Water Hyacinth Adaptation to Higher Saline Levels

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Abstract One of the world's most noxious pests, water hyacinth (Eichhornia crassipes) causes many ecological, physical, and economical problems. Water hyacinth growth occludes waterways, lowers the amount of sunlight available to other aquatic organisms, and reduces aquatic activities. This plant shows the amazing ability to live in toxic waters and tolerate extremes of nutrient load, pH, and temperature. The reach of water hyacinth is limited by high saline levels. Water hyacinth is unable to establish itself and proliferate at saline levels greater that 6 ppt. However, previous research believes that the water hyacinth will soon adapt to life at a higher saline level. Some researchers believe this process is already underway. I believe there exists a new ecotype that morphologically looks like any traditional water hyacinth plant, but is genetically adapted to aid the plant's ability to grow increasingly salt tolerant. This experiment took place in a greenhouse, and plants in an early growth stage were placed in either a nutrient solution (control) or a salt solution (test). The saline levels tested were 5, 6, and 7 ppt. For each salt level, the plants were tested for four different lengths of time; 7, 14, 21, and 28 days. Results indicate that beyond the oligosaline level (>5 ppt) plants will experience damage and the mesosaline level (>7 ppt) will impede growth. These results will be useful in assessing the possibility of a new ecotype as well as the use of salinity as biological prevention for reducing water hyacinth growth.

Introduction

Water hyacinth (*Eichhornia crassipes*) is a perennial aquatic plant that originated in the Amazon Basin, and is presently found in North America, Africa, Australia, China, India, and Japan. It is a very resilient plant with unique structure, able to endure a wide range of water quality and reproduce using more than one method. (Tag El Seed, 1975; Julien et al., 2001). Water hyacinth grows quickly and has two main methods of reproduction. In the first method is vegetative, the plants float on the surface of the water in mats and extend rhizomeswith a new plantlets already on the end, the mat often becomes so dense it shades any other submerged species. The second method of reproduction is through flowering; the hyacinth is self-fertilizing and can also be insect and bird-pollinated. Each hyacinth rosette produces up to 12,000 seeds viable for at least twenty years (Julien et al., 2001; Barrett, 1980). These factors enable the water hyacinth to out-compete many native plant species, and it has rapidly emerged as one of the world's most noxious pest. It was introduced to California in the early 1900's as an ornamental plant and has become a common and vexing problem in many California water bodies including the San Francisco Bay Delta area.

Water hyacinth causes physical obstruction of water ways, alters the food web, lowers overall pH, and out-competes more sensitive native species (Masifwa, et. al. 2001). If this new ecotype begins to invade the San Francisco Bay area, not only will it disrupt the entire waterway, but also cause a number of other socio-economic problems. The area is used for a number of recreational activities. Water hyacinth could cause the water body to become so occluded that it would retain no recreational value (Penfound and Earle, 1948). Parts of the bay used for a costly hydropower generation could soon be rendered useless.

Water hyacinth has shown the ability to withstand extremes of nutrient levels, temperature, pH, and toxic waters. Salinity is one parameter that water hyacinth has shown sensitivity to. At 8 ppt water hyacinth experience compete necrosis (Muramoto and Oki, 1988). However, there is concern and increased studies believing that hyacinth is becoming more salt tolerant. (De Casabianca and Laugier, 1995; Penfound and Earle, 1948). Penfound and Earle (1948) found water hyacinth from the Mississippi River could not survive 8 days in a salt solution at 6ppt. De Casabianca and Laugier (1995) compared their results to the ones of Penfound and Earle and found a progressive adaptation of water hyacinth to salinity. They varied salt tolerance in a population of water hyacinth from France. They discovered that at 6 ppt, the plants remained viable, and it wasn't until 9 ppt when the plants' damages became irreversible. This suggests a geographic variation in tolerance and or physiology to salinity. Should this new ecotype enter and survive in the San Joaquin River area, we could soon see a new ecotype invading areas of the Delta originally thought to be too saline for survival (Tung, 2004).

