THE REPRODUCTIVE ECOLOGY OF *VALLISNERIA AMERICANA*: THE EFFECT OF SEED PRE-TREATMENT AND SALINITY OF GERMINATION AND THE EFFECT OF SALINITY ON SEEDLING GROWTH AND SURVIVAl

A Thesis

Submitted to the Graduate Faculty of the
University of South Alabama
in partial fulfillment of the
requirements for the degree of

Master of Science

in

The Department of Biology

by
Richard Duane Clark Jr.
B.S., Troy State University, 1994

May, 1999

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COLLEGE OF ARTS AND SCIENCES

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In Memory of Rayna Denise Clark and Gregory Allen Worthington
for their love and support. Though they are not with us now,
they will always be missed.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Clark Jr., Richard Duane. M.S., University of South Alabama, May 1999. The reproductive ecology of *Vallisneria americana*: The effect of seed pre-treatment and salinity on germination and the effect of salinity on seedling growth and survival.

The number of prolific *Vallisneria americana* beds has declined over the past 30 years. Experiments on how salinity effects *Vallisneria* seed germination, seedling survival and seedling growth were conducted to determine the best method of establishing *Vallisneria* beds in Mobile Bay. Studies revealed no significant difference in the percent germination of seeds in salinities of 0, 2, 4, 6 and 12ppt, although there was a slight decrease in percent germination at 12ppt. Three methods of pre-treating *Vallisneria* seeds were tested to see which was the best method for stimulating the seeds to germinate. Seed coat abrasion with sand paper showed a significantly higher percent germination than the control (no pre-treatment), the cold shocked or acid shocked treatments. Seedling growth and survival was monitored for 56 days at salinities of 0, 2, 4, 6 and 12ppt. Seedling growth was highest at the lower salinities of 0, 2, and 4ppt. The data presented shows the 6ppt plants had the highest growth at the end of the experiment, however, the smaller plants at this salinity were dying off leaving the tallest seedlings to be measured. Seedling survival was highest in the 12ppt seedlings initially because very few seedlings were actually germinated at this salinity. Seedling survival was highest at the lower salinities of 0-4ppt.

BACKGROUND AND LITERATURE SURVEY

Morphology

Vallisneria americana is a common, submergent aquatic macrophyte found in the upper portions of Mobile Bay, Alabama. Historically, this plant thrived further south in Mobile Bay around Point Clear, Alabama., but currently there are no plants living in that area and the reasons for its disappearance are not known. Studies performed on the reproductive ecology of *Vallisneria americana* could provide insight into why this plant has disappeared in certain areas, whether due to physical or biological reasons or to some combination of the two.

Vallisneria americana, referred to as wildcelery, tapegrass, or eel-grass, is a dioecious perennial plant with fibrous roots and is found submerged in freshwater to moderately saline water (salinity 0-12ppt) (Twilley and Barko, 1990). *Vallisneria* is found primarily in eastern North America, occurring west from Nova Scotia to South Dakota and south to the Gulf of Mexico (Lowden, 1982) (Figure 1). *Vallisneria americana* plants have linear submerged or floating leaves that are strap- or tape-shaped extending up to 2 meters or more. The stem is vertical with a short axis that may bear stolons. Lowden, (1982) recognized that *Vallisneria americana* may have a narrow or broad-leaved form. The narrow-leaved form has a leaf that is approximately 10mm wide while the broad-leaved form is approximately 10-25mm wide. The margins of the blades are

