

# Positive and negative consequences of salinity stress for the growth and reproduction of the clonal plant, *Iris hexagona*

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## Summary

1 Salinization is a growing environmental stress in wetland ecosystems world-wide. Several models have been proposed that predict clonal plant responses to stress, including that environmental stress stimulates sexual reproduction.

2 We conducted a common-garden experiment to investigate the effects of salinity on 10 natural populations of *Iris hexagona*, a clonal perennial endemic to freshwater and brackish wetlands of the North American Gulf Coast.

3 Salinity reduced vegetative growth but either increased or had neutral effects on sexual reproduction, consistent with the clonal stress hypothesis. Salinity of  $4 \mu\text{g g}^{-1}$  more than doubled the number of seeds produced compared with freshwater controls, but flower number and seed mass were unaffected.

4 Salinity reduced total below-ground mass by nearly 50% compared with controls, with no significant change in rhizome numbers.

5 Plants from 10 randomly selected *I. hexagona* populations differed dramatically in growth and reproduction, independent of salinity. Total biomass that accumulated over the 20-month experiment ranged across all treatments from 52 to 892 g, and flower numbers varied from 2.3 to 11.3 per replicate.

6 Populations did not respond differently to salinity, except with respect to above- : below-ground ratios, thus providing no conclusive evidence for local adaptation to salinity stress.

7 Our results concur with published models of plant reproductive strategies in variable environments, in that environmental stress stimulated sexual reproduction at the expense of growth. However, these models do not predict the observed sharp decline in seed production at near lethal salinity levels.

*Key-words:* clonal, environmental stress, facultative reproduction, fecundity, plasticity

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## Introduction

Plant salinity stress is an escalating global problem, particularly in coastal areas, which are vulnerable to influxes of high salinity caused by natural and anthropogenic forces (Boesch *et al.* 1994; Rogers & McCarty 2000). Salinity also impacts agricultural regions, where it has severely degraded the landscape (Yeo 1999). Salin-

ization in Louisiana wetlands (Grace & Ford 1996; Howard & Mendelssohn 1999a,b) has caused widespread mortality in freshwater plant populations (Allen *et al.* 1997; Krauss *et al.* 2000). However, relatively few studies have investigated the effects of salinity stress on natural plant populations in these areas.

Plants typically alter allocation to growth and reproduction in response to environmental challenges (Bazzaz *et al.* 1987; Sultan 1987; Hutchings 1988; Crawley 1996). Species from different habitats and with different evolutionary histories respond to environmental stress in diverse ways. For example, osmotic stress due to drought can increase (Boot *et al.* 1986), decrease (Steyn *et al.* 1996) or have no effect (Cox & Conran 1996) on flower production, and allocation to sexual reproduction is influenced by a variety of other abiotic

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and biotic stresses, including overcrowding (Harper 1977; Abrahamson 1980; Sackville Hamilton *et al.* 1987), interspecific competition (Mandujano *et al.* 1998; Weiner 1988), nutrient limitation (Reekie 1991; Sugiyama & Bazzaz 1997), and herbivory (Reekie 1991; Mandujano *et al.* 1998).

Many plants that occur in habitats with elevated salinity reproduce primarily via rhizomes or other clonal structures (Silander 1985; Mitsch & Gosselink 1993), but like other clonal species, they also have the ability to reproduce sexually (Silander 1985). The relatively low incidence of sexual reproduction in these species has been attributed to reduced germination (e.g. Shumway & Bertness 1992) or high seedling mortality (Abrahamson 1980; Ungar 1991). In addition, reproductive plasticity would allow shifts in allocation between sexual or asexual propagules should salinity conditions warrant (Adam 1990), but the occurrence of this strategy is poorly understood in wetland plants.

Analytical and simulation models have been developed to predict the facultative, plastic responses of clonal plants to changing environmental conditions (Loehle 1987; Sakai 1995; Gardner & Mangel 1999). (Note that these models are different from life-history models (e.g. Grime 1977, 1979; Sackville Hamilton *et al.* 1987), which attempt to model the types of life-history strategies that would evolve in given environments.) Loehle's (1987) model predicts that productive habitats should decrease the relative cost of seeds, thereby stimulating sexual reproduction. Alternatively, less productive habitats that inhibit seed germination and establishment should reduce plant allocation to sexual reproduction. In contrast, the models of Sakai (1995) and Gardner & Mangel (1999) predict that favourable habitats should promote asexual growth over sexual reproduction. However, all three models predict that seed production should be favoured when their establishment probability is greater than that of asexual ramets, i.e. if the mortality threat to the ramet is sufficiently high. We tested these hypotheses in a long-term common garden experiment where we altered the habitat quality of the clonal perennial *Iris hexagona*. We measured several different responses to characterize allocation to sexual and asexual reproductive effort, and compared our results with the qualitative predictions of these three models.

