis. Hyacinth is capable of producing organic matter at a rate of 28g of carbon/sq m/day under ideal growing conditions (Tag El Seed, 1975).

The high productivity and absorption capacity of the hyacinth creates a big problem for freshwater lakes and rivers. Water hyacinth causes damage by: obstructing navigation, impeding drainage, destroying wildlife resources, reducing outdoor recreation, and constituting a hazard to life. The choking of streams and the obstruction of run-off increases backwater and flood conditions in many areas which affects water transportation and navigation (Penfound, 1948). In many systems, hyacinth can be considered an ecosystem engineer (Toft, 2003). Hyacinth has the capacity to modify and change its surrounding habitat. The dense floating mat and projecting leaves above the surface provide a habitat for other species. Under the surface, decomposing organic matter can cover the benthic zone which leads to low dissolved oxygen levels (Toft, 2003). The low dissolved oxygen level eliminates all fishes and eliminates many of the food plants for wildlife (Penfound, 1948). However, hyacinth has also been shown to have some positive effects on water quality. *E. crassipes* has been known to assist in the purification of water because of its settlement action and absorption capacity. The high productivity rate of

water hyacinth is one of the main reasons why it has been commonly used in Southern France in the treatment of industrial waste waters (De Casabianca *et al.*, 1995).

Water hyacinth is an immense problem to any freshwater or brackish water ecosystem. Some attempts have been taken to control the growth of hyacinth. Some of the methods used to control the hyacinth include mechanical shredding, biological treatments, and chemicals (Penfound, 1948). The use of chemical 2,4-dichlorophenoxyacetic acid (2,4-D) was found to be useful for controlling growth but failed in inhibiting seed germination (Tag El Seed, 1975). Studies conducted by Penfound (1948) and De Casabianca and Laugier (1995) show that salinity does have an effect on water hyacinth yield. Water hyacinth is sensitive to salinity; however, very limited research has been done on this topic (De Casabianca and Laugier, 1995). Researchers believe that water hyacinth is adapting itself to salt water since they are now more prevalent in brackish water as well as shorelines of salt water bodies (Penfound, 1948).

Research conducted on water hyacinth collected from Louisiana found that water hyacinth could not tolerate salinity levels higher than one percent salt (Penfound, 1948). Hyacinth is only able to survive in salinity ranges between freshwater and oligosaline conditions (0-5 ppt). Necrosis and death was observed in mesosaline and polysaline conditions (5 ppt-18 ppt). Similar research conducted in France confirms the results of the experiment carried out in Louisiana. Hyacinth collected in Southern France revealed that hyacinth grown in a salinity of 9.2 g/l and 13.7 g/l resulted in necrosis (De Casabianca and Laugier, 1995).

I hypothesize that water hyacinth collected from Antioch, C.A. is becoming more salt tolerant to brackish waters. If this is true, then the ecotype may eventually become a pest in other regions of the San Francisco Bay. The fresh water currents in the Bay and the water hyacinth's survivability could result in the invasion of hyacinth in other regions of the Bay. There is approximately 250,000 acres of wetland in the Bay; most of which is suitable for water hyacinth (Wetlands 2003, elect. comm.) My study focuses on the effects of salinity on water hyacinth found around the San Joaquin River in California. Specifically, I will be looking at water hyacinth's productivity and growth at varying salinity levels. This information would be useful in assessing the risk of water hyacinth infestation as well as possible methods of control.

