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The Effect of Salt on Germination of Samphire Species

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ABSTRACT

Nine *Halosarcia** plant species from Lake Carey, Western Australia, were tested to determine the effect of salt on seed germination. These species were *Halosarcia* 'Angel Fish Island' (B. Davey 4)** *Halosarcia calyptrata* Paul G. Wilson, *Halosarcia halocnemoides* (Nees) Paul G. Wilson, *Halosarcia halocnemoides* (Nees) Paul G. Wilson subsp. *caudata* Paul G. Wilson, *Halosarcia indica* (Willd.) Paul G. Wilson subsp. *bidens* (Nees) Paul G. Wilson, *Halosarcia peltata* Paul G. Wilson, *Halosarcia pergranulata* (J. M. Black) Paul G. Wilson, *Halosarcia pruinosa* (Paulsen) Paul G. Wilson and *Halosarcia undulata* Paul G. Wilson. All species were subjected to salt concentrations of 0, 10, 20 and 30 g/l NaCl both in the laboratory in Petri dishes and under outdoor conditions, either buried or on the surface of soil from the lake margin. The nine species were also tested for their ability to recover after exposure to high NaCl concentrations. To test the ability to recover, the seeds were germinated in fresh water. For the majority of species, increased NaCl concentrations resulted in decreased germination percentages both in the laboratory and outdoors. In contrast, *H. halocnemoides* showed a slight increase in germination percentages at higher NaCl concentrations. In laboratory trials, the greatest reduction in germination percentage was 81% for *H.* 'Angel Fish Island'; the average reduction was 37% across all species. In outdoor experiments, germination only reached a maximum of 25%, which could have been due to lack of moisture, scarification of seeds, or temperature. Averaged across all the species, there was an increase of 58% in germination of the remaining seeds once salt was removed by flushing with fresh water.

INTRODUCTION

The effect of the saline environment can be the dominant factor that determines the ability of halophytes to reproduce and perpetuate their existence (Waisel 1972), whilst also influencing the zonation and inhabitation of samphire species. Salty environments can prevent the uptake of water by the seed due to the high osmotic potential of the medium, or the embryo may become poisoned due to the toxic effects of certain ions (Waisel 1972). Germination

inhibition is proportional to the external osmotic potential, which occurs at high salt concentrations (Waisel 1972).

Samphires belong to the family Chenopodiaceae and represent the group of succulent shrubs, which include the genus *Halosarcia*. These plants are generally associated with saline environments such as salt lakes and pans, salt marshes and samphire flats with the availability of water governing their life cycles (Datson 2002), hence samphires have adapted to survive in different zones of saline wetlands. Samphire seeds along with other flora and fauna of these saline environments only have a small window of opportunity to reproduce and germinate. Previous experiments have determined that seed germination is reduced at high NaCl concentrations for some *Halosarcia* species but that germination can increase once salt is flushed from the seeds (Barrett 2000). Malcolm (1964) found temperature was a vital factor for germination, with the highest germination recorded at a fluctuation of 5-35°C. However very little information is available on germination for the majority of *Halosarcia* species, except for *Halosarcia pergranulata*, which has been studied for use in revegetation programs. Barrett 2000 conducted germination testing and found that removing a small portion of the testa was beneficial for germination of *Halosarcia pergranulata*.

In Lake Carey, located in the eastern Goldfields region of Western Australia, saline groundwater is being released onto the lake playa on a continual basis. The groundwater is abstracted from a number of mine sites to ensure safe and dry working conditions. The volume of water is not enough to alter water levels of the lake but concerns have been raised that this flush of hypersaline groundwater is increasing the concentrations of salt within the soil and preventing germination of samphire species. We have observed that samphire species at Lake Carey germinate when rain flushes salt from the surface soil.

[* Since this paper was written the genus *Halosarcia* has been renamed *Tecticornia* after genetic testing (Shepherd & Wilson 2007). The species names remain the same. ** Taxonomic synonym of *Tecticornia mellaria* (K.A. Sheph.).]