Differences in microhabitat conditions can often lead to local adaptation to specific environmental regimes or environments (Galen *et al.* 1991; Sork *et al.* 1993). Local adaptation is also possible in response to large environmental differences between sites such as mine contaminated soils (Shaw 1999), or plants growing in saline environments (Thompson *et al.* 1991). It is common for salt-marsh populations to exhibit wide variation in salinity tolerance (Silander 1985; Allen *et al.* 1997; Hester *et al.* 1998; Seliskar & Gallagher 2000), indicating the potential for the formation of locally salt-adapted ecotypes. Therefore, we also explored the potential for different iris populations to

exhibit variation in adaptation to salinity stress. Evidence for differential adaptation to salinity would be indicated by a significant population–salinity interaction and by populations that outperformed others in growth or reproductive traits in elevated salinity treatments.

## Materials and methods

### STUDY SPECIES AND SITE

*Iris hexagona* is part of the Louisiana iris species complex. Louisiana irises have stimulated ecological and evolutionary research for decades (Viosca 1935; Riley 1938; Cruzan & Arnold 1993; Burke *et al.* 2000; Johnston *et al.* 2001). *Iris hexagona* (Walter) is the most widespread Louisiana iris, and is indigenous to freshwater and brackish wetlands along the Gulf Coast (Bennett & Grace 1990). This clonal species is self-compatible, but requires insect pollinators to set seed (Emms & Arnold 2000; P. Van Zandt, personal observation). Influxes of high salinity from tropical storms have killed some *I. hexagona* populations, but other populations are tolerant of elevated levels of salinity (P. Van Zandt, M. Tobler, S. Mopper, personal observations).

Marsh Island (29.79 °N, 91.78 °W) is a 300-km<sup>2</sup> uninhabited wildlife refuge 10 km south of the Louisiana coast. Erosion and other geological processes separated the island from the mainland in the past 5000 years, changing the habitat from freshwater to intermediate salt marsh (Orton 1959; Törnqvist *et al.* 1996). Mainland *I. hexagona* populations are typically large (> 3000 plants) and continuous, but island saltmarsh populations are usually small (< 300 plants) and isolated, perhaps owing to severe selection from increasing salinization. Average Marsh Island water salinity varies seasonally from about 1.0–9.0 µg g<sup>-1</sup> (higher in drought years), and is at its lowest level during April–June when flowers and seeds are produced (Van Zandt & Mopper 2002).

### THE COMMON GARDEN EXPERIMENT

In March 1997, we randomly collected *I. hexagona* plants from each of 10 isolated sites on Marsh Island, Louisiana, and transported them 80 km to the University of Louisiana Research Center in Carencro, Louisiana. The distance between sites ranged from 1.2 to 9.0 km, and population size ranged from 71 to 261 individual ramets. We weighed, marked and planted irises in 30 227-L plastic containers (Rubbermaid Products, Wooster, OH, USA) that were sufficiently large to avoid crowding as plants grew. The experimental containers were placed approximately 1 m apart in an open field, and filled with Mississippi alluvial topsoil. Each container contained plants from three randomly selected populations and plants were randomly assigned their positions within containers, thereby reducing container position effects or competitive effects of neighbours. There were three replicate plants for each

population  $\times$  salinity combination. Individual plants were labelled with coloured nylon twine to distinguish population identities. We used Instant Ocean™ (Aquarium Systems Inc., Mentor, Ohio, USA), a phosphate-free synthetic seawater mixture, to establish and maintain treatments at target salinity levels of 2 and 4  $\mu\text{g g}^{-1}$ . The control represented freshwater conditions and did not receive supplemental salt.

Plants can tolerate pulses of salinity much better than sustained long-term exposure (Flynn *et al.* 1995; Howard & Mendelssohn 1999a,b), and we therefore took a conservative approach in applying salinity in order to avoid mortality or excessive stress, particularly during the critical reproduction periods. Weekly salinity readings during the 20-month experiment, taken with an Orion 125™ meter (Orion Research Inc., Beverly, MA, USA), indicated that salinity levels in the control, 2 and 4  $\mu\text{g g}^{-1}$  treatments averaged  $0.2 \pm 0.4$  SE,  $2.0 \pm 1.2$  SE, and  $3.7 \pm 1.8$  SE  $\mu\text{g g}^{-1}$ , respectively, over the experimental period.

We conducted a separate, smaller scale experiment in 2001 to test the effect of higher salinity specifically on seed production, using doubled target salinities of 4 and 8  $\mu\text{g g}^{-1}$ . The 12-month salinity averages for the three treatments were  $0.2 \pm 0.002$ ,  $4.49 \pm 0.24$  and  $7.3 \pm 0.17$   $\mu\text{g g}^{-1}$ .

