Water Hyacinth: Assessing Recovery from Salinity Induced Stress

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Abstract   Water hyacinth, *Eichhornia crassipes,* recognized as one of the world's most noxious aquatic invasive species is currently invading the San Francisco Bay Delta. Although water hyacinth can tolerate a wide range of environmental conditions, salinity is known to limit its spread in estuaries and tidally influenced streams suggesting that a better understanding of water hyacinth's tolerance to salinity may facilitate its control. While extensive information exists on the salinity levels needed for water hyacinth mortality (6.0-8.0 ‰), virtually no information exists on water hyacinth recovery when the plant experiences temporary exposure to lethal levels of salinity. This study determined the recovery ability of water hyacinth as a function of three factors: exposure duration, salinity level, and recovery time. Water hyacinth were exposed to salinity levels of 5.0 ‰, 6.0 ‰, and 7.0 ‰ for 7, 14, 21, and 28 day periods. After exposure the water hyacinth were allowed 7 and 14 days to recover in non-saline environments. Wet plant weight and leaf chlorophyll were used as indictors of water hyacinth health. The multiple linear regression of weight on salinity, exposure duration, recovery, and interactions explained 77% of the variability in weight and was highly significant ($p < 0.0001$). Recovery did not significantly affect leaf chlorophyll. Recovery rates were greatest for water hyacinth experiencing the shortest exposure times at the lowest salinity levels. This study determined that the base level needed for water hyacinth mortality where no recovery would occur is an exposure of 21 days at 7 ppt salinity.

Introduction

Recognized as one of the world's most noxious aquatic weeds, *Eichhornia crassipes* (Mart) Solms is native to the Amazon River Basin and now occupies numerous new tropical and sub-tropical habitats throughout the world (Penfound and Earle 1948, Barret 1980). Known more commonly as water hyacinth, this species is recognized by the California Invasive Plant Council (Cal-IPC) as a "most invasive wildland pest plant." Water hyacinth has an incredible rate of growth: populations can double their numbers in two weeks through vegetative reproduction alone (Penfound and Earle 1948, Barret 1980, Harley 1990). Through water hyacinth's engulfing presence, large amounts of sunlight are blocked, thorough oxygen exchange is prevented and dissolved oxygen levels drop, the food web is altered, habitat for water fowl and other organisms is either destroyed or changed, and the biological diversity of the invaded area is greatly reduced (Denny et al. 2001, Brendonck et al. 2003). Water hyacinth can be a problem economically as it negatively affects fisheries, slows or even prevents water traffic, impedes irrigation, reduces the water supply, obstructs water ways, and slows hydropower generation (Denny et al. 2001, Brendonck et al. 2003).

Despite water hyacinth's ability to survive in wide ranges of sunlight, pH, and water supply (Tag El Seed 1975), elevated levels of salinity inhibit its health and vitality. Salinity levels become lethal for water hyacinth between 6.0 and 8.0 ‰ with no individuals able to persist concentrations greater than 8.0 ‰ (Muramoto and Oki 1988). While there is adequate information on the fatal levels of salinity for the water hyacinth when the exposure duration is indefinite (Earle and Penfound 1948, Haller et al. 1974, Muramoto and Oki 1988, Casabianca and Laugier 1995), very few studies have provided information on the plants recovery abilities from salinity stress after temporary exposures to these salinity levels. Although Casabianca and Laugier (1995) looked at water hyacinth recovery from saline stress in a petroliferous wastewater medium, information on recovery in naturally saline environments is still lacking. The current project investigates water hyacinth's performance given 7 and 14 days to recover in a non-saline solution after 7, 14, 21, and 28 days of continuous exposure to salinity solutions of 5.0 ‰, 6.0 ‰, and 7.0 ‰.

An understanding of the combination of factors (exposure duration, concentration, and recovery time) that lead to water hyacinth death will be of value in the development of plausible control methods in environments where salinity levels can be manipulated. In the San Francisco

Bay Delta salinity varies between 0.1 ‰ and 33 ‰ due to tidal, seasonal, and anthropogenic influences. These techniques may be used separately or in combination with other control methods that are currently in use, such as chemical spraying, biological control using a weevil, or mechanical removal. Further study will need to be done in order to assess the effects of higher salinity concentrations on an environment and determine which combination of control methods will maximize hyacinth control while minimizing ecosystem damage.