We conducted germination trials to establish the effect of salt on the germination of nine salt tolerant species from the genus *Halosarcia* present at Lake Carey. The results will determine whether the continued release of saline groundwater onto lake playas from surrounding mine sites has a detrimental affect on samphire germination. This trial was carried out within two environments to gain a more thorough understanding of expected germination rates. The laboratory trial was carried out within a controlled environment to observe germination rates absent of variables such as temperature, whilst the nursery trials provided a more realistic environment that would be expected within the natural habitat of the samphires.

MATERIALS AND METHODS

Seed Collection and Viability Testing

Trials were carried out in the laboratory trials for four weeks and six weeks for nursery trials, from September to November 2007. Seeds from nine species of *Halosarcia* (Table 1) were collected in October 2006 from Lake Carey and cleaned to remove any foreign material. Cleaning methods included hand rubbing over a screen, putting seed through a de-huller screen and seed air separator. *H. pergranulata* was scarified to increase germination (Barrett 2000). Seeds were subjected to salt concentrations of 0, 10, 20 and 30 g/l NaCl in either tap water (outdoor settings) or deionized water (laboratory trials). The upper saline concentration of 30 g/l NaCl was chosen because previous experiments by Barrett (2000), and English et al. (2002) demonstrated that germination success for *Halosarcia pergranulata* decreased above this concentration. To reduce the risk of contamination all seeds were surface sterilized by soaking for ten minutes in a 1% solution of sodium hypochlorite followed by rinsing with deionized water (Sauer 1986). This is a standard procedure also reflected within the International Rules for Seed

Testing (ISTA 1985). However, sodium hypochlorite treatment did not prevent mold, particularly at salinities of 20 and 30%.

Seed viability was assessed using the tetrazolium (TZ) salt biochemical test (Barrett 2000). Two replicates of 20 seeds were randomly selected and cut using a scalpel and placed in the TZ solution for 24 hours. Replicates of 20 seeds were used to represent the sample size of the replicates in the trials. After 24 hours the seeds were removed from the TZ solution and the embryos checked for pink staining under a microscope. The average of the two replicates was recorded as percentage viable.

Laboratory Trial

Five replicates of 20 seeds from each species were placed on filter paper (7 layers of Whatman No 1 filter paper) within Petri dishes. The treatment solution was added to each dish to ensure saturation of the filter paper without runoff. All dishes were secured in plastic bags and randomly positioned in a controlled temperature room at 20-25°C to represent the optimum temperature for germination (Shepherd 2007). Numbers of germinated seeds were recorded at weekly intervals for four weeks and removed together with contaminated seed. Contaminated seeds were those subject to mold, and were removed to avoid cross contamination to remaining healthy seeds. Saline water representing each concentration was added as required to maintain moisture using a dropper to avoid over saturation and seed movement. The seeds were scored as having germinated when the radicle emerged from the testa. The germination success per trial was expressed as a percentage of clean seed used in the trial.

Table 1–*Halosarcia* species, preferred habitat and viability (Datson 2002).

Species	Code	Habitat	Viability
<i>Halosarcia</i> 'Angelfish island'	HA	Well-drained saline soils. Never found in wet run-off areas preferring the dunes beside the lake.	80.0%
<i>Halosarcia calyptrata</i>	HC	Slightly higher rises in wet saline areas with good drainage.	65.0%
<i>Halosarcia halocnemoides</i>	HH	Wettest and most saline parts of the wetland.	22.5%
<i>Halosarcia halocnemoides</i> subsp. <i>caudata</i>	HHC	Wettest and most saline parts of the beach profile.	32.5%
<i>Halosarcia indica</i> subsp. <i>bidens</i>	HIS	Well drained saline soils at upper margins of lake.	40.0%
<i>Halosarcia peltata</i>	HPT	Lowest part of wetland in wet areas, run off areas and gutters. Seems to tolerate high salinity.	55.0%
<i>Halosarcia pergranulata</i>	HP	Lower parts of the wetland in wet areas that are not necessarily very saline.	60.0%
<i>Halosarcia pruinosa</i>	HPR	From lake playa through beach profile and into wetter depressions in dunes. Tolerates high salinity, water logging and dry conditions.	65.0%
<i>Halosarcia undulata</i>	HU	Lower part of wetland.	67.5%