#### MEASURING PLANT RESPONSES

During the flowering season in the spring of 1998, we counted the number of flowers produced by each replicate population. To assess maximum potential seed production in the absence of pollen limitation, we hand pollinated flowers and harvested seeds when they were mature. We used the number of flowers, number of seeds and total and average seed mass as estimates of sexual reproduction. Plants were excavated in October 1998, 20 months after planting. We weighed plants to determine total biomass (both above- and below-ground), and counted the total number of ramets. As an estimate of asexual reproduction, we subtracted the original number of ramets at planting from the final number of ramets at excavation to obtain the number of ramets produced during the experiment. We subtracted initial plant weight from weight at excavation to obtain accumulated vegetative biomass. Our experimental results were obtained from living irises, therefore we sacrificed 24 plants to obtain correlations between fresh and oven-dried plant material. Leaf area was used to estimate above-ground biomass. Below-ground biomass was determined by subtracting the estimated above-ground biomass from accumulated vegetative biomass. This method may underestimate above-ground biomass produced during the experiment because of normal foliage turnover.

Salinity was a fixed effect in this split-plot design, whereas population and the population–salinity interaction were random effects. We used mixed model analysis of variance with linear unbiased estimators for

fixed effects, and restricted maximum likelihood statistics for random effects (PROC MIXED; SAS 2001). Salinity degrees of freedom were corrected using Satterthwaite's procedure; population, and population  $\times$  salinity were tested with likelihood ratio chi-square statistics with one degree of freedom (Littell *et al.* 1996). Most response variables were normally distributed and homoscedastic. We transformed raw data when necessary to meet parametric assumptions, and used both non-transformed data and log transformations to compare plant size and fecundity relationships. All graphs present non-transformed data. Biomass was included as a covariate in statistical analyses when it was significant at  $P < 0.1$ .

For the second experiment (plants established in 2001), we collected and weighed seeds in 2002 from 8, 7 and 4 containers from control, 4 and 8  $\mu\text{g g}^{-1}$  treatments, respectively. The number of replicates differed between treatments because of plant mortality. We used a MANOVA to analyse the effect of salinity on seed number and seed mass, and Tukey adjusted pairwise comparisons to test individual treatment effects. The data were normally distributed.

#### SIZE–REPRODUCTION RELATIONSHIP

We examined the relationship between plant size and reproduction (both sexual and asexual) in 10 *I. hexagona* populations grown in the common garden. Plant size was defined as total biomass accumulated during the experiment, not including seeds, flowers or flower stalks. Clonal reproduction was estimated using the number of ramets produced during the experiment. We conducted parametric regression analysis (after Klinkhamer *et al.* 1990) with non-transformed and log transformed size and reproduction data to assess whether plant reproduction was a function of plant size. All zero values were replaced with '1's to facilitate transformation. We also conducted a logistic regression (SAS PROC GENMOD) to compare the relationship between flower number and plant mass. This regression assumes that number of flowers is a categorical variable with a Poisson distribution. Plants that produced no flowers were included in the analysis.

## Results

#### EFFECTS OF SALINITY ON IRIS GROWTH AND REPRODUCTION

Measurements of living plants were highly correlated with dried samples. Based on information from 24 sacrificed plants, the area of living leaves was highly correlated with wet leaf mass ( $r = 0.98$ ,  $P < 0.0001$ ), as was fresh and dry leaf mass ( $r = 0.99$ ,  $P < 0.0001$ ), and fresh and dry below-ground mass ( $r = 0.96$ ,  $P < 0.0001$ ).

Salinity significantly reduced *I. hexagona* biomass by 20% and 48% in the 2 and 4  $\mu\text{g g}^{-1}$  groups, respectively,

**Table 1** Mixed model ANOVA and ANCOVA testing the effects of salinity (fixed), population (random), and salinity × population (random) on *I. hexagona* grown in a common garden. Initial biomass was included as a covariate when it was significant at  $P < 0.1$ 

Performance variable	Source	d.f.	$F$ (fixed) or $\chi^2$ (random)	$P$
Total biomass	Salinity	2, 18	7.1	0.0053
	Population	1	12.0	0.0005
	Salinity × population	1	0.7	0.4028
Above-ground biomass	Salinity	2, 18	14.7	0.0002
	Population	1	19.3	0.0001
	Salinity × population	1	0.3	0.5839
Below-ground biomass	Salinity	2, 18	7.0	0.0055
	Population	1	9.9	0.0017
	Salinity × population	1	1.1	0.2943
Above- : below-ground ratio	Salinity	2, 18	4.4	0.0277
	Population	1	10.2	0.0014
	Salinity × population	1	6.9	0.0086
	Biomass	1, 32	2.2	0.1456
Accumulated ramets	Salinity	2, 18	2.8	0.0849
	Population	1	2.3	0.1294
	Salinity × population	1	< 0.01	> 0.99
	Biomass	1, 32	151.4	0.0001
Number of flowers	Salinity	2, 18	1.0	0.3766
	Population	1	7.8	0.0052
	Salinity × population	1	0.1	0.7518
	Biomass	1, 32	5.0	0.0324
Number of seeds	Salinity	2, 18	5.2	0.0162
	Population	1	3.6	0.0578
	Salinity × population	1	< 0.01	> 0.99
	Biomass	1, 32	4.2	0.0494
Individual seed mass	Salinity	2, 18	1.7	0.2207
	Population	1	9.7	0.0018
	Salinity × population	1	< 0.01	> 0.99
	Biomass	1, 32	1.5	0.2450