Methods

To increase population-level variability within the test specimens, the water hyacinth plants that were used in this experiment were collected from two separate populations: the Dow Wetlands in Antioch, CA and the Stone Lakes National Wildlife Refuge in Elk Grove, CA (Fig. 1). Only pre-flowering daughter rhizomes (i.e. young individuals arising from vegetative reproduction) were collected to ensure that all specimens were in roughly the same developmental stage. Plants for the 5.0 ‰ and 6.0 ‰ exposures were collected on September 15, 2004 and plants for 7.0 ‰ exposure were collected on October 29, 2004. The plants were collected in buckets and immediately transferred (one hour drive from Antioch and a two hour drive from Stone Lakes) to a greenhouse at the University of California, Berkeley's Oxford tract where the experiment was conducted.
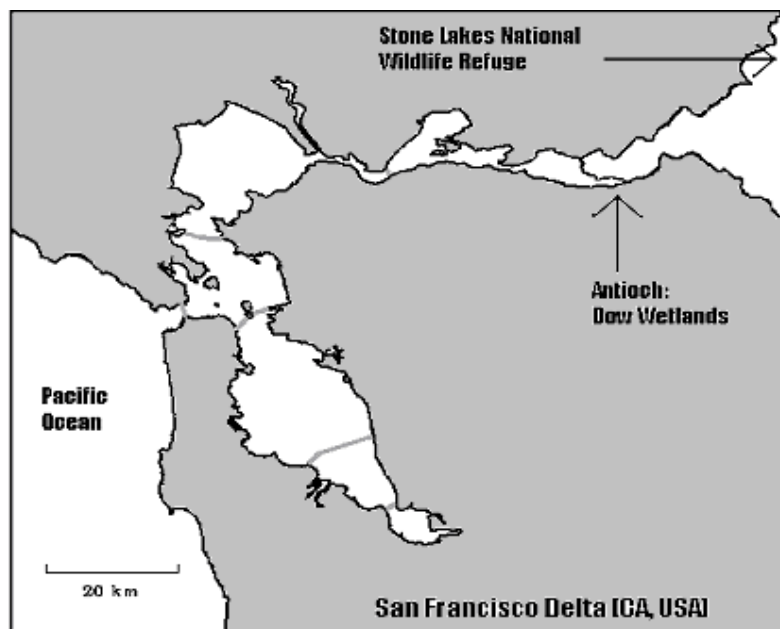

Figure 1. The location of the two water hyacinth collection sites

Once in the greenhouse, all water hyacinth were placed in nutrient solution for an initial seven day stabilization period to ensure that all plants started the experiment in similar health conditions. Three plants from each location (n=6) were placed in 38 L holding containers. Treatments were then tested with combinations of three factors: salinity level, exposure duration, and recovery. All possible combinations of salinity, exposure duration, and recovery were tested. The plants were exposed to four salinity levels (0.0 ‰, 5.0 ‰, 6.0 ‰, and 7.0 ‰) for four exposure durations (7, 14, 21, and 28 days) and allowed to recover for two time periods (7 and 14 days) in a non-saline nutrient solution. Treatments exposed to 5.0 ‰, 6.0 ‰, and 7.0 ‰ consisted of three replicates while treatments exposed to 0.0 ‰ consisted of 1 replicate (Table 1).

		Exposure Duration			
		7 days	14 days	21 days	28 days
	0.0 ‰	1	1	1	1
Salinity	5.0 ‰	3	3	3	3
Level	6.0 ‰	3	3	3	3
	7.0 ‰	3	3	3	3

Table 1. Number of replicates per treatment (combination of salinity and exposure) where each replicate consist of three individuals from each of the two locations.