Outdoor Trial

To assess the effects of salinity within a more realistic environment representative of natural habitats, seeds were germinated on nursery benches. To prevent rain or watering from diluting the applied salinity concentrations, two nursery benches were covered in plastic with seedling trays placed underneath to represent a hot house. The plastic covering was adjusted to ensure that the maximum temperature did not go beyond 35°C as previous studies have shown this as the optimal temperature for similar samphires and members of the Chenopodiaceae family (Malcolm 1964). This was achieved by adjusting the amount of air flow through the hot house. Seeds were germinated in soil collected from Lake Carey, which is silty clay with gypsum crystals (Coleman 2003). Salt was leached from the soil prior to the addition of seeds by saturating the soil in a wheelbarrow until the salt content was reduced to 0.6 parts per thousand (ppt) which was determined using an Electrical Conductivity (EC) meter (Hanna Combo pH & EC meter). The trays were lined with mesh to avoid loss of soil. The nursery trials were two weeks longer than the laboratory trials, as it took longer for initial germination. Most of the nursery plots formed a salt crust during the trial due to evaporative wicking.

Three replicates of 40 seeds from each species were placed into slide holders on filter paper and secured with a polypropylene fleece ('Plant Cover'—PP Non Woven Fleece (17 g/m²) to prevent seeds from being lost. Ten seeds were placed in each slide holder with two holders buried in the soil and two placed on top of the soil to establish if light also affected germination. Seeds were watered with saline water representing each concentration on a regular basis with watering increasing over time as required. Watering began at weekly intervals and then increased to every second day for two weeks, followed by daily watering for two weeks to maintain soil moisture. Number of seeds germinating were recorded on a weekly basis for six weeks and removed. Mean germination was calculated by dividing the total number of germinants per concentration by the number of clean (non-contaminated) seeds.

After six weeks the seed trays were watered three times a day with fresh water to flush the salt and test the ability of all seeds to recover for a further two weeks. All germinants were counted at the end of this time and the average calculated by dividing the number of NaCl concentrations (4) by total mean germination.

$$\frac{\sum x^1}{\sum c^1} = y$$

where x^1 = total mean germination
 c^1 = number of concentrations

Statistical Analyses

The statistical package Minitab 14 was used to analyze the data. The data was not transformed because not all species had the same response to salinity of the irrigation water and a significant relationship was achieved without transformation for all of the laboratory trials. For the laboratory trials the entire data set was examined for interaction between species and salinity using an ANOVA two-way test. For individual species a regression analysis was completed between proportion of seeds germinated against salinity with only the probability and R² being reported. The main issue with the nursery trials was the low germination. An ANOVA analysis of the nursery data was not done because of the large number of zero scores. Instead, these data were analysed only with regression analysis as described above.

RESULTS

Viability Trials

Five of the nine species had a seed viability of 60% or greater. *Halosarcia* 'Angel Fish Island' had the highest germination at 85% and *H. halocnemoides* was the species with the lowest seed viability of 22.5%.

Laboratory Trials

With increasing NaCl concentrations there was a marked decrease in germination for all species except *H. halocnemoides*, which increased by 11% at higher NaCl concentrations (Figure 2). The majority of species had some germination at all concentrations. However, *H. indica* subsp. *bidens* seeds did not germinate at 20 or 30 g/l NaCl, *H.* 'Angelfish Island' seeds did not germinate at 30 g/l NaCl, and *H. halocnemoides* seeds did not germinate at 0 g/l NaCl. Seeds of *H.* 'Angelfish Island' showed the greatest salt sensitivities; seed germination decreased 81% between 0 and 30 g/l NaCl (Figure 2). *H. calypttrata* seeds showed the greatest salt tolerance with 42% seed germination at 30 g/l NaCl (Figure 2). Of the nine species, *H. pergranulata*, *H. indica* subsp. *bidens* and *H. halocnemoides* had the poorest germination in the laboratory trials.