**Table 2** Salinity effects on *I. hexagona* growth and reproduction. Presented are the least squares means, standard error (in parentheses), and  $P$ -values ( $\leq 0.05$  in bold) determined by Tukey-Kramer adjusted pairwise comparisons. Average sample non-transformed data presented; some tests were conducted on transformed data

	Means (SE)			$P$ -values		
	Control	2 $\mu\text{g g}^{-1}$	4 $\mu\text{g g}^{-1}$	Control vs. 2 $\mu\text{g g}^{-1}$	Control vs. 4 $\mu\text{g g}^{-1}$	2 $\mu\text{g g}^{-1}$ vs. 4 $\mu\text{g g}^{-1}$
Total net mass final-initial (g)	894 (97)	712 (85)	469 (67)	0.2700	<b>0.0039</b>	0.1076
Below mass final (g)	1158 (85)	1004 (74)	770 (60)	0.3255	<b>0.0042</b>	0.0826
Above mass final (g)	132 (11)	98 (9)	71 (7)	<b>0.0183</b>	<b>0.0001</b>	0.0732
Allocated below (%)	90 (0.5)	91 (0.4)	92 (0.5)	0.1763	<b>0.0261</b>	0.5290
No. rhizomes	3.5 (0.4)	3.8 (0.3)	4.7 (0.4)	0.8428	0.0867	0.1859
No. flowers	4.6 (0.9)	5.5 (0.9)	6.6 (1.0)	0.7754	0.3450	0.6862
No. seeds	33 (12)	73 (11)	81 (12)	<b>0.0460</b>	<b>0.0200</b>	0.8185
Individual seed mass (mg)	14 (1)	13 (1)	12 (1)	0.6560	0.1950	0.5500

compared with the control group (Tables 1 and 2). Both above- and below-ground tissue decreased, and the above- : below-ground allocation ratio increased with salinity (Table 1, Fig. 1). Post-treatment biomass was not correlated with pre-treatment planting biomass ( $r = 0.001$ ,  $P = 0.72$ ,  $n = 90$ ).

Salinity significantly increased fecundity (Tables 1 and 2). Plants in the 4  $\mu\text{g g}^{-1}$  salinity treatment produced more than twice as many seeds as the control plants (Fig. 2). Although seed number strongly increased in response to salinity, neither flower number or seed

mass varied significantly among salinity treatments (Table 1, Fig. 2). Salinity had a positive but weaker effect on clonal reproduction. Ramet numbers increased in response to salinity, but the difference between treatments was not statistically significant when biomass was included as a covariate (Table 1). There was a significantly positive correlation between sexual (seed number) and asexual (ramet number) reproduction ( $R^2 = 0.267$ ,  $P = 0.002$ ,  $n = 90$ ). In agreement with the ANCOVA results, the logistic regression analysis indicated that populations differed significantly

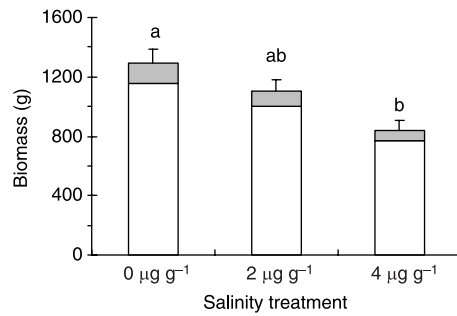


Fig. 1 Above- and below-ground biomass of *I. hexagona* (shaded and open bars, respectively) after 20 months of exposure to salinity in a common garden. Means ( $\pm 1$  standard error) shown. Letters above bars indicate significant differences at  $P < 0.05$ , based on Tukey adjusted pairwise comparisons.

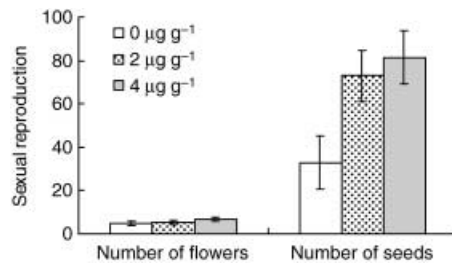


Fig. 2 Effect of salinity on sexual reproduction. Means ( $\pm 1$  standard error) shown. Significance tests between salinity treatments shown in Table 1.

in flower production ( $\chi^2 = 27.53$ ,  $P < 0.0001$ ), but salinity did not affect flowering ( $\chi^2 = 2.81$ ,  $P = 0.24$ ).