Using experimental design similar to that proposed by past ES 196 student Jack Cheng, I am looking for the possible existence of a new ecotype of water hyacinth. Cheng compared his own results to the previous results found on salinity tolerance of hyacinth populations in 1948 by Penfound and Earle and the 1995 study conducted by De Casabianca and Laugier and concluded that the California population was no different from others. I will examine and compare two populations of water hyacinth from California and assess how my results compare to previous studies. My two populations stemmed from California's San Francisco Bay Delta region (Fig. 1). The salt concentrations for population one varies between freshwater (0 ppt) and oligosaline (3 ppt). The saline variance, found in the Tidal Marsh of Dow Wetlands, is due to an influx of freshwater during high tide from the San Joaquin River. The other hyacinth population is found in a freshwater (0 ppt) tributary of the San Joaquin River in an area called Stones Lake. I hypothesize that the population with a varying saline concentration (Dow Wetlands) because of the fluctuation, is already adapting to higher saline levels and therefore will be able to tolerate higher salinity levels. In addition to testing for a possible ecotype and I will help develop a biological control method using a control population that is similar geographically and chronologically

The data from this experiment will shed light on the possible development of a new ecotype and the usability of salt as a biological control method. While there are already numerous methods of controlling the invasive plant (Wade, 1990) this study can be a stepping stone for further testing of biological methods of controlling hyacinth populations. If the possibility of a new ecotype is discovered, new initiatives would need to be taken to examine the hyacinth genetically and stop the possible further invasion of the water hyacinth.

Methods

Study sites Approximately 500 plants were collected from Stones Lake near Sacramento (Fig. 1), and another 500 from the Dow Wetlands in Antioch (Fig. 1), for a total of 1000 plants. Both populations stem from the San Francisco Bay Delta. All the water hyacinth was brought to the greenhouse on Oxford and placed in a nutrient solution for one week to counteract any shock that may have occurred during transfer. The plants are hand collected from each site placed in bins with water from the site then driven from each of these locales to Berkeley where the drive ranges from 45 minutes to and hour and a half depending on traffic. Then I will analyze and observe the effect of salt concentrations of 5, 6, and 7 ppt on the two different populations. I will collect data for each plant pertaining to its biomass, chlorophyll level, and petiole length.

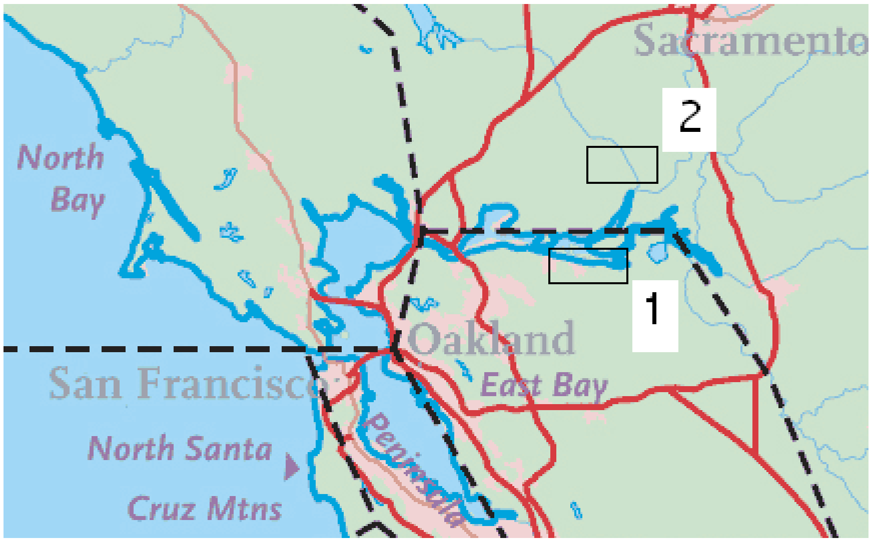

Figure 1. The location of the two study sites (in the rectangles) in reference to the overall bay area as well as each other. Dow's Wetland Preserve (1) and Stones Lake (2) Source: http://www.virtualparks.org/states/california.html

Preliminary trials Preliminary data were collected during spring and summer of 2004. The preliminary work was done to ensure that testing would be done at appropriate salinity levels and that the experiment could be executed in the time frame allotted. These data will be excluded from the final analysis.