Two health indicators were used to gauge the health of the water hyacinth: leaf chlorophyll and wet weight. In this project, which pioneered the use of chlorophyll as a health indicator, leaf chlorophyll was measured because higher levels of chlorophyll correspond to higher levels of photosynthesis, which is crucial to the health of a plant. The chlorophyll measurement was taken from all the leaves on the plant using the non-destructive Minolta SPAD 502 chlorophyll meter and data were expressed as a relative percentage of chlorophyll content within the leaf. Preliminary data resulted in chlorophyll measurements being consistently taken from the bottom right segment of the leaf where chlorosis was more likely to occur and the chlorophyll meter would provide the most reliable results. Each plant received an overall chlorophyll score calculated as the mean from the chlorophyll measures of the individual leaves on each plant. Asexually produced daughter rhizome plants were considered to be separate individuals and not measured by the SPAD. Wet weight was found to be a good indicator of water hyacinth health by Muramoto and Oki (1988) and Casabianca and Laugier (1995). To measure wet weight, plants were removed from the solution and allowed to drip water for three seconds before

weighing. Baseline data on these two health measures were collected prior to any salinity treatment and, subsequently, at seven day intervals during the salinity treatment and recovery stages.

A multiple linear regression was used to determine what threshold combination of salinity, exposure duration, and recovery time impacted the recovery ability of the water hyacinth. The two health proxies, wet weight and plant chlorophyll, were analyzed individually and regressions were calculated separately. In addition, regression lines were produced for each recovery time (0, 7, and 14 days) at each salinity level to determine trends in weight and chlorophyll change and to more precisely observe recovery.

Results

Chlorophyll Individual linear regressions of chlorophyll on exposure for varying levels of salinity show that recovery was not a consistently significant factor influencing chlorophyll at any level of salinity (Fig. 2). However, the y-intercepts of the 14 day recovery regressions for 5.0 ‰ and 6.0 ‰ were higher than the y-intercepts at 0 days and 7 days recovery (Fig. 2). Water hyacinth at all salinities experienced a linear decline in chlorophyll levels each week. Chlorophyll levels dropped by ~25 % each week from day zero so that by day 21 there was a ~75 % drop in chlorophyll levels and by day 28 there was a ~95 % drop in chlorophyll from the initial collection week (Fig. 2).
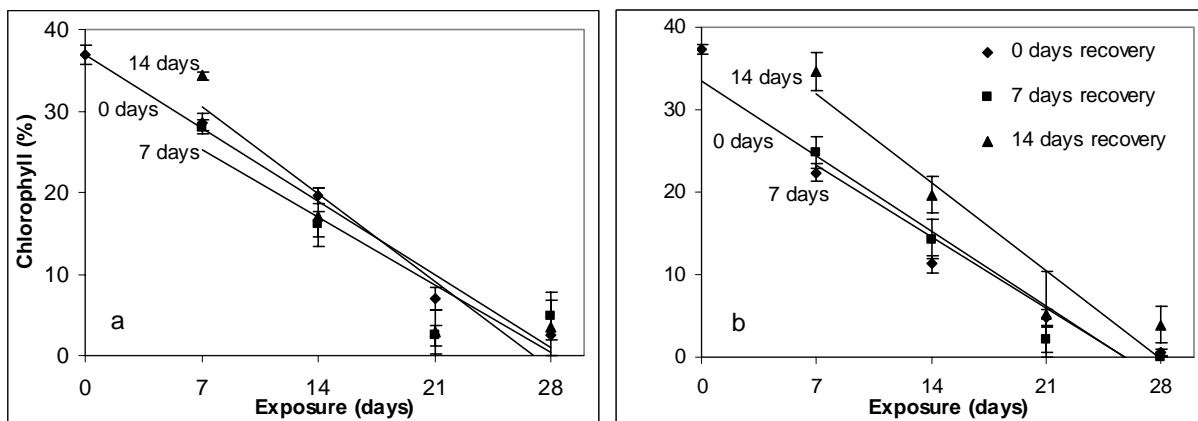

Figure 2. Simple linear regressions of chlorophyll on exposure for a) 5 ‰ and b) 6 ‰

Table 2. Linear regression statistics for chlorophyll

Salinity	Recovery	R^2	p-value	Slope
	0 days	0.94	<.0001	-1.32
5.0 ‰	7 days	0.79	0.0001	-1.19
	14 days	0.82	<.0001	-1.50
	0 days	0.91	<.0001	-1.44
6.0 ‰	7 days	0.89	<.0001	-1.24
	14 days	0.80	<.0001	-1.52