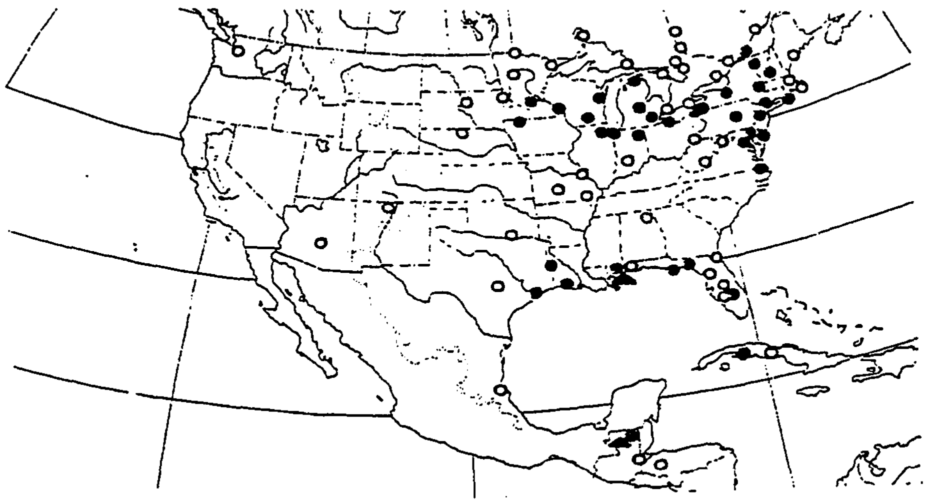

Figure 1. Distribution of *Vallisneria americana* in North America: bisexual populations with solitary female flowers (solid circles), umbel or spike-like inflorescences (triangles), and representative unisexual or sterile collection (open circles) (Lowden, 1982).

entire to slightly toothed. Hunt, (1963) found the optimal depth for *Vallisneria* growth in the Detroit River was 30cm, and Carter and Rybicki, (1985) indicated that it could grow at light levels as low as 10% of surface irradiance. It has been known to grow in gravel to hard clay and can be present in both lotic and lentic environments.

Reproductive Phenology

Vallisneria is capable of both asexual and sexual reproduction. The interplay between sexual reproduction and clonal growth in dioecious plant species has been the subject of recent investigations. Lowden, (1982) determined the distribution of three reproductively distinct populations of *Vallisneria americana* in North America (Figure 1). Doust and Laporte, (1991), also looked at population sex ratios and population mixtures of *Vallisneria* and found that vegetative clonal growth was strongly favored over sexual reproduction. *Vallisneria* tends to show spatial segregation between male and female beds, rarely with male and female plants cohabiting the same area. Additionally, as with most submerged macrophytes, *Vallisneria* exhibits aggressive clonal growth and spreading. Clonal growth in *Vallisneria* may be accomplished by stolon growth and the production of winter buds.

Most vegetative reproduction in this species occurs by stolon reproduction, although some consider this merely vegetative growth of one plant rather than reproduction. A plant will produce a stolon horizontally through the substrate and at the tip of the stolon a new leaf rosette and root system will form. This is probably the most effective means for *Vallisneria* propagation because the new tissue is protected from displacement by currents since it remains connected to the parent plant until established or until the stolon is broken.

Winter buds, sometimes called turions or tubers (Figure 2), are produced in the late summer from stolons that grow down into the sediment. The remaining rosette is separated from decomposing stem tissue. One plant can produce as many as four winter buds, which add to the aggressive nature of vegetative spread. The plant overwinters as a winter bud and emerges from the substrate in the spring. It has been noted that ducks will feed preferentially upon winter buds, and on leaves to a lesser extent (Korschgen and Green, 1988). Stolon reproduction, as well as winter bud formation may well be the cause of the spatial distribution or colony patchiness of male and female plants.

Vallisneria americana populations can be divided into two ecotypes, a northern and a southern (Smart *et al.*, 1996). The northern ecotype produces perennating winter buds while the southern ecotype does not produce winter buds. However, the geographic limits of these populations have not been established.

Samples taken in the fall and winter in Mobile Bay, Alabama have failed to reveal the production of winter buds, possibly because the winters are not cold enough to stimulate winter bud production. In areas of Mobile Bay that are not exposed at low tide, green leaves can be found year round (personal observation).

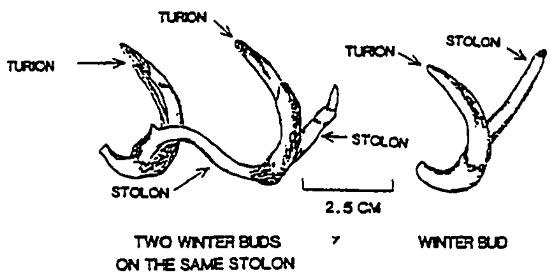

Figure 2. Gross morphology of *Vallisneria americana* winter buds (Donnermeyer, 1982).