Water Hyacinth Collection Following the method employed by Cheng (2004), a total of 200-300 individuals were collected from each of the two sites, Dow Wetlands and Stone Lake Park (Fig 1). Although individual plants can grow to over 150 cm, younger plants ranging from 4 cm to 30 cm were selected, to ensure that they were being tested during the early stages of growth. Plants were collected in plastic bucket (by researchers wearing waders) and driven 1-2 hours to the greenhouse. The plants were then placed in a nutrient solution for a two week recuperation period.

Parameters Weekly data were collected for each plant. This data included petiole length, percentage of dead leaves, wet weight, and chlorophyll level. These are all parameters indicative of plant health. In previous studies leaf length was measured over petiole length, however because the leaves had a tendency to mature too quickly while petiole would progressively mature, therefore changes in growth was best monitored through petiole measurements. The petiole length was measured in centimeters with a flexible ruler from the base of the stem to where the leaf begins (Fig. 2). The percentage of dead leaves is found by dividing the number of

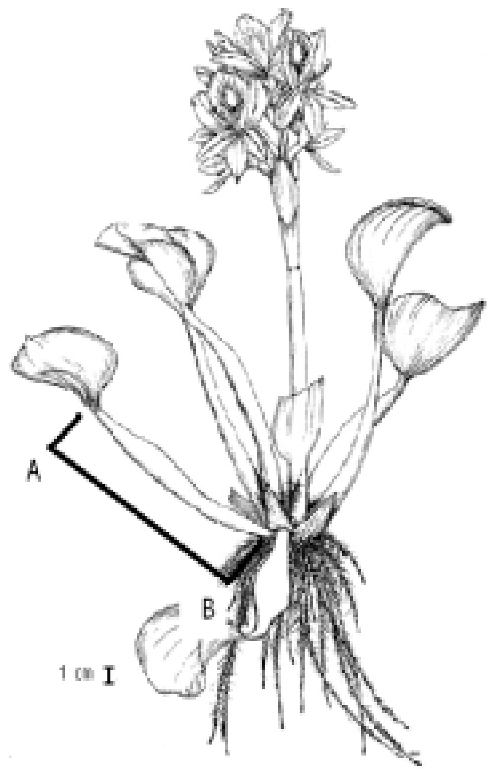

Figure 2. taken from
http://www.homestead.com/fapms/main.html
(A) where the leaf begins (B) the base of the stem. From A to B is the total length measured

dead leaves into the total number of leaves. Dead leaves were determined by the chlorophyll level and discretion of the researcher: brown coloration and softened, dehydrated stems were indicators of dead leaves. The dual method of determining whether the leaf is dead is necessary because of varying levels of chlorophyll throughout the leaf. Using a non-destructive SPAD-502 chlorophyll meter by GENEQ Inc. I will be measuring the amount of chlorophyll before, during, and after treatment. The amount of chlorophyll recorded was used to gauge the plant's ability to photosynthesize. This will be useful in determining whether the plant is viable or still growing. The wet weight is measured by removing the plant for three seconds from its solution and then weighing it in grams. The three second standardization is necessary because for each plant it would be too time consuming to wait for all the excess water to drip off each plant. The wet weight of the plant allows me to measure the gain or loss in biomass. These parameters are used to gauge the overall growth and photosynthetic abilities of each plant.

Experimental Design This experiment was conducted for three time trials at each of three different salinity levels: 5ppt, 6ppt, and 7ppt (Table 1). These concentrations were chosen based upon previous work (Cheng 2004, De Casabianca and Laugier 1995), which found that water hyacinth were unable to establish themselves and spread at around 5-6 ppt and underwent total necrosis at 8-9ppt.

Table 1. The experimental design set up of sixteen bins at one salinity level, to be repeated twice for the other saline levels. X in the first column corresponds to the salt concentration, either 5, 6, or 7 ppt. Each time length includes one control group and three test replicates; A, B, and C.