## Methods

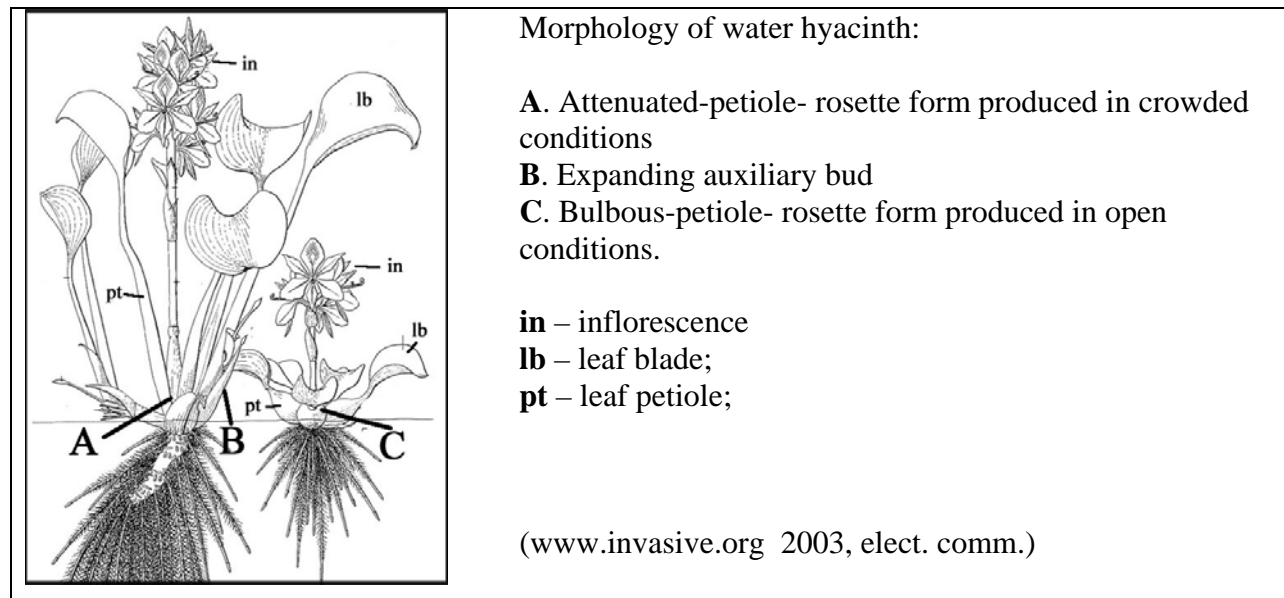


Image 1. Morphology of water hyacinth

**Data Collection** Water hyacinth was collected from the Dow Wetland Preserve in Antioch, California. Bulbous petioles were collected if they had not undergone reproduction and were in healthy condition (Image 1). Plants were collected randomly from two different locations. Half the plants were collected from the Antioch Marina and the other half was collected from the tidal marsh at the Dow Wetland Preserve. The background salinity level in these two bodies of water was approximately 0.01% (0.1 ppt). Hyacinth was selected based on the maturity level. After the shoots were collected, they were brought back to Berkeley where they were cleaned and placed in a nutrient rich solution (Appendix A). The plants were placed in a nutrient solution for a week to encourage growth and to make sure that the plants were healthy before placing them in a salt solution.

**Trials** Two trials of the experiments were carried out in a greenhouse at the University of California, Berkeley Oxford Tract. The first trial was conducted from March 21<sup>st</sup> through March 28<sup>th</sup>. The second trial was from March 28<sup>th</sup> until April 4<sup>th</sup>. Both trials took place over seven days and data was collected three times a week. Data was collected the first, third, and last day of each trial. The temperature of the greenhouse was kept at approximately 65°F- 70°F and the humidity in the greenhouse was on average 70%. The water hyacinth was exposed to approximately sixteen hours of light each day both through sunlight and greenhouse lights.

**Procedure** After a week of incubation, hyacinth shoots were selected and placed into eighteen 15L tanks. Each tank contained quarter strength nutrient water to ensure that the availability of nutrients would not be the limiting factor of growth. The nutrient water consists of both micro and macronutrients (Appendix A). Each container also contained varying amounts of kosher salt (NaCl). Six hyacinth plants were placed in each container: four small plants, one medium, and one large. Plants were categorized based on leaf number and size. The four smaller plants were labeled 1-4 while the medium and large plants were numbered 5 and 6 respectively. Smaller hyacinth had approximately 2-3 leaves and were about 7-8 cm in length. Medium and large hyacinth generally had 5-6 leaves and typically had leaves 12-18 cm in length. After the plants were selected they were all placed in the tanks of nutrient water. Kosher salt was then added to make the saline solution. The experiment had one control and five treatments replicated three times. Salinity concentration for the control was 0 ppt while the treatments had concentrations of 0.5 ppt, 2.25 ppt, 4.5 ppt, 9 ppt, and 18 ppt. A concentration of 0 ppt. is a freshwater environment. Salinity concentrations of 0.5 ppt, 2.25 ppt, and 4.5 ppt. fall into the range of oligosaline while a concentration of 9 ppt. and 18 ppt. is mesosaline and polysaline respectively (Cowardin, 1979). There were a total of three duplicates of hyacinth and salinity tanks. In total there were 108 plants, and 18 tanks.

**Parameters** Leaf number, length, width, and plant color were the parameters measured as an indicator for growth. Leaf number was done with a visual count. Leaf counts were taken before they were placed into salt water and leaf counts were taken again at the three day marker and at the end of the week. The number of leaves at the start was then subtracted from the number of leaves observed at the end of the week. Any leaf that had budded out was considered positive growth. A leaf that rotted or wilted off was considered negative growth. Leaf length was measured from the base of the root until the apex of the leaf blade of the largest leaf of each plant (Image 1). The width of the leaf was measured from widest points of the largest leaf. Data and measurements collected were from the larger healthier leaves. Each plant was measured once for leaf number, length, width, and color. Visual observations and plant color were also used to determine the health of the hyacinth. The health of the hyacinth was determined by leaf color. Leaves fell into one of the two categories. They were either green (healthy) or yellow (unhealthy). The Munsell Color Chart for Plant Tissue was used to determine the color of the hyacinth. Since color coding is subjective, hyacinths were classified into two distinct groups.

Any plant tissue that was not on the 5GY (Green-Yellow) Munsell color chart fell into the 2.5Y (Yellow) Munsell color chart (Munsell, 1977). Leaves that had similar colors to the 5GY Munsell color chart were considered healthy, while those found on the 2.5Y Munsell color chart were considered unhealthy.

**Statistics** Foliage growth was calculated by subtracting the leaf count on the last day from the leaf count from the first day. Leaf growth was also measured the same way. The length and width of the leaves on the last day was subtracted from the length and width on the first day. The difference in leaf count and size was then used to determine if different salinities had an effect on growth rates. These parameters were then plotted against salinity and tested for correlations. An ANOVA was first used to evaluate whether there was a difference in the means. The ANOVA concluded that there was a difference in the group mean with a p-value < 0.001. The Tukey Honestly Significant Difference (HSD) test was then used to examine the differences in foliage growth and leaf growth. Tukey's multiple comparison test can be used to determine which means amongst a set of means differ from the rest.