Vallisneria also reproduces sexually and Figure 3 shows the morphological differences between male and female flowering plants. The pistillate flower is born on a pedicel that increases in length by cell elongation to carry the flower to the air-water interface for pollination. The male inflorescences originate submerged in the axils of leaves. The male inflorescence consists of about 2,000 flowers, each less than 1mm long (Wylie, 1917). When the male inflorescence matures, it will break open at the base of the plant and the tiny male flowers will float to the surface to be dispersed by air or water currents. At the water's surface, the male flower may encounter a female flower. The male flower may be captured by a female flower during temporary immersion by a wave, or simply get blown into the female flower by wind or wave action (Korschgen and Green, 1988).

After pollination occurs, the pedicel contracts below the water surface where fruit development takes place. The fruit contains approximately 250 seeds, 1-2mm in length, embedded in a gelatinous matrix and covered by an outer resistant tissue layer. The fruit can either rupture and release the seeds and the gelatinous matrix that surrounds them, or the fruit can break free and settle to the bottom where germination takes place within the fruit (Kaul, 1978). Choudhouri, (1966) determined that 30-35°C is the optimum germination temperature for *Vallisneria* in more tropical zones. Sexual reproduction of *Vallisneria* is influenced by water depth and turbidity, pH, salinity, type of substrate, temperature, longevity of seeds, and herbivory (Korschgen and Green, 1988).

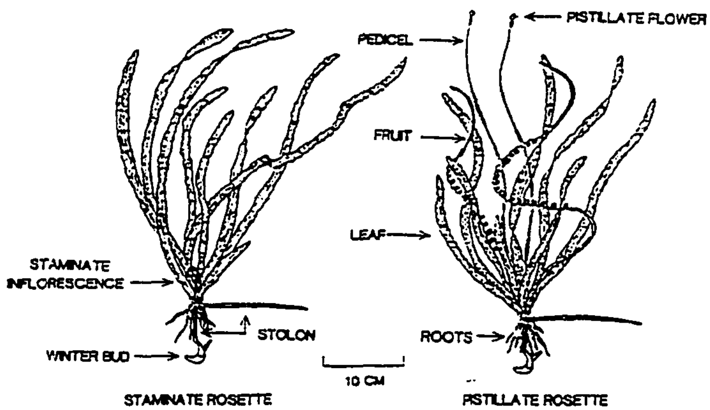

Figure 3. Gross morphology of male and female plants of *Vallisneria* (Donnermeyer, 1982).

Reproductive Ecology

Water depth and Reduced Light Intensity

Vallisneria has limited elongation potential which results in an inability to concentrate photoreceptive material at or near the water surface in low light environments and deep water (Barko *et al.*, 1984). Through physiological adaptation to low light regimes, *Vallisneria* grew at 4.5% surface light intensity in Trout Lake, Wisconsin (Spence and Chrystal, 1970a,1970b). Meyer *et al.*, (1943) found that *Vallisneria* exhibited a constant decrease in photosynthesis with increasing depth of immersion, but maintained an appreciable rate of photosynthesis to a depth of 10m where light intensity was 0.5% of that at the surface.

Doust and Laporte, (1991) found that *Vallisneria* plants that did not flower were approximately one half the biomass of those plants that did flower. It is possible that light-limited plants may not reach the biomass necessary for reproduction.

Another problem associated with deep-water reproduction is that *Vallisneria* needs to be able to get its female flower to the water surface for pollination (Donnermeyer, 1982) and if the water is too deep, pollination may not occur.

Water pH

Titus and Stone, (1982) found that the dissolved inorganic carbon (used in photosynthesis) uptake rates by *Vallisneria* declined with increasing pH, declining by 61% from pH 8 to 9. They also showed that a low pH did not directly affect *Vallisneria* growth but the lowered bicarbonate levels due to low pH may result in carbon becoming a major limiting nutrient. Titus and Hoover, (1993) found that seed production in

Vallisneria decreased with decreasing pH and, at a pH of 5, seed production was severely restricted. They also found that winter bud formation was restricted with decreasing pH. This suggests that the problems associated with acid rain or lake acidification might affect the reproductive ecology of a *Vallisneria* population.