Time Trials	**7 Days**	**14 Days**	**21 Days**	**28 Days**
Control (0 ppt)	● ● o o ● o	o ● o o ● ●	● o o ● ● o	o ● o ● ● o
Test A (x ppt)	● o o ● ● o	● ● o o o ●	o ● o ● ● o	o ● ● ● o o
Test B (x ppt)	● o o ● ● o	● o o ● ● o	o ● o o ● ●	● ● o o ● o
Test C (x ppt)	o ● ● ● o o	o ● o ● ● o	o ● o ● ● o	o ● o ● ● o
● = Dow Water Hyacinth O = Stones Lake Water Hyacinth				

Two trials began on October 6, 2004 for salinity levels of 5 ppt and 6 ppt. To avoid any confusion between the two populations, each plant was tagged in a dual color and number system to identify its origin. (Table 2) The plants would be exposed to the given salt concentration for different lengths of time: 7, 14, 21, and 28 days (Table 1). For every duration set, there were three test bins filled with 15 liters of salt solution and one control bin with 15 liters of nutrient solution and no salt. After the experimental plants were exposed to saline condition for the designated amount of time, they were reintroduced to nutrient solution for a two week rebound session. During this rebound session data collection and observation continued on a weekly basis. Trial two was conducted solely at 7ppt. The same process is repeated for this second trial. The experiment was split into two trials to be more time effective.

Statistical Analysis Following the work of Cheng (2004), I will be using the one way analysis of variance (ANOVA) to test the plant responses to each factor and their interactions. I will use the JMP Start Statistics software to perform the ANOVA test and also JMP as well as Microsoft Excel will be used to create a linear regression, displaying the means of the parameters in a graph which I will obtain an analysis from. Multiple linear regressions were performed on each parameter and crosses of all the parameters in order to look for possible correlations as well as confounding factors. These results will allow me examine the separate and interacting effects of each population of water hyacinth, varying time length of exposure, and exposure to the different saline levels.

Parameter Focus Due to time constraints and parameter reliability, I chose to analyze only wet weight. Previous research (De Casabianca and Laugier, 1995; Muramoto and Oki, 1988) also used weight as the sole measurement of plant health. Wet weight is the most reliable of the parameters measured. Within each plant there are large discrepancies between petiole lengths and chlorophyll levels, all which is encompassed in wet weight. With one plant there could be one healthy petiole while another which has been partially submerged has lost all rigor mortis. This ultimately affects the photosynthetic rate (chlorophyll level) because the plant has experienced a change in source-sink relationship, the chlorophyll will be allocated elsewhere giving a pseudo-higher reading of chlorophyll in leaves being measured (Kessler and Baldwin 2002). Wet weight in contrast, will include all these factors and will only be slightly affected by factors such as submersion.

I expect a counterintuitive trend in the increasing wet weight in relation to the amount of time exposed to a saline level. While the plant may be damaged during exposure time and appear to lose a substantial amount of weight, the plant actually gains weight. This increase in wet weight is due to the increased density of water being taken up by the plant as well as other osmotic factors (Muramoto and Oki, 1988)

Results

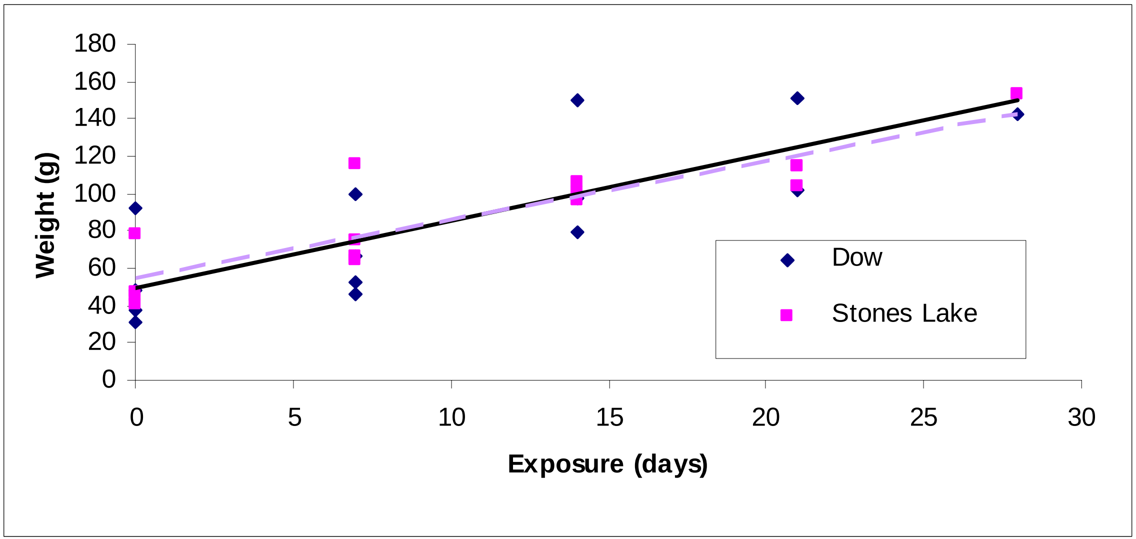

Figure 3 Trends for the control (0 ppt) of the Stones Lake hyacinth population (dotted line) and the Dow Wetlands population (continuous line). Plotted are the mean weights of the control replicates measured within a week.