## **Results**

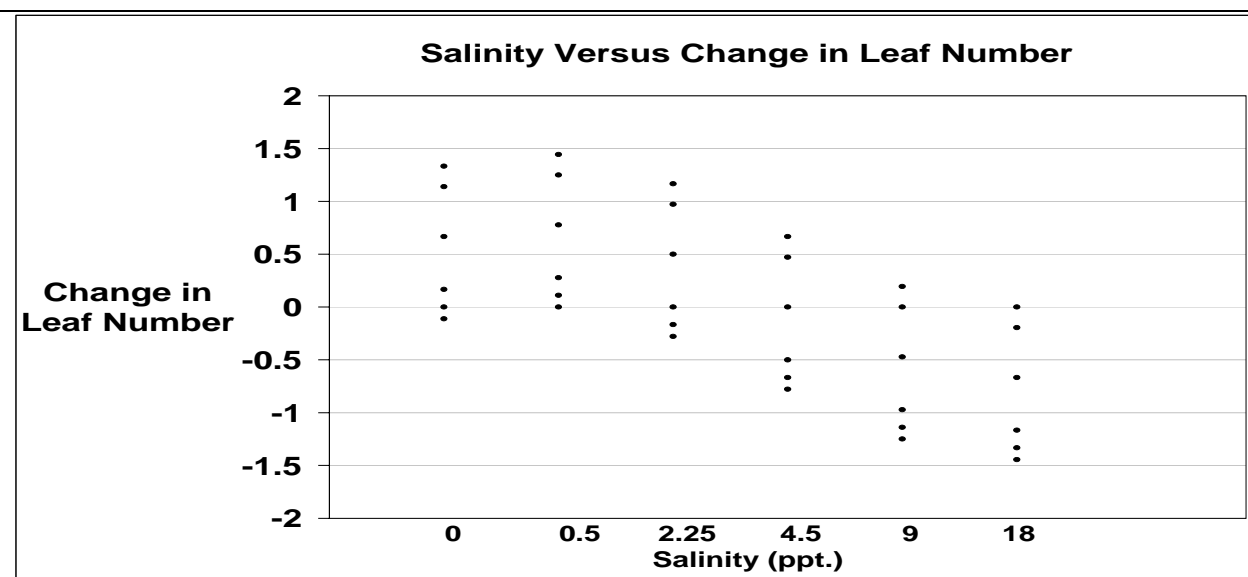
**Observations** All the hyacinths were healthy and green on the first day they were placed into varying salinity tanks. By day three most of the hyacinths that were placed in a salt solution showed some wilting on the larger leaves. The stems of the plants in 9 and 18 ppt. began to soften and lose its buoyancy after day three. At the end of the week, there were drastic changes in leaf color and tissue stability in the 9 and 18 ppt. solution. The older leaves and stems in 9 and 18 ppt. became yellow and soft after a week of exposure to salt water. New shoots and leaves began to wilt and turn brown at the apex of the leaves in concentrations higher than 4.5 ppt. after a week of exposure.

**Color** Changes in leaf color were compared against the Munsell Color Charts for Plant Tissue. All plants started off with an initial color of 5GY. Treatments with salinities greater than 9 ppt. experienced a dramatic change in color. Hyacinth at salinities greater than 9 ppt. went from a green-yellow color leaf to a yellow colored leaf (2.5Y). Table 1 shows that approximately ten percent of hyacinth in 9 ppt. and twenty percent of hyacinth in 18 ppt. experienced a drastic change in leaf color and health. There were no signs of severe color change in any of the other salinities.

	0 ppt.	0.5 ppt.	2.25 ppt.	4.5 ppt	9 ppt.	18 ppt.
5G	100%	100%	100%	100%	89%	82%
2.5 Y	---	---	---	---	11%	18%

Table 1. Percentage of leaf color

**Leaf Number** The Tukey HSD test was used to determine if the mean number of leaves grown or lost was significantly different from the other salinities. The control revealed that it was significantly different from hyacinth grown in salinities greater than 9 ppt. We can be 95% confident that the change in leaf number in 0.5 ppt. was different from those with a salinity level greater than 4.5 ppt. Hyacinth grown in the 2.25 ppt concentration was shown to be significantly different from hyacinth in the 9 and 18 ppt solution. Only hyacinth grown in the 4.5 ppt. salinity showed a difference from hyacinth grown in the 0.5 ppt. solution. Figure 1 shows a general decrease in leaf number as salinity increases. With higher concentrations of salt, leaf productivity begins to fall. Salinity appears to hinder hyacinth growth by discouraging new leaf growth.



Comparison for all pairs using the Tukey test

	0 ppt.	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-0.72066	-0.60955	-0.55399	-0.05399	0.418229	0.612673
0.5 ppt.	-0.60955	-0.72066	-0.44288	0.057118	0.52934	0.723784
2.25 ppt.	-0.55399	-0.44288	-0.72066	-0.22066	0.251562	0.446007
4.5 ppt.	-0.05399	0.057118	-0.22066	-0.72066	-0.24844	-0.05399
9 ppt.	0.418229	0.52934	0.251562	-0.24844	-0.72066	-0.52622
18 ppt.	0.612673	0.723784	0.446007	-0.05399	-0.52622	-0.72066

Positive values show means that are significantly different

Figure 1. Salinity Versus Change in Leaf Number

**Leaf Width** The Tukey HSD test was unable to detect any significantly different changes in leaf width. The average changes in leaf width were all similar in all six salinity concentrations. Salinity levels cannot account for the growth or shrinkage of leaves. The graph in figure 2 does not appear to have any trends. As salinity increases, leaf width begins to decrease. This does not appear to be significant due to the fluctuations in the change of leaf width.