Substrate

Vallisneria grows in substrates ranging from gravel to hard clay, but grows best in silty sand (Hunt. 1963). Hunt believed that the only substrate unsuitable for *Vallisneria* establishment was a soft, shifting one. In the Potomac River, the majority of winter buds were between 10 and 20cm deep in silty clay and between 5 and 15cm in sand (Rybicki and Carter. 1986). Carter *et al.*, (1985), in laboratory experiments, showed that the emergence of winter buds was affected by the depth of substrate. Most winter buds emerged from the substrate when buried by 15cm of sediment, only 25% emerged when covered by 20cm of sediment and none emerged when covered with 25 to 55cm of sediment. Therefore, a heavy influx of sediment could destroy a *Vallisneria* bed if the winter buds become buried too deeply.

Temperature

The growth of *Vallisneria* becomes restricted at temperatures below 20°C (Hunt, 1963). *Vallisneria* grew at temperatures of 19 to 31.5°C in the Detroit River (Hunt, 1963) and from 22.7 to 26.3°C in Lake Erie (Meyer *et al.*, 1943). These differences in temperature tolerance ranges between the two sites are probably due to the genotypic differentiation between the two populations of *Vallisneria*. Typically, temperatures below

19°C arrested growth and temperatures of 40°C or higher caused *Vallisneria* to become limp and disintegrate (Wilkinson, 1963). The only part of *Vallisneria* to overwinter in cold temperate environments are the tubers or winter buds, and perhaps the seeds. In warm temperate and subtropical environments, green shoots can be found year round and winter bud production has not been observed. A decrease in temperature, or day length, or both, might trigger winter bud production, but the major controlling factor has not been determined.

Herbivory

Herbivory is one of the more important aspects of *Vallisneria* reproductive ecology because the principal edible part of the plant is the winter bud. Many ducks, muskrats, red-bellied sea turtles, and carps feed on *Vallisneria*, especially the winter buds. The blades are grazed upon by snails and by many other micro- and macroinvertebrates. The consumption of buds is restricted to vertebrates and invertebrates that can dive and uproot the winter buds from deep within the sediment. In upper Mobile Bay, coots have been known to eat the entire plant in shallow and exposed areas during the late fall (personal observation). Korschgen and Green, (1988) found that canvasback ducks consumed as much as 40% of the standing crop of *Vallisneria*, but it is not known if the ducks graze upon the fruits. Juvenile blue crabs and small shrimps, however, have been known to feed upon seagrass seeds (McMillan, 1991).

Salinity

Although *Vallisneria* is considered a freshwater plant. it will grow in water with elevated salt concentrations. Mature plants can survive at salt concentrations up to 12ppt if allowed to acclimate, but will perish if the salt concentrations get any higher (Hunt. 1963; Twilley and Barko, 1990). Twilley and Barko referred to *Vallisneria* as a halophyte because it can tolerate a wide range of salinities as long as the salinity was constant or if the salinity change was gradual. Their studies revealed that mature *Vallisneria* plants, when acclimated to salinity increases of 1ppt per day, showed no significant difference in leaf biomass or number of inflorescences in salinities of 0, 2, 4, 6, and 12ppt. Studies by Bourn. (1932, 1934), and Haller *et al.*, (1974) demonstrated the effect of a sudden increase in salinity when adult plants were taken from fresh water and immediately exposed to higher salinity treatments. Haller *et al.*, (1974) reported that growth occurred over a range of 0.17ppt to 3.33ppt, but that no growth occurred above 6.66ppt. Bourn, (1932, 1934) showed that growth peaked at 2.8ppt and that no growth occurred above 8.4ppt. These studies show how storm driven or other sudden influxes of saltwater can damage a *Vallisneria* bed, but a gradual change up to 12ppt would not have the same significant effect on the adult plants. *Vallisneria* may be unable to use the short term method of osmoregulation in which potassium is used as an inorganic osmoticum (Rhodes and Hanson, 1993). Increasing the potassium level over long periods could inhibit metabolic activity so that *Vallisneria* might be better adapted to using organic osmotica, such as proline, glycine betaine or dimethyl sulfonioproprionate (DMSP). This has not been demonstrated for *Vallisneria*, but has been reported in studies of other halotolerant plants (Rhodes and Hanson, 1993).