Control For both the Dow Wetlands and the Stones Lake population, the wet weight of the individual plants increase linearly with time (Fig. 3). The slopes of the equations (Table 2) inclusive of standard error are the same. Wet weight and growth appear to be the same. The R squared value is lower for the Dow population, with exposure accounting for only 61% of the variation in wet weight as compared to the 76% as found in the Stones Lake population. There are significant differences between the means within both populations indicated by the p values which are lower by 0.05.

.**Table 2.** First three rows are the statistical results for the Dow population, the last three are for the Stones Lake population. Listed are the equations for the trend lines, R squared values, standard error, and p values.

Control – 0 ppt
(Dow) Weight = (49.6±10.9) + (3.6±0.82)Exposure
$R^2 = 0.61$
P = 0.0010
(Stones Lake) Weight = (60.0±2.7) + (3.1±0.2)Exposure
$R^2 = 0.\ 76$
P < 0.0001

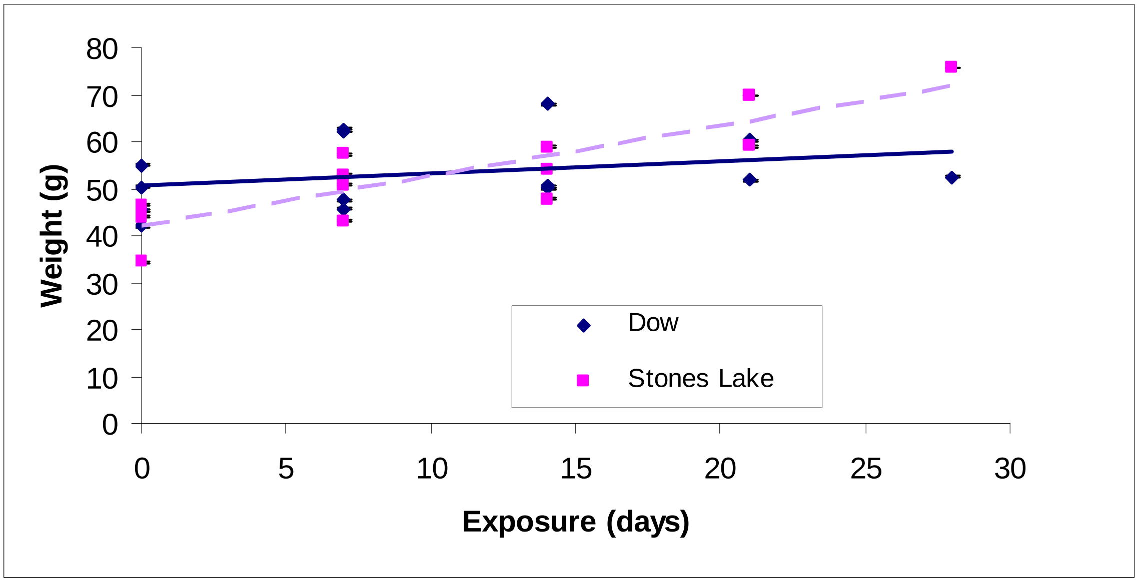

Figure 5 Trends for the Stones Lake hyacinth population (dotted line) and the Dow Wetlands population (continuous line) during the 5 ppt trial. Plotted are the mean weights for the 5 ppt replicates measured within a week.

5 ppt Once again the weight increases linearly with exposure to the saline water for both populations. The slope for the Stones Lake population is higher than the Dow Wetlands population (Fig. 5, Table 3). Both slopes are significantly lower than the slopes found from the control population. The R squared values is again lower for the Dow Wetlands population, and it is also much lower than the Dow population from the control (Table 3). The P value is significant only for the Stones Lake population.

Table 3. First three rows are the statistical results for the Dow population, the last three are for the Stones Lake population. Listed are the equations for the trend lines, R squared values, standard error, and p values.