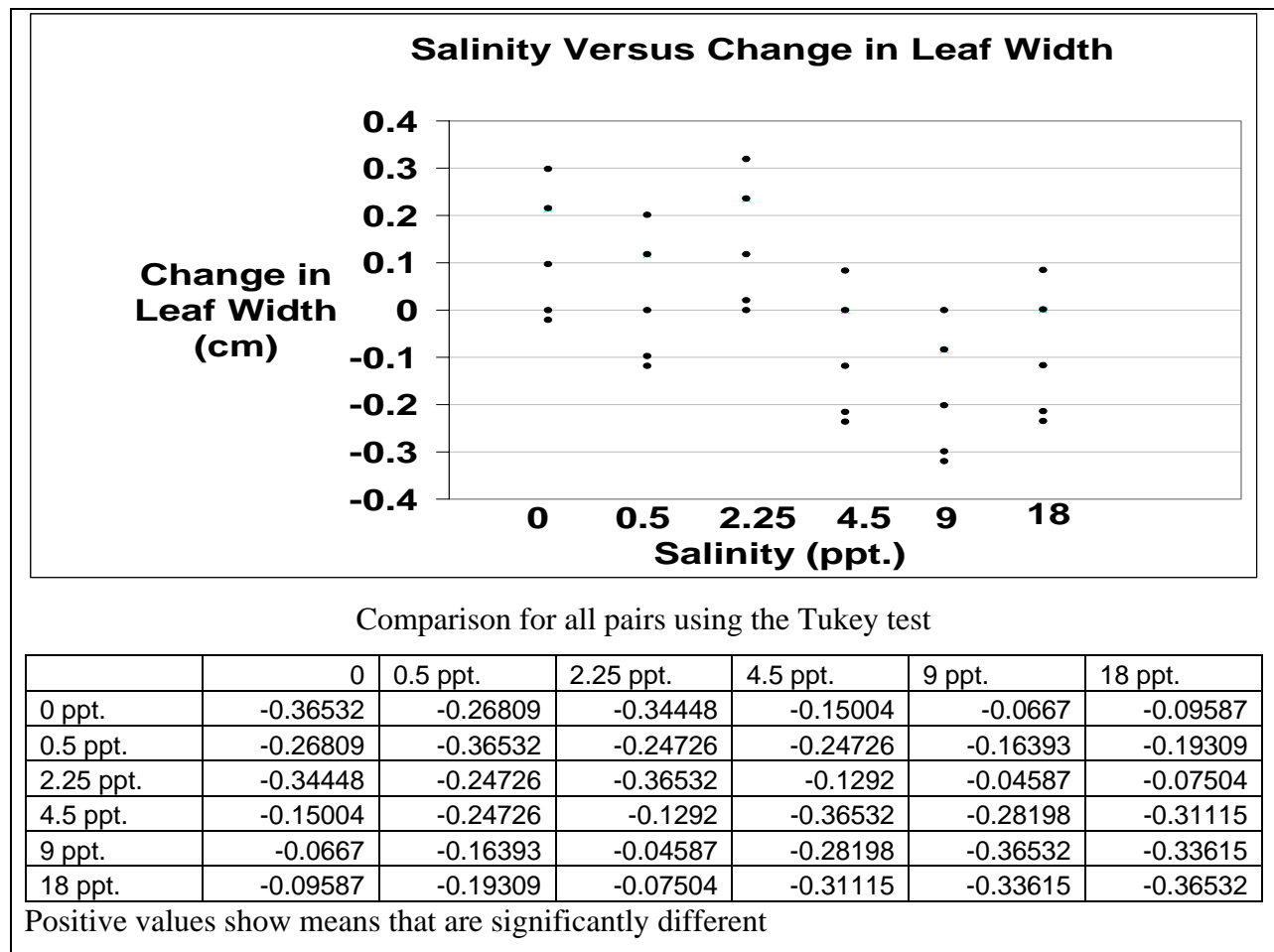


Figure 2.

**Leaf Length** The only significantly different pair was between 0.5 ppt. and 9 ppt. The mean length grown in 0.5 ppt. is larger than the mean growth that occurred in tank 9 ppt. No detectable differences could be found between salinities 0 ppt., 2.25 ppt., 4.5 ppt., and 18 ppt. Figure 3 also reveals a general decrease in leaf length as salinities increase. Increased salinity levels results in decreased leaf growth. Hyacinth in the higher salinity ranges generally had less growth than those in the lower salinity ranges.