In 2002, there was a significant overall treatment effect of salinity, this time on seed mass and seed number (Wilks' Lambda = 0.365,  $F_{4,30} = 4.91$ ,  $P = 0.004$ ); however, seed number and seed mass were not significantly correlated ( $r = -0.322$ ,  $P = 0.21$ ), in agreement with the previous experiment. A subsequent univariate analysis showed that salinity only marginally affected seed number ( $F_{2,19} = 3.01$ ,  $P = 0.077$ ). The response of seeds towards salinity appeared to be non-linear, as seed production averaged  $69.6 \pm 16.2$  in  $0 \mu\text{g g}^{-1}$ ,

$95.6 \pm 27.7$  in  $4 \mu\text{g g}^{-1}$ , and  $12.3 \pm 4.2$  in  $8 \mu\text{g g}^{-1}$  salinity. The  $4 \mu\text{g g}^{-1}$  and the  $8 \mu\text{g g}^{-1}$  differed at the  $P = 0.06$  level, but other comparisons were not significant. The univariate model for average seed mass showed a strong effect of salinity ( $F_{2,19} = 6.28$ ,  $P = 0.0097$ ). The 0 and  $4 \mu\text{g g}^{-1}$  plants produced similar-sized seeds ( $0.15 \text{ g} \pm 0.01$  SE and  $0.12 \text{ g} \pm 0.01$  g, respectively,  $P = 0.213$ );  $4 \mu\text{g g}^{-1}$  and  $8 \mu\text{g g}^{-1}$  ( $0.09 \text{ g} \pm 0.01$ ) seeds did not differ significantly ( $P = 0.149$ ), but  $0 \mu\text{g g}^{-1}$  seeds were significantly larger than  $8 \mu\text{g g}^{-1}$  seeds ( $P = 0.008$ ). Natural bumblebee pollinators were scarce in 2002, and the low sample sizes (see methods) combined with high variance reduced the power of the experiment.

#### RESPONSE OF IRIS POPULATIONS TO SALINITY

There were large differences among Marsh Island iris populations in growth and reproduction (Tables 1 and 3). Plants from different populations varied widely in growth (accumulated biomass) during the 20-month experiment, which ranged from 52 g to 892 g per replicate. Furthermore, populations differed markedly in allocation to sexual and asexual reproduction. For example, some populations did not produce any new rhizomes, whereas most reproduced both sexually and asexually (Fig. 3). Populations also differed significantly in above : below-ground allocation ratios under different salinity treatments (Fig. 4).

#### SIZE-REPRODUCTION RELATIONSHIP

Plant size (total vegetative biomass) and fecundity (number of seeds) were not correlated when analysed using the full regression model, which included salinity, population, and the salinity-population interaction (Table 4). Similarly, there was no significant relationship between plant size and fecundity in the decomposed model with experimental factors and interactions removed. Log transformed size and fecundity data produced results qualitatively similar to the non-transformed data in both the full and simple models. These results are not surprising as salinity had opposite effects on

Table 3 Growth and reproduction of 10 Marsh Island *I. hexagona* populations grown in a common garden. Data are sample least square means with standard errors (in parentheses)

Population	Growth (accumulated biomass, g)	Number of flowers	Individual seed mass	Proportion below-ground	Net number of ramets
1	650 (217)	3.3 (1.7)	0.07 (0.04)	0.93 (0.01)	4.3 (2.1)
2	749 (250)	4.7 (3.3)	0.10 (0.05)	0.91 (0.02)	5.3 (2.9)
3	498 (250)	5.0 (3.6)	0.07 (0.04)	0.93 (0.01)	5.0 (2.7)
4	892 (41)	11.3 (5.5)	0.14 (0.01)	0.91 (0.01)	4.3 (1.9)
5	389 (230)	2.3 (1.2)	0.07 (0.03)	0.92 (0.02)	1.7 (1.2)
6	463 (234)	9.3 (5.8)	0.09 (0.04)	0.92 (0.00)	5.0 (2.5)
7	52 (46)	5.3 (1.2)	0.09 (0.02)	0.92 (0.04)	0.0 (0.0)
8	192 (97)	3.7 (0.3)	0.16 (0.03)	0.91 (0.01)	0.3 (0.3)
9	472 (235)	11.0 (4.5)	0.09 (0.00)	0.89 (0.01)	4.7 (2.4)
10	339 (133)	3.3 (2.0)	0.09 (0.04)	0.92 (0.01)	3.3 (1.9)