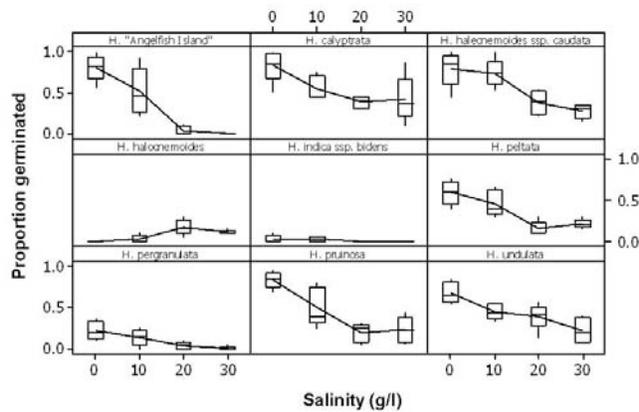


Figure 1—The effects of salinity on seed germination for the 9 study species in the 4 week laboratory trials.

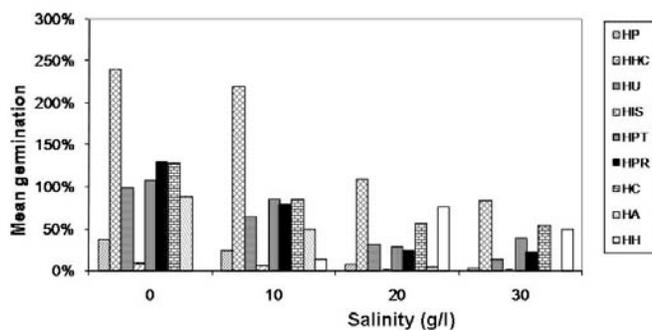


Figure 2—Germination of nine samphire species, expressed as a percentage of seed viability (from Figure 1) for 0, 10, 20 and 30 g/l NaCl concentrations in 4 week long laboratory trials. See text for an explanation of values > 100%. Error bars show standard error.

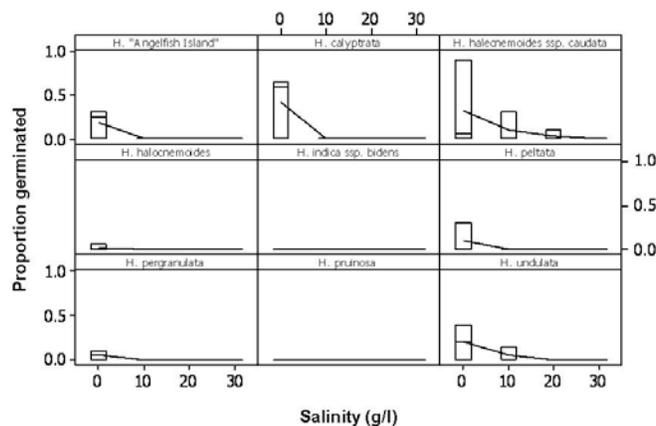


Figure 3—The effects of salinity on seed germination for the 9 study species grown in outdoor trials. The box plot display consists of a rectangular box representing roughly the middle 50% (interquartile range) of the data, and lines ("whiskers") extending to either side indicating the general extent of the data. The median value is shown as a cross bar inside the box and the line connects the mean values.

The Two-way ANOVA analysis of the entire data set showed that there was a significant relationship between salinity and germination, species and germination and an interaction between the factors:

Source	DF	SS	MS	F	P
Species	8	6.6757	0.83447	48.47	0.000
TDS (g/l)	3	3.9852	1.32839	77.17	0.000
Interaction	24	2.6574	0.11072	6.43	0.000
Error	144	2.4789	0.01721		
Total	179	15.7972			

In all cases other than *H. halocnemoides* there was negative linear relationship between germination and salinity of the irrigation water (Table 2). *H. halocnemoides* had the highest proportion of seeds germinate at 20 g/l. *H. indica* subsp. *bidens* had a very poor germination rate at all salinities which may explain the significant probability but a low R^2 of 17%.