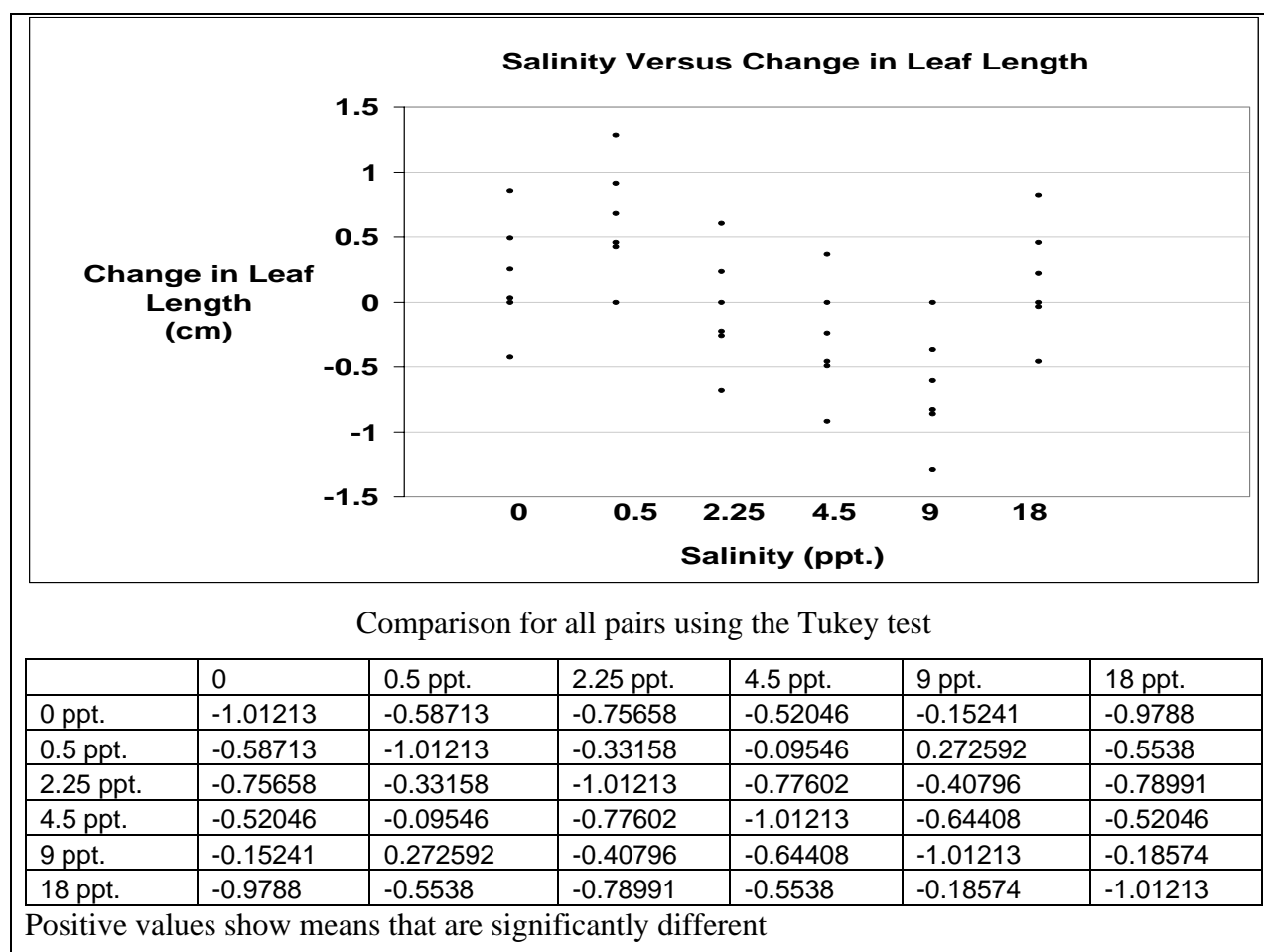


Figure 3. Salinity Versus Change in Leaf Length

**Plant Sizes** The number of leaves that died or sprouted in the smaller hyacinth proved to be significantly different (appendix B.1.1). Hyacinth from the control and the 2.25 ppt. solution were both different from hyacinth in the 18 ppt. concentration. In addition, hyacinth from the 0.5 ppt. solution were different from those grown in salinities greater than 4.5 ppt. There were also significant differences in the change in plant length (appendix B.2.1). The amount of change that took place in the control was different from hyacinth grown salinities greater than 9 ppt. There were no detectable differences when comparing the mean width growth of the small hyacinth in all six concentrations (appendix B.3.1). Growth in leaf width in the smaller hyacinth was all statistically insignificant. There were no significant differences in both the width and length of medium sized plants. The only parameter that showed a difference was the change in leaf number (appendix B.1.2). The control and the 0.5 ppt. salinities were both different from salinities greater than 9 ppt. The larger sized plants showed no difference in leaf length and

width as well. The only parameter that revealed a difference was the change in leaf number (appendix B.1.3). Hyacinth in the control and 2.25 ppt. were different from hyacinth found in the 18 ppt. solution.

## **Discussion**

**Hyacinth Health** By day three most of the hyacinth in the salt solutions showed some wilting on the larger leaves. The stems of hyacinth grown in the 9 ppt. and 18 ppt. solutions also began to lose its firmness as it began to wilt. At the end of the week only hyacinth in the 9 ppt. and 18 ppt. had undergone drastic changes. Most large and medium sized leaves began to shrivel and the leaves showed signs of unhealthiness. Signs of stress were even apparent in the younger shots. The tips of the fresh leaves were now beginning to wilt and showing indication of necrosis. Longer exposure to salt would have probably resulted in the death of the hyacinth plant.