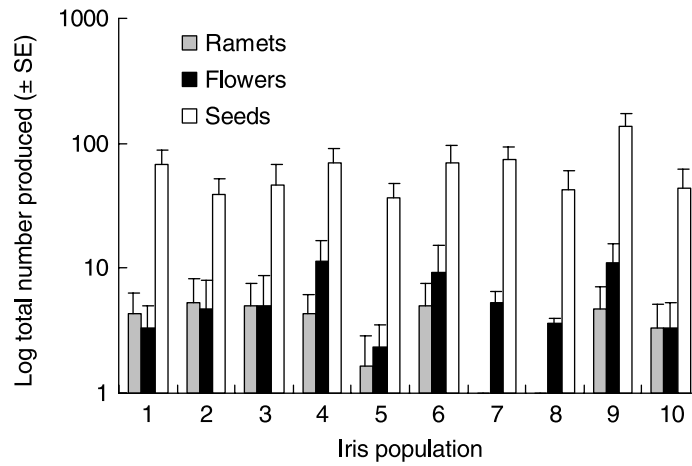


Fig. 3 Differential allocation to sexual and sexual reproduction by 10 *I. hexagona* populations grown in a common garden.

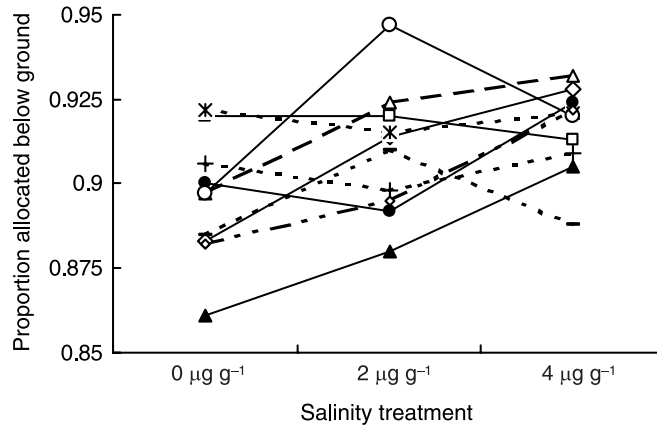


Fig. 4 Reaction norm plot of the below-ground biomass allocation of 10 *I. hexagona* populations in response to salinity. There are significant differences among populations ( $\chi^2 = 10.2$ ,  $P = 0.0014$ ) and significant salinity-by-population interactions ( $\chi^2 = 6.9$ ,  $P = 0.0086$ ).

Table 4 Linear regressions of sexual (A) and asexual (B) reproduction on plant size (vegetative mass) using raw and log-transformed data with standardized regression estimates. Salinity, population (pop) and their interaction are included. Size and logsize interactions with salinity and population were not significant and are not included in table

	$r^2$ adj	$\beta_0$	$P$	$\beta_1$	$P$	$\beta_2$	$P$	$\beta_3$	$P$	$\beta_4$	$P$
A. Fecundity models											
seeds = $\beta_0 + \beta_1$ (size)	-0.015	78.9	0.0001	0.002	0.911						
logseeds = $\beta_0 + \beta_1$ (logsize)	-0.004	1.9	0.0001	-0.055	0.388						
seeds = $\beta_0 + \beta_1$ (size) (control plants only)	-0.05	51.8	0.0212	0.006	0.98						
seeds = $\beta_0 + \beta_1$ (size) + $\beta_2$ (salinity) + $\beta_3$ (pop) + $\beta_4$ (salinity $\times$ pop)	0.128	87.9	0.0001	-0.191	0.289	-0.140	0.547	-0.248	0.318	0.249	0.432
logseeds = $\beta_0 + \beta_1$ (logsize) + $\beta_2$ (salinity) + $\beta_3$ (pop) + $\beta_4$ (salinity $\times$ pop)	0.111	2.1	0.0001	-0.305	0.233	-0.313	0.535	-0.726	0.309	1.01	0.504
B. Ramet models											
ramets = $\beta_0 + \beta_1$ (size)	0.48	1.3	0.148	0.698	0.0001						
logramets = $\beta_0 + \beta_1$ (logsize)	0.451	-1.4	0.0001	0.678	0.0001						
ramets = $\beta_0 + \beta_1$ (size) + $\beta_2$ (salinity) + $\beta_3$ (pop) + $\beta_4$ (salinity $\times$ pop)	0.508	0.552	0.443	0.785	0.0001	0.060	0.801	-0.007	0.978	-0.079	0.7784
logramets = $\beta_0 + \beta_1$ (size) + $\beta_2$ (salinity) + $\beta_3$ (pop) + $\beta_4$ (salinity $\times$ pop)	0.446	-1.19	0.0042	0.598	0.0001	-1.972	0.103	0.373	0.707	-0.122	0.938

plant size (negative) and fecundity (positive). However, not even the control plants, which were not exposed to salinity stress, displayed a significant correlation between size and fecundity (seeds =  $51.8 + 0.0005$  (size),  $R^2$  adj. =  $-0.05$ ,  $F_{1,20} < 0.001$ ,  $P = 0.98$ ). In all comparisons, the y-intercepts were significantly different from zero, indicating a minimum size at which *I. hexagona* can produce seeds (Table 4). This supports field observations that *I. hexagona* does not become reproductive until the second year (S. Mopper, personal observation).