The multiple linear regression of chlorophyll on salinity, exposure, recovery, and their interactions revealed that recovery period was not a significant factor influencing chlorophyll percentages at any level of salinity or exposure duration (p<0.12). The factors that best explain variability in chlorophyll were salinity, exposure, and their interactions and the best fit was obtained by performing a multiple regression with these two factors and their interactions (Table 3). The regression explained 89 % of the variation in leaf chlorophyll. Salinity, exposure, and their combination were all highly significant (p<0.0001).

Table 3. Parameters for the multiple linear regression that best explains chlorophyll variability

Summary of Fit	R^2	p-value	N	
	0.89	<0.0001	264	
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	54.4	0.8	66.7	<0.0001
Salinity	-3.76	0.13	-29.8	<0.0001
Exposure	-0.920	0.037	-24.7	<0.0001
Salinity * Exposure	-0.245	0.012	-17.7	<0.0001

Wet Weight The multiple linear regression of wet weight on salinity, exposure, recovery, and their interactions explained 77 % of the variability in wet weight. Salinity, exposure duration, recovery, and their combinations were all significant (Table 4).

Table 4. Parameters for the multiple linear regression that best explain variability in wet weight

Summary of Fit	R^2	p-value	N	
	0.77	<0.0001	264	
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	94.5	3.5	27.2	<0.0001
Salinity	-8.00	0.55	-16.3	<0.0001
Exposure	1.28	0.16	7.85	<0.0001
Recovery	2.48	0.28	8.73	<0.0001
Salinity * Exposure	-0.734	0.061	-12.1	<0.0001
Salinity * Recovery	-0.466	0.106	-4.42	<0.0001
Exposure * Recovery	-0.0918	0.0311	-2.95	0.0034
Salinity * Exposure * Recovery	-0.0334	0.0115	2.90	0.0041