Trial – 5 ppt
(Dow) Weight = (50.6±3.1) + (0.3±0.2)Exposure
$R^2 = 0.09$
P = 0.2918
(Stones Lake) Weight = (42.2±2.3) + (1.1±0.2)Exposure
$R^2 = 0.\ 76$
P < 0.0001

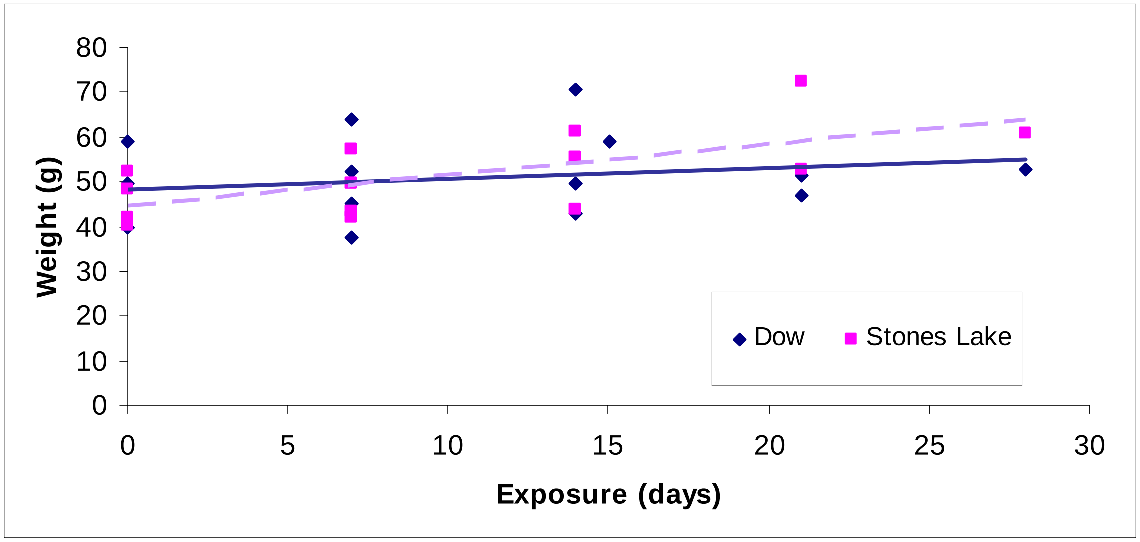

Figure 6 Trends for the Stones Lake hyacinth population (dotted line) and the Dow Wetlands population (continuous line) for the 6 ppt trial. Plotted are the mean weights for the 6 ppt replicates measured within a week.

6 ppt The weight for both populations increase linearly with exposure to the saline water. The slope is higher in the Stones lake population but the slopes between the two populations only differ slightly (Fig. 6, Table 4), not to the degree of difference found in the 5 ppt trial. Both slopes are significantly lower than the slopes found from the control population, but similar to the slopes found in the 5 ppt trial. The R squared values is again lower for the Dow Wetlands population, and it is also much lower than the Dow population from the control (Table 3). The P value is significant only for the Stones Lake population.

Table 4. First three rows are the statistical results for the Dow population, the last three are for the Stones Lake population. Listed are the equations for the trend lines, R squared values, standard error, and p values.

6 ppt
(Dow) Weight = (47.7±3.6) + (0.4±0.3) Exposure
R^2 = 0.14
P = 0.19
(Stones Lake) Weight = (44.8±3.0) + (0.9±0.2)Exposure
R^2 = 0.76
P = 0.0106