In contrast to fecundity, ramets were highly correlated with plant size (Table 4). Log transformed ramet and size data also produced a significant relationship, as well as a significant y-intercept. Although ramets are the primary mechanism of clonal reproduction, it is difficult to interpret these results because plant size (vegetative biomass) and ramet numbers are not independent. The logistic regression analysis indicated that mass was a significant predictor of the number of flowers produced ( $\chi = 5.08$ ,  $P = 0.024$ ), and estimated a 0.05% increase in flower production for every 1 g increase in plant biomass.

## Discussion

### SALINITY AND PLANT PERFORMANCE

Salinity had both negative effects on *I. hexagona* growth and positive effects on sexual reproduction in our common garden experiment, indicating that even at low concentrations, salinity is a strong environmental cue altering growth and reproduction. Irises exposed to salinity were substantially smaller than control plants (Fig. 1), with moderate levels of salinity ( $2\text{--}4 \mu\text{g g}^{-1}$ ) reducing both above- and below-ground biomass, and increasing the above-: below-ground allocation ratio. These results are consistent with other studies showing that osmotically stressful environments inhibit plant growth (Adam 1990; Blits & Gallagher 1991; Stanton *et al.* 2000), and stimulate resource allocation to roots (McKee 1995; Bell & Sultan 1999). However, while below-ground biomass in *I. hexagona* is dominated by rhizome tissue (Fig. 1), it should be noted that our below-ground measurements included both root and rhizome mass.

In the first experiment, the sharp reductions in biomass with salinity were accompanied by a corresponding increase in fecundity. The results of the higher salinity experiment were consistent with the first experiment for the control and  $4 \mu\text{g g}^{-1}$  salinity levels. However, at the highest ( $8 \mu\text{g g}^{-1}$ ) salinity treatment, seed production declined sharply, suggesting a non-linear allocation to sexual reproduction with increasing salinity stress. Furthermore, in neither experiment was there a trade-off between seed numbers and seed size (Table 1) as observed in other species (Harper 1977; Primack 1987). Despite the decline in below-ground

biomass with increasing salinity, the number of ramets marginally increased ( $P = 0.085$ ; Table 1), suggesting a proliferation of asexual reproductive structures in higher salinity.

### COMPARISON WITH ALLOCATION MODELS

Many properties of clonal plants make it difficult to apply simple reproductive model predictions, thus necessitating the creation of models specific to plasticity in allocation for clonal plants. For example, there are inherent trade-offs between asexual propagation and sexual reproduction in clonal plants that are not apparent in annual plants or non-clonal perennials (Gardner & Mangel 1999). Furthermore, because clonal plants exhibit more complexity in their genetic and physiological identities, it is difficult to determine the individual level at which selection is occurring, making the assessment of fitness for clonal plants a difficult task (Harper 1977; Wikberg 1995; Pan & Price 2001).

Three specific models have been proposed for the allocation to sexual and asexual reproduction in clonal plants (Loehle 1987; Sakai 1995; Gardner & Mangel 1999). While each of these models predicts that sexual reproduction should be favoured when the quality of the local environment decreases or when the threat of mortality to the parent plant (ramet or genet) increases, their predictions for plants in favourable sites differ. Loehle's (1987) model predicts that more favourable conditions will decrease the cost of sexual reproduction, leading to increased seed output, but that harsh conditions that would decrease seedling establishment would curtail seed production. Our data are not in agreement with these predictions, because plants in the lowest salinity produced the greatest biomass, but lower numbers of seeds than the  $4 \mu\text{g g}^{-1}$  treatment. Furthermore, as salinity leads to linear decreases in iris seed germination and seedling establishment (Van Zandt 2001), Loehle's model would predict lower seed numbers in the  $4 \mu\text{g g}^{-1}$  salinity treatment. None of the models accurately predict the allocation to flower production of irises, as plants flowered equally in all three salinity treatments in the first experiment.