Individual regressions of wet weight on exposure for each salinity level show, for example, that results for wet weight depend not only on salinity and exposure duration but also on recovery (Fig. 3). At 0 ‰ (control), none of the wet weight regression slopes for the different recovery periods were significantly different from each other and there was a continuous weight increase with time (Fig 3, Table 5). At 5 ‰, the regression slope for 0 days recovery was not significantly different from the regression slopes of 7 days and 14 days recoveries; however, the slopes of 7 days and 14 days recoveries were significantly different. The y-intercept for the 7 days recovery regression is greater than the y-intercept of the 0 days recovery. During all exposure durations at 5 ‰ there was an increase in weight for plants during salinity exposure (0 days recovery) and an equal decrease in weight for both 7 and 14 days recovery periods (Fig 3, Table 5). At 6 ‰, the regression slopes for 0 days and 7 days recovery were not significantly different while the regression slope for 14 days recovery was significantly different than the slopes of 0 and 7 days recovery. Plant weight continually increased during salinity exposure (0 days recovery), slightly decreased during 7 days recovery, and decreased greatly during 14 days recovery and (Fig 3, Table 5). During exposure at 7 ‰, none of the wet weight regression slopes for the different recovery periods were significantly different from each other and there was a continuous weight increase during salinity exposure (0 days recovery) and 7 days recovery while plants allowed 14 days of recovery showed no weight change over all exposure durations (Fig 3, Table 5).
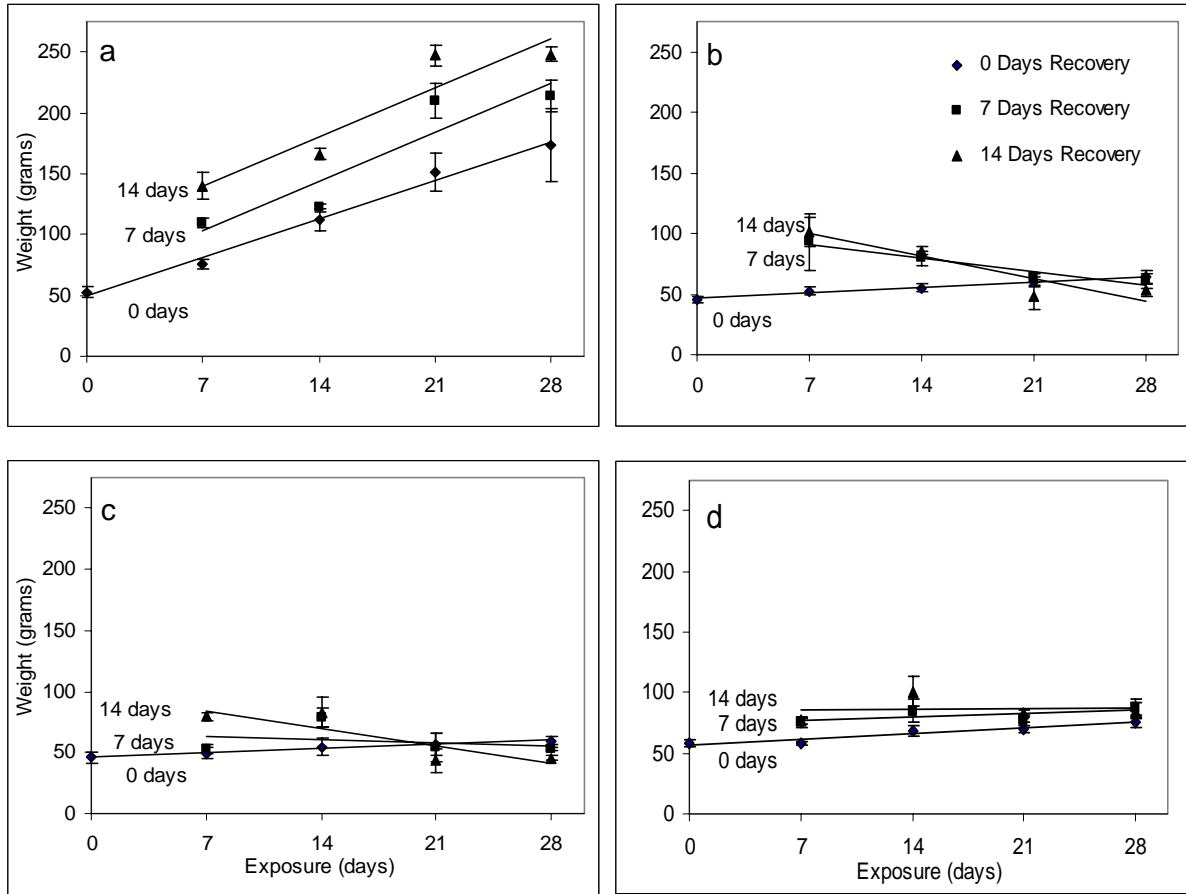
Figure 3. Simple linear regressions showing wet weight recovery for a) 0 ‰, b) 5 ‰, c) 6‰, and d) 7 ‰

Table 5. Linear regression statistics for wet weight

Salinity	Recovery	R^2	p-value	Slope	Significantly Different
	0 Days	0.73	<0.0001	4.52	a
0 ‰	7 Days	0.79	<0.0001	5.76	ab
	14 Days	0.84	<0.0001	5.81	b
	0 Days	0.27	0.0004	0.656	c
5 ‰	7 Days	0.31	0.058	-1.57	d
	14 Days	0.65	0.0015	-2.64	d
	0 Days	0.07	0.0807	0.530	e
6 ‰	7 Days	0.04	NS	-0.366	e
	14 Days	0.54	0.0067	-2.00	f
	0 Days	0.30	0.0002	0.665	g
7 ‰	7 Days	0.10	NS	0.425	g
	14 Days	0.003	NS	0.0894	g

Discussion

The trends in leaf chlorophyll and wet weight show that there are no threshold levels of salinity, exposure, recovery, or their interactions, as might be expected, that would result in a rapid and immediate change in the two health proxies. Remarkably, for water hyacinth under salinity stress, leaf chlorophyll and wet weight, and therefore, plant health, change linearly over time. This was not a priori expected, and suggests that the response to these factors as well as their interaction might be reliably predicted, allowing for development of a more dependable and effective control method.