Germination was tested against the viability levels of each species (Figure 4). The viability-adjusted germination for *H. halocnemoides* subsp. *caudata* at all concentrations far exceeds that expected with a viability of only 32.5%. Germination of *H. peltata*, *H. pruinosa* and *H. calypttrata* also exceed their viability at 0 g/l NaCl. Viability results were therefore defective for some species.

Outdoor Trials

In contrast to the laboratory trials, seed germination in the outdoor trials was very low. Only seeds of two species buried in the soil germinated at 10 and 20 g/l NaCl and no germination occurred at 30 g/l NaCl (Figure 4). There was no germination of the seeds placed on the surface. *H. halocnemoides* subsp. *caudata* and *H. undulata* seeds both germinated at 10 g/l NaCl and *H. halocnemoides* subsp. *caudata* and *H. pruinosa* had 1 to 2 germinants at 20 g/l NaCl. The highest germination across all salinities was for *H. calypttrata* followed by *H. halocnemoides* subsp. *caudata*. *H. halocnemoides* subsp. *caudata* is the most salt tolerant and *H. indica* subsp. *bidens* being the most salt sensitive with no germination recorded.

The regression statistics relating germination to salinity are shown in Table 2. The probability and regression did not have the same frequency of significance as for the laboratory trials but the low germination rate would be major factor in this result. The results of the analysis showed that germination of four species was negatively affected by salinity, germination of three species was not and the remaining two did not have any seeds germinate.

All species had the ability to recover from high salt concentrations once flushed with fresh water (Figure 5). The species with the greatest increase in germination, once flushed, was *H. ‘Angelfish Island’*. *H. peltata* seeds had a great increase in germination on the surface. The species with the least ability to recover was *H. halocnemoides*. Averaged across all the species, there was an increase of 58% in germination once salt was removed.

Table 2—Regression analysis relating the germination of different samphire species to salinity.

	Lab. trial		Soil trial	
	Probability	R ²	Probability	R ²
<i>H. ‘Angelfish Island’</i>	0.000	75%	0.040	29%
<i>H. calypttrata</i>	0.002	38%	0.039	30%
<i>H. halocnemoides</i> subsp. <i>caudata</i>	0.000	63%	0.141	12%
<i>H. halocnemoides</i>	0.002	39%	0.192	8%
<i>H. indicia</i> subsp. <i>bidens</i>	0.042	17%	na	na
<i>H. peltata</i>	0.000	58%	0.192	8%
<i>H. pergranulata</i>	0.000	57%	0.056	25%
<i>H. pruinosa</i>	0.000	64%	na	na
<i>H. undulata</i>	0.000	60%	0.039	30%

DISCUSSION

There is a significant relationship between reduced germination of samphire species present at Lake Carey and salinity. The two trials (laboratory and outdoor) supported similar results in that all seed germination decreased with increased NaCl concentrations. The strong results from the controlled laboratory trials, however, were not replicated by the soil trials. Germination for the nine studied samphire species will occur at 0 g/l and 10 g/l NaCl followed by a rapid decline at 30 g/l NaCl. Seed germination dramatically increased following flushing with fresh water.

The decrease in germination for all species (except *H. halocnemoides* subsp. *caudata*) within the laboratory trials suggests that the concentration of salt does have an effect on seed germination; the higher the NaCl concentration the lower the germination. *H. halocnemoides* subsp. *caudata* was the only species where germination increased, likely because its habitat is the most saline parts of Lake Carey (Table 3). For the three species that had low germination (*H. indica* subsp. *bidens*, *H. pergranulata* and *H. halocnemoides*), the temperatures used in the experiment may not have been optimal (Malcolm 1964). Although

H. pergranulata seeds were scarified, there was little germination for this species. A test between non-scarified and scarified seeds was not undertaken although the experiments completed by Barret (2000) concluded that there was an increase in germination from scarification.