Hyacinth raised in salinities higher than 9 ppt. were the only tanks that undergone major color change. The hyacinth grown in salinities less than 4.5 ppt. showed no signs of color change. All of the hyacinth began with a similar healthy green color. At the end of the week approximately twenty percent of the hyacinth from 18 ppt. had changed color to a paler shade. Ten percent of plants from 9 ppt. had changed color as well. Change only occurred in treatments with salinities higher than 9 ppt. Water hyacinth can tolerate salinities up to 9 ppt. After that point, the hyacinth begins to undergo serious discoloration and loses its structure. It is possible for hyacinth to ride freshwater currents in the bay without incurring major damage to its own health. Under certain tides and weather conditions, a hyacinth plant may be able to float along a freshwater current less than 9 ppt. for approximately one week before necrosis or severe wilting occurs. If the currents from the San Joaquin River provide the right conditions for the water hyacinth to become mobile, hyacinth may begin to spread to other regions of the bay.

**Hyacinth Growth** High salinity greatly hinders the productivity of water hyacinth. Hyacinth grown in salinities greater than 9 ppt. had limited leaf growth. Salinity may be impeding the growth of new leaves. As the salinity concentrations increased, the amount of foliage growth generally decreased. The data gathered as well as the general trend suggests that salinities close to that of salt water, discourages growth and limits the size of the plants. Any hyacinth that gets carried away with a salt water current will most likely die if it were to ride the current for an extended amount of time.

The average changes in leaf length and width were similar in all six salinity concentrations. Both leaf length and width had a negative relationship with salinity, but salinity does not appear to have a large impact on leaf size. There was no significant difference between any of the salinity concentrations which implies that leaf length and width may not be greatly impacted by salinity. However, it was observed that leaves that were in contact with the water did not shrink as much as those that were exposed above the surface. Leaves above the surface experienced wilting but were able to recuperate and expand after submerging the leaf in water. Even hyacinth grown in salinities greater than 9 ppt. experienced severe wilting and were still capable of returning to its fully hydrated state. There were signs of some damage such as discoloration and some permanent folds on the leaf, but the hyacinth still survived. Contact with water rejuvenated and restored the leaf. If the leaf were to remain above the surface, it may have eventually wilted off due to dehydration. If leaves were to not come into contact with water over a week, salinity may actually damage the leaf to the point where it can no longer hydrate itself. Any hyacinth that washes up on the shore or another wetland may actually perish if it does not come into contact with freshwater. Hyacinth may be able to move to a new location, but if there is no freshwater available, the hyacinth will continue to dehydrate and eventually perish.

**Hyacinth Sizes** Analysis of leaf number revealed that smaller hyacinth plants were the most affected by salinity when compared to medium and large sized plants. Since most of the differences were found in the smaller plants the data suggests that smaller plants are more susceptible to salinity levels. Medium and large sized hyacinth only showed two differences each. The lack of detectable differences implies that large and medium sized plants are not affected as much as the smaller plants. The medium and large hyacinth experienced more new growth in comparison to the smaller hyacinth.

The large and medium hyacinth may be more salt tolerant than the younger smaller plants. Smaller hyacinth was more susceptible to salinity than the medium or larger plants. Younger plants underwent more change than larger plants which implies that salinity affects younger plants more drastically. Since hyacinth is more susceptible to high salinities when it is younger, applying methods of controls may be the most useful during earlier stages of development. Penfound (1948) found that hyacinth begins its growing season around March, and does not reach its maximum growth until May. Treatments applied during this time period would be the most effective because of hyacinth's susceptibility to salinity. Once hyacinth reaches its

maximum growth, hyacinth will not be as vulnerable to treatments. Control methods applied after May may not be as effective as methods applied before May.

**Future Studies** In the study conducted by De Casabianca, biomass was the only parameter that they measured (De Casabianca and Laugier, 1995). The study of biomass could have been flawed because as hyacinth is placed in higher salinity solutions they are more likely to lose water to its surroundings. They assumed that five percent of wet weight is equal to the dry weight. This may be a bad assumption because a stronger salt solution would cause a plant to lose more water and therefore weigh less. The change in leaf number and leaf size are probably better for the measurement of growth. Future studies should incorporate leaf counts and measurements as well as modified biomass techniques. The biomass assumption can be made at the initial weighing of the hyacinth, but only the final weight at the end of the experiment should be taken. The hyacinth should be placed in an oven and dehydrated then weighed. This method would be more appropriate than making daily assumptions on total biomass.

A possible flaw in the design of this experiment was the selection of the largest and most mature leaf as the leaf to observe. Since the leaf selected was already mature, there would be little room for growth. During the experiment it was noted that younger shoots would experience faster growth rates than the larger leaves. It may be possible that since the larger leaves were closer to maturity, more of the resource and nutrients were focused on developing the younger leaves. Some of the factors that could be improved upon in future studies would be the selection of a younger medium sized leaf rather than selecting the largest leaf as the leaves to observe and measure. Medium sized leaves allow for more growth and are more representative of the average leaf condition.

**Conclusion** The mobility of hyacinth from the Antioch, C.A. is still limited by the saline conditions of the San Francisco Bay. The fresh water currents in the Bay and the water hyacinth's survivability still pose a threat to other wetlands in the bay. A hyacinth plant would need to survive the journey along the freshwater current and then find a suitable place for growth before it can successfully invade a new environment.