Chlorophyll levels were affected by salinity level and exposure duration and became more damaged as salinity levels were increased and exposure periods were extended (Fig. 2, Table 3). Recovery period was not a statistically significant parameter indicating that there was not a consistent effect of recovery across all exposure durations. The greater y-intercepts for 14 days recovery suggest that for 5.0 ‰ and 6.0 ‰ salinities, leaf chlorophyll recovery occurred after 14 days following short exposure times and chlorophyll recovery did not occur for plants experiencing longer exposure times. No chlorophyll recovery occurred during exposure to 7.0 ‰.

Although this result is robust, it does not account for the recovery visually observed during the experiment. For longer exposure periods, recovery tended to occur in the production of daughter rhizomes and the production of new shoots with new leaves on the original plant; recovery of existing leaves was very rare. Chlorophyll measurements were not made on the daughter rhizomes, which were predetermined to be separate individuals, and the leaves generated by the new shoots tended to be too small after 14 days of recovery for the chlorophyll meter to measure. Recovery after shorter exposure periods generally occurred on preexisting leaves and this would explain the higher intercepts of 14 day recovery at 5‰ and 6‰ (Fig. 2).

The wet weight of the plants was affected by salinity, exposure duration, recovery, and their interactions (Table 4). As membranes in a stressed plant stopped functioning after experiencing higher levels of salinity for longer exposure durations the plant gained weight due to the intake of higher density water and water logging that comes from damage. As a plant recovered and membranes began to function properly, the plant lost weight as it took in less dense water and became less water logged from improved health (Feldman, pers. comm). This behavior is

characteristic of what was found by Muramoto and Oki (1988) and explains why there is a negative slope with recovery and a positive slope when recovery does not occur.

Weight gain during 0 ‰ (control) occurred at a positive rate ~25 grams per week and can be attributed to normal weight gain as was observed through the production of plant material. This weight gain should not be confused with weight gain occurring during salinity stress. Weight gain during salinity stress and before recovery occurred at rates between ~3 grams to ~5 grams per week depending on salinity level and exposure duration. Plant growth was halted during stress and this weight gain can be credited to water logging and the intake of salt (not the healthy production of plant material) that occurred after membrane breakdown during salinity stress. Plant weight after 14 days recovery following 28 days of salinity exposure returned to original pre-stress weight levels because loss of weight during recovery was due to increased membrane function that expelled excess water gained during stress. Production of daughter rhizomes and offshoots during recovery were trivial weight gains compared to the weight of the expelled water. If recovery past 14 days were observed, plant weight would be expected to increase at normal rates (from plant material production) as seen in 0 ‰.

There was greater wet weight recovery at lower salinities and shorter exposure periods. During 5.0 ‰, the regression slopes for 7 days and 14 days recovery were not significantly different indicating that there was a consistent recovery after all exposure durations after 7 days (Fig. 3). At 6.0 ‰, the regression slope for 7 days recovery was not significantly different for 0 days recovery but had a higher y-intercept. This indicates that after 7 days of salinity exposure, recovery was not consistent over all exposure periods, yet, recovery did occur after shorter exposure periods. The regression slope for 14 days recovery was significantly different from 0 and 7 days recovery indicating that there was a consistent recovery after all exposure periods after 14 days (Fig. 3). At 7.0 ‰, the regression slopes for 0 days, 7 days, and 14 days recovery were not significantly different. This signifies that consistent recovery did not occur at all for any exposure duration at 7.0 ‰ (Fig. 3).

Using the indicators of chlorophyll and wet weight to gauge the health of the water hyacinth, it is clear that the water hyacinth is a hardy plant and can recover after limited stress. Nevertheless, it is obvious that to control the water hyacinth, long exposure periods of at least 21 days and high salinity levels of at least 7.0 ‰ are needed. Salinity levels and exposure durations may potentially be reduced if other treatments are integrated and applied simultaneously.

Further research directed at the application of control methods using salinity and the effects of sustained increases in background salinity levels of natural environment will be needed.

Acknowledgments

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