Sakai (1995) and Gardner & Mangel (1999) predict that sexual reproduction should be minimized and asexual growth maximized in the most favourable habitats, in agreement with our findings. However, all three models predict that clonal plants should increase sexual reproduction in habitats as the mortality rate of the clone increases. While we did not observe a decrease in ramet survival with increasing salinity in the first experiment, the second experiment utilized treatment levels that substantially increase the probability of mortality (Wang *et al.* 2001). Therefore, because iris seed production appears to be non-linear (stimulated at intermediate salinity and strongly curtailed at higher salinity), they appear to be more sensitive to alterations in their local salinity environments than is predicted by these models. Our results

suggest modifications in the models because while seed production increases with intermediate levels of stress, it drops considerably at salinities that approach the limits of plant tolerance. Future modelling efforts of investment in sexual and asexual reproduction in clonal plants should incorporate this non-linear response, which may reflect different physiological tolerance maxima for sexual reproduction and individual ramet subsistence.

Interestingly, natural populations of *I. hexagona* flower and develop seed from April–June, when salinity at Marsh Island is at the lowest levels, around  $2 \mu\text{g g}^{-1}$  (Van Zandt & Mopper 2002). The physiological impact of salinity stress could be affected by the pattern of exposure as well as the intensity. For example, plants can recover more rapidly from pulses of salinity than from sustained long-term exposure (Flynn *et al.* 1995; Howard & Mendelssohn 1999a,b).

There are limited data regarding the plastic reproductive strategies of clonal plants exposed to salinity stress. The clonal perennials *Sporobolus virginicus* and *Hordeum jubatum* tend to decrease sexual reproduction in higher salinity sites (Blits & Gallagher 1991; Wang & Redmann 1996), in support of the Sakai (1995) and Gardner & Mangel (1999) models. The exception demonstrated by irises in this study suggests that alternative strategies may emerge from plants with different life histories and environmental conditions (Loehle 1987; Stanton *et al.* 2000).

#### SIZE–REPRODUCTION RELATIONSHIP

Life history models predict a positive linear relationship between plant reproduction and vegetative biomass (Samson & Werk 1986; Weiner 1988), but these predictions are less clear for clonal plants because of trade-offs between sexual and asexual reproduction (Hartnett 1990; Gardner & Mangel 1999). Furthermore, the relationship between plant size and reproduction can be influenced by species identity, genotype, competition and environmental conditions (Aarssen & Taylor 1992; Schmid & Weiner 1993). Our study found no correlations between size and fecundity in *I. hexagona* (Table 3). This cannot be attributed solely to a stress-induced reduction in biomass because there was no size–fecundity correlation in the control plants grown in fresh water. There was strong evidence for a minimum size for sexual reproduction, indicated by highly significant y-intercepts (Table 4). This is common in plants (Schmid *et al.* 1995) and consistent with published *Iris* life history information that plants do not reproduce until the second year of growth (Caillet & Mertzweiler 1988).

#### RESPONSE OF POPULATIONS AND INDIVIDUALS TO ENVIRONMENTAL STRESS

*Iris hexagona* populations showed wide variation in growth and reproduction when grown in a common garden (Table 1, Figs 3 and 4), consistent with mole-

cular evidence for genetic differentiation. Both RAPD (randomly amplified polymorphic DNA) and SSR (single sequence repeats, or microsatellites) DNA markers indicate that *I. hexagona* populations are differentiated (unpublished data, Jerry He and Susan Mopper, University of Louisiana Lafayette, and Alan Meerow, USDA-ARS-SHRS, National Germplasm Repository, respectively).

The significant variation among populations in above- : below-ground allocation lends support to theoretical models describing genotypic selection in clonal plants, which can occur when genetic traits are associated with differential ramet production (Pan & Price 2001). However, evidence for local adaptation is inconclusive at this time. It is likely that sexual and clonal reproduction play important roles in the survival and persistence of *I. hexagona*, which invests heavily in both modes of reproduction. Identifying the mechanisms underlying genetic structure is difficult in our study system because geographical distance and environmental variation are confounded. Microsatellite surveys and local adaptation common garden experiments are currently underway to separate these factors.

*I. hexagona* has no specialized structures to compartmentalize or excrete excessive sodium, and it accumulates in all tissues (including flowers) except rhizomes (Wang *et al.* 2001). Plants begin to senesce and will die if salinity is sustained at  $10 \mu\text{g g}^{-1}$  (Wang 2002; S. Mopper, personal observation). Salinity induces the production of osmoprotectants in *I. hexagona*, including betaine, proline and dimethylsulphoniopropionate (DMSP), in addition to magnesium and manganese, which ameliorate the deleterious effects of salts (Wang 2002). In addition, foliage nitrogen and stress-response hormones are altered by salinity stress (Mopper *et al.* 2003). Individual variation in physiological salinity tolerance, combined with salinity-induced seed and/or ramet production, could select for novel salt adapted *I. hexagona* genotypes, and ensure continuing survival as wetland environments increase in salinity. As salinization is occurring in many regions of the world, more research is needed to understand plant responses to this growing environmental stress.

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