The results from this experiment show that the water hyacinth found in Antioch, C.A. are just as tolerant of salt water as those found in Louisiana and France. Experiments carried out in Louisiana and France revealed that hyacinth found in those regions were tolerant of salt water conditions up to the mesosaline range (9 ppt.-18 ppt.). Research conducted by Penfound (1948)

and De Casabianca (1995) show that hyacinth would only tolerate salinities less than 10 ppt. The results from my experiment confirm the findings by Penfound and De Casabianca. From this study, we were able to confirm the limitations of water hyacinth's survivability in salt water conditions. Water hyacinths found near the San Joaquin River are as vulnerable to brackish water as those found in Louisiana and France.

Nevertheless, the results of my study have important implications in the treatment of water hyacinth infested areas. My study did reveal that smaller plants are more vulnerable to salt water conditions than larger plants. The practice of shredding hyacinth in conjunction with a dry and drought seasons between March and May could be an effective way of reducing the prevalence of water hyacinth. Continual shredding of hyacinth before dry seasons may be a possible way to control and limit the growth of hyacinth.

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## Appendix A

### 1/2 STRENGTH "HOAGLAND'S" SOLUTION CONSTITUENT CONCENTRATIONS

#### MACRONUTRIENTS

CHEMICAL	CONCENTRATE IN BARRELS		FINAL CONCENTRATION WHEN APPLIED TO PLANTS	
	g/L	g/L	mol/L	
<b><u>Solution #1 - 1:100 barrel*</u></b>				
1a. $\text{NH}_4\text{NO}_3$ - Ammonium nitrate	5.284	0.053		$1.26 \times 10^{-3} \text{ M}$
1b. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ - Calcium nitrate	62.087	0.620		$5.09 \times 10^{-3} \text{ M}$
1c. Iron chelate - Ciba-Geigy 138 Fe Sequestrene (6% Fe)	0.0538 Fe	$5.38 \times 10^{-4}$ Fe		$2.07 \times 10^{-5} \text{ M Fe}$
or " " 330 Fe Sequestrene (10% Fe)	0.0528 Fe	$5.28 \times 10^{-4}$ Fe		$2.03 \times 10^{-5} \text{ M Fe}$

CHEMICAL	CONCENTRATE IN BARRELS		FINAL CONCENTRATION WHEN APPLIED TO PLANTS	
	g/L	g/L	mol/L	
<b><u>Solution #2 - 1:200 barrel*</u></b>				
2a. $\text{KNO}_3$ - Potassium nitrate	68.692	0.343		$6.86 \times 10^{-3} \text{ M}$
2b. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - Magnesium sulfate	47.556	0.238		$1.83 \times 10^{-3} \text{ M}$
2c. $\text{KH}_2\text{PO}_4$ - Monopotassium phosphate	13.210	0.066		$9.71 \times 10^{-4} \text{ M}$
2d. Micronutrients (details follow)				

#### MICRONUTRIENTS

CHEMICAL	8 L. SOL'N	BARREL	FINAL CONCENTRATION WHEN APPLIED TO PLANTS	
	g/L	g/L	g/L	mol/L
<b><u>Combined with Solution #2 - 1:200 barrel</u></b>				
3a. $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ - Magnesium chloride	5.083	0.134	0.000670	$6.32 \times 10^{-6} \text{ M}$
or $\text{NaCl} \cdot 6\text{H}_2\text{O}$ - Sodium chloride	2.920	0.077	0.000385	$4.38 \times 10^{-6} \text{ M}$
3b. $\text{H}_3\text{BO}_3$ - Boric acid	2.863	0.076	0.000380	$1.19 \times 10^{-5} \text{ M}$
3c. $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ - Manganese sulfate	1.525	0.040	0.000200	$2.41 \times 10^{-6} \text{ M}$
3d. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ - Zinc sulfate	0.223	0.006	0.000030	$2.03 \times 10^{-7} \text{ M}$
3e. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ - Copper sulfate	0.079	0.002	0.000010	$7.90 \times 10^{-8} \text{ M}$
3f. $\text{MoO}_3$ - Molybdenum oxide	0.018	0.0005	0.000003	$4.50 \times 10^{-8} \text{ M}$

## Appendix B.1

### Size comparison- Leaf Number Versus Salinity

#### 1. Small Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-0.78752	-0.45419	-0.70419	-0.2419	-0.3752	0.08748
0.5 ppt.	-0.45419	-0.78752	-0.37085	0.12915	0.29581	0.42081
2.25 ppt.	-0.70419	-0.37085	-0.78752	-0.28752	-0.12085	0.00415
4.5 ppt.	-0.20419	0.12915	-0.28752	-0.78752	-0.62085	-0.49585
9 ppt.	-0.03752	0.29581	-0.12085	-0.62085	-0.78752	-0.066252
18 ppt.	0.08748	0.52081	0.00415	-0.49585	-0.66252	-0.78752

#### 2. Medium Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-1.6348	-1.4681	-1.1348	-0.8015	0.3652	0.1985
0.5 ppt.	-1.4681	-1.6348	-1.3015	-0.9681	0.1985	0.0319
2.25 ppt.	-1.1348	-1.3015	-1.6348	-1.3015	-0.1348	-0.0319
4.5 ppt.	-0.8015	-0.9681	-1.3015	-1.6348	-0.4681	-0.6348
9 ppt.	0.3652	0.1985	-0.1348	-0.4681	-1.6348	-1.4681
18 ppt.	0.1985	0.0319	-0.3015	-0.6348	-1.4681	-1.6348