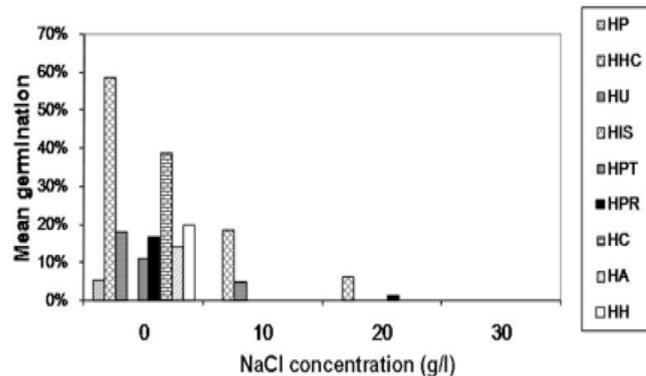


Figure 4—Viability-adjusted germination in outdoor settings across all species at differing NaCl concentrations with standard errors. Error bars show standard error.

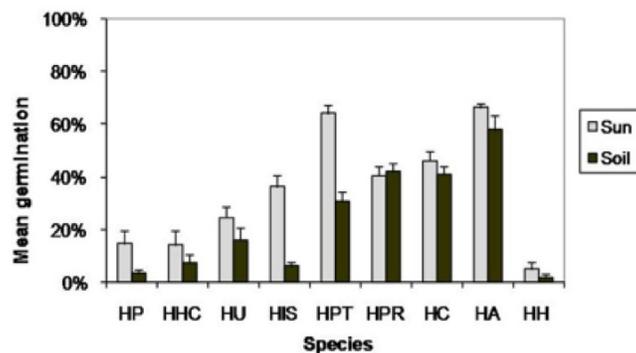


Figure 5—Comparison of ability to recover once flushed with fresh water for seeds in soil and sun in outdoor trials for all 9 study species. Error bars show standard error.

There was very little germination of seed either on the surface or buried in the soil in the outdoors trials, which makes it difficult to determine which species were the most salt sensitive or salt tolerant in the natural soil of the area. *H. halocnemoides* subsp. *caudata* had the most germination over 10 g/l NaCl and naturally occurs in the most saline areas, although there was little germination for *H. halocnemoides*, which inhabits similar areas. The main difficulty with the nursery trials was controlling the salt content of the plots and therefore the salinity of the pore water. The salt crusts that formed likely had a negative impact on germination.

A lack of moisture may also have contributed to the limited germination within the outdoor trials although some seeds receiving 0 g/l NaCl concentration still germinated. The irrigation frequency remained the same for all treatments. When watering frequencies were increased there was no direct increase in germination.

Once the viable seeds were watered three times a day with fresh water to flush the salt from the soil, there was a substantial increase in germination across all species. This suggests that a flush of fresh water is required to increase germination once seeds have been exposed to salt concentrations above 20 g/l NaCl. This supports previous findings at Hannan Lake, Western Australia that halophytes primarily germinate in periods of lowered soil salinity; an adaptive trait that ensures favorable growth conditions for young seedlings (English et al. 2002). We have observed that samphire species at Lake Carey germinate when rain flushes salt from the surface soil. In this case an analysis of the recruitment versus rainfall showed that there was a strong but not significant relationship between total recruitment to rainfall at all sites monitored over seven years.

It is inferred from this experiment that if the addition of hypersaline water to the lake reduces the period when seeds are exposed to freshwater conditions in the top soil at Lake Carey's edge, the germination of these samphires will be reduced. However the evidence is that once a suitable flushing event occurs, significant recruitment will occur.

This experiment has shown that the samphires tested in this trial require freshwater to germinate but that they will accommodate hypersaline conditions for a period and recover to germinate when the salinity drops to a low salinity.

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