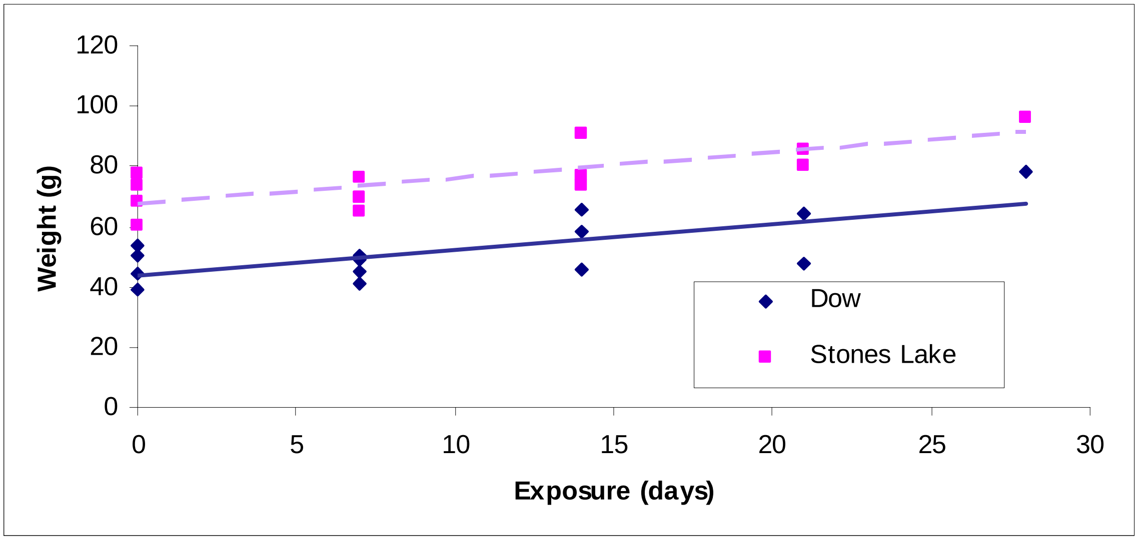

Figure 7 Trends for the Stones Lake hyacinth population (dotted line) and the Dow Wetlands population (continuous line) for the 7 ppt trial. Plotted are the mean weights for the 7 ppt replicates measured within a week.

7 ppt The weight for both populations increase linearly with exposure to the saline water. The slope inclusive of standard error is the same for both populations (Fig. 6, Table 5). The intercept is higher in the Stones lake population due to heavier starting plants (Fig. 6). Both slopes are significantly lower than the slopes found from the control population. The R squared values do not differ to the degree that it does in the two previous trials (5 and 6 ppt) however the Dow Wetlands R squared value is again lower and slightly lower than the Dow population from the control (Table 3). The P value is significant for both populations.

Table 5. First three rows are the statistical results for the Dow population, the last three are for the Stones Lake population. Listed are the equations for the trend lines, R squared values, standard error, and p values.

7 ppt
(Dow) Weight = (43.9±3.3) + (0.6±0.3)Exposure
R^2 = 0.50
P = 0.006
(Stones Lake) Weight = (67.4±2.7) + (0.7±0.2)Exposure
R^2 = 0.59
P = 0.0013

Factors Affecting Overall Weight After performing a multiple linear regression on all the factors and crosses of all the factors, I found the overall R squared value = 0.62**.** The factors in

Table 6 are listed in order starting with the parameters with the most leverage, to the least significant factors. The sign indicates whether the slope of the relationship is positive or negative. There is a negative value for salinity because the mean weights for the control population are much heavier than the trial plants. After eliminating control the slope became positive. If the P value is significant it had some leverage on the variation found in wet weight.

Table 6. List of parameters and interactions (first column) and explanation of any contribution to the change in wet weight (last three columns)

Factor	Sign	Significant	P value
Salinity*Exposure	+	Yes	<0.0001
Salinity	–	Yes	<0.0001
Exposure	+	Yes	<0.0001
Population	+	Yes	0.045
Salinity*Population	+	No	0.10
Exposure*Population	0	No	0.64
Exposure*Salinity*Population	0	No	0.45

Discussion

The establishment, proliferation, and effect of water hyacinth on the Dow Wetlands have been immense. This may be due to the potential new ecotype that has been found within these results. When comparing the slopes of the equations for 5, 6, and 7 ppt, the Dow trend lines are very similar to one another. In contrast, the trend lines for the Stones lake population are found to increase and then merge back to a similar slope value as Dow. This indicates that while both populations are affected similarly, the Stones Lake water hyacinth population is slightly more sensitive to changes in salinity. This trend is also seen in the R squared values where, in the control population they start off as very similar values, then the R squared value is much lower for Dow than it is for Stones Lake. At 5 ppt, only about 14% of the variation in wet weight is due to the exposure in the saline waters, while in the Stones lake population exposure accounts for 76% of the change in wet weight. It isn't until 7 ppt, when both plants are beginning to show equal vulnerability to the saline level.