#### 3. Large Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-2.4064	-1.9064	-2.2397	-1.5730	-0.5730	0.2603
0.5 ppt.	-1.9064	-2.4064	-2.0730	-2.0730	-1.0730	-0.2397
2.25 ppt.	-2.2397	-2.0730	-2.4064	-1.7397	-0.7397	0.0936
4.5 ppt.	-1.5730	-2.0730	-1.7397	-2.4064	-1.4064	-0.5730
9 ppt.	-0.5730	-1.0730	-0.7397	-1.4064	-2.4064	-1.5730
18 ppt.	0.2603	-0.2397	0.0936	-0.5730	-1.5730	-2.4064

Positive values show means that are significantly different

## Appendix B.2

### Size comparison- Leaf Length Versus Salinity

#### 1. Small Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-0.71040	-0.52290	-0.36665	0.03332	0.34168	0.12293
0.5 ppt.	-0.52290	-0.71040	-0.55415	-0.22082	0.15418	-0.06457
2.25 ppt.	-0.36665	-0.55415	-0.71040	-0.37707	-0.00207	-0.22082
4.5 ppt.	-0.03332	-0.22082	-0.37707	-0.71040	-0.33540	-0.55415
9 ppt.	0.34168	0.15418	-0.00207	-0.33540	-0.71040	-0.49165
18 ppt.	0.12293	-0.06457	-0.22082	-0.55415	-0.49165	-0.71040

#### 2. Medium Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-2.8389	-1.6306	-2.5889	-1.8806	-2.3389	-2.6306
0.5 ppt.	-1.6306	-2.8389	-1.3806	-0.6723	-1.1306	-1.8389
2.25 ppt.	-2.5889	-1.3806	-2.8389	-2.1306	-2.5889	-2.3806
4.5 ppt.	-1.8806	-0.6723	-2.1306	-2.8389	-2.3806	-1.6723
9 ppt.	-2.3389	-1.1306	-2.5889	-2.3806	-2.8389	-2.1306
18 ppt.	-2.6306	-1.8389	-2.3806	-1.6723	-2.1306	-2.8389

#### 3. Large Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-4.7854	-2.6938	-4.6938	-4.0688	-4.3354	-1.8604
0.5 ppt.	-2.6938	-4.7854	-2.7854	-3.4104	-2.2438	-3.9521
2.25 ppt.	-4.6938	-2.75854	-4.7854	-4.1604	-4.2438	-1.9521
4.5 ppt.	-4.0688	-3.4104	-4.1604	-4.7854	-3.6188	-2.5771
9 ppt.	-4.3354	-2.2438	-4.2438	-3.6188	-4.7854	-1.4104
18 ppt.	-1.8604	-3.9521	-1.9521	-2.5771	-1.4104	-4.7854

Positive values show means that are significantly different

## Appendix B.3

### Size comparison- Leaf Width Versus Salinity

#### 1. Small Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-0.37117	-0.31908	-0.36075	-0.14908	-0.21492	-0.25867
0.5 ppt.	-0.31908	-0.37117	-0.32950	-0.24617	-0.26700	-0.31075
2.25 ppt.	-0.36075	-0.32950	-0.37117	-0.20450	-0.22533	-0.26908
4.5 ppt.	-0.19408	-0.24617	-0.20450	-0.37117	-0.35033	-0.30658
9 ppt.	-0.21492	-0.26700	-0.22533	-0.35033	-0.37117	-0.32742
-0.25867	-0.31075	-0.26908	-0.30658	-0.30658	-0.32742	-0.37117

#### 2. Medium Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-1.0608	-0.7692	-0.9775	-0.7275	-0.7692	-0.2692
0.5 ppt.	-0.7692	-1.0608	-0.6858	-1.0192	-1.0608	-0.5608
2.25 ppt.	-0.9775	-0.6858	-1.0608	-0.6442	-0.6858	-0.1858
4.5 ppt.	-0.7275	-1.0192	-0.6442	-1.0608	-1.0192	-0.6025
9 ppt.	-0.7692	-1.0608	-0.6858	-1.0192	-1.0608	-0.5608
18 ppt.	-0.2692	-0.5608	-0.1858	-0.6025	-0.5608	-1.0608

#### 3. Large Plants

	0	0.5 ppt.	2.25 ppt.	4.5 ppt.	9 ppt.	18 ppt.
0 ppt.	-1.0054	-0.9220	-0.9220	-0.7554	-0.1304	-0.9637
0.5 ppt.	-0.9220	-1.0054	-0.8387	-0.8387	-0.2137	-0.9637
2.25 ppt.	-0.9220	-0.8387	-1.0054	-0.6720	-0.0470	-0.8804
4.5 ppt.	-0.7554	-0.8387	-0.6720	-1.0054	-0.3804	-0.7970
9 ppt.	-0.1304	-0.2137	-0.0470	-0.3804	-1.0054	-0.1720
18 ppt.	-0.9637	-0.9637	-0.8804	-0.7970	-0.1720	-1.0054

Positive values show means that are significantly different