As further evidence to this potential ecotype, both of these populations have plants that continue to grow beyond 8 days at the saline level of 6ppt. Subsequently, the plants also remain viable at 7 ppt. This is in contradiction to the original belief of the toxicity level at 6 ppt for 8 days (Penfound and Earle, 1948). However these results are similar to the more recent results found in the 1995 study performed by De Casabianca and Laugier, where plants remained viable at 6ppt, but underwent total necrosis at 9 ppt. Because some of the plants of this experiment managed to produce new offspring, this study has found plants capable of growing beyond the believed toxicity level. While all this is proof of a new ecotype, I cannot determine whether or not the differences I found are due to other factors such as the controlled environment the experiment was conducted in, or perhaps other physiological factors I didn't account for.

Through mechanisms at the cell, tissue, and organ level in coordination with salt stress, water hyacinth can adapt to salinity stress. Some of these mechanisms require a great deal of anatomical organization to avoid excess ion concentrations (Greenway and Munns, 1980). So while I may have found sufficient evidence pointing to a potential new ecotype, there would need to be a much more in-depth look at each plant to really discover whether or not the ability to tolerate the higher saline level is indeed due to genetics.

Potential Pitfalls There are complications with the proper collection of regeneration data. Some plants chosen for experimentation have large, expansive root systems which contribute a great deal to the wet weight of the plant. During the plant's time in the saline solution, some plants will lose a large portion of their roots systems, affecting the overall wet weight. In these plants we find new growth and regeneration, but this growth may not completely compensate for weight of the lost roots. Some of the wet weight data will not accurately portray a resuscitated plant regaining its health. In addition to factors affecting wet weight, water hyacinth can be self-fertilized and vegetative reproductive, is also insect and bird pollinated. So in nature, the increase of wet weight could have been more dramatic with the increased abilities of reproduction. However, in the controlled environment of the greenhouse, this factor affecting wet weight is not taken into account.

During our first trial for 5 and 6 ppt, a large percentage of Dow plants were injured during the transfer into the greenhouse. Many plants had bent or broken stems, as well as ripped leaves. This could contribute to a smaller percentage of successful Dow plants or could explain why the plants were not as dramatically different as were expected. However healthier plants were used for 7 ppt, so from there I can make conclusions about the results of Dow plants for 5 and 6 ppt.

Future Studies This experiment is a good stepping stone in the discovery of a new ecotype. I would suggest studying individual plants as well as the progeny of the plants, to look for genetic changes. Previous studies indicate that correlations are found between whole plants and salinity at the cellular level (Naik and Widholm, 1993) In addition to studying the progeny of the plant I would also examine the plant at the cellular level to look at what the change in osmotic pressure does to the cell. Also to ensure the changes aren't due to the change in environment, I recommend simulating the experiment in the actual ecosystem. Stabilize bins within Dow's Tidal Marsh and keep bins filled with the water from the area and add salt as needed. This method will allow for all methods of reproduction including insect and bird-pollination and also this will account for season and temperature variation.

Another potential use of these results includes looking into new potential biological control method of water hyacinth invasion. At the Dow Wetlands the area overrun with hyacinth has a varying salinity level due to tidal fluctuations. If the levee separating the Dow Wetlands from it's freshwater source (the San Joaquin river), through evaporation you can eventually raise the salinity level to 8-9 ppt killing off the hyacinth. However, the new research would have to involve the raised salinity level's effect on the surrounding ecosystem, and the other organisms in the water body besides hyacinth.

Conclusions The ocean, with salinity levels upwards of 20 ppt, still remains a distant possibility as a habitat for water hyacinth. However, the San Francisco Bay Delta region is vulnerable to hyacinth invasion. These studies show that in comparison with previous studies, overall the water hyacinth of both populations show an increased tolerance of salinity. The difference in rate of growth in relation to salinity and exposure display results which agree with my hypothesis, that there is potentially a more salt-tolerant ecotype in the San Francisco Bay Delta region. This possible ecotype will have the increased ability to survive and adapt to brackish water conditions, posing a threat to the entire delta ecosystem. However I cannot conclusively determine that this increased tolerance is due to genetics and advise further examination at the cellular level and the progeny to without a doubt determine if there exists a new ecotype.

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