Sexual reproduction is an important means of spreading for *Vallisneria americana*, but the effects of salinity on *Vallisneria americana* seed germination and seedling survival have not been studied. This may be a critical factor in survival and colonization in estuaries with significant tidal influences. Estuarine populations have been poorly studied and their ecology is not well understood.

RESEARCH HYPOTHESIS

The effects of increasing salinity on the sexual reproduction of *Vallisneria americana* has not been previously examined. Various laboratory and field experiments were implemented to test the following four hypotheses:

H_{01}: Germination of *Vallisneria americana* seeds is similar in salinities of 0, 2, 4, 6 or 12ppt.

H_{02}: Germination of *Vallisneria americana* seeds is similar when the seeds are given no pre-treatment or when they are pre-treated with acid shock, cold shock, or abrasion.

H_{03}: Seedling survival of *Vallisneria americana* is similar in salinities of 0, 2, 4, 6 or 12ppt.

H_{04}: Average seedling growth rates are similar in salinities of 0, 2, 4, 6 or 12ppt.

Pilot studies

Water level, salinity, and light intensity were monitored every 15 days during 1995 and 1996 at three sites (Figure 4). Two experimental sites, Montrose and Point Clear, where *Vallisneria* beds once thrived (personal communication with property owners) and one control site at Meaher State Park where *Vallisneria* beds flourish. For 1995 and 1996, the mean salinity level off the shore of Montrose, Alabama was $5.45^{0}/_{00}$ and at Point Clear, Alabama it was $9.01^{0}/_{00}$. However, after Hurricane Opal, on September 27, 1995, these two areas were flushed with a saltwater storm surge of $18.5^{0}/_{00}$ at Montrose and $20.4^{0}/_{00}$ at Point Clear (personal observation). When these areas were sampled one month

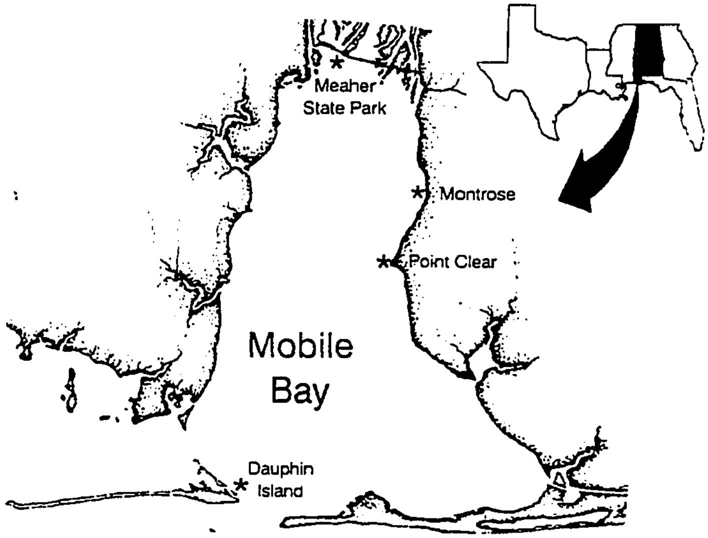

Figure 4. Map of the collection site and sites where *Vallisneria* had previously flourished.

later, the salinities were at $5.2^0/_{00}$ and $10.9^0/_{00}$ respectively. It was not known how long these areas were flooded with water of higher salinity. These two areas once supported large *Vallisneria* beds, however, higher salinity levels may inhibit recolonization by *Vallisneria* sexual propagules. We know from the literature that adult *Vallisneria* plants can tolerate up to 12 ppt. but the tolerance levels for *Vallisneria* seeds and seedlings has not been demonstrated. The low tolerance level for oyster survival is 10 ppt. (Tatum *et Alabama,* 1995), and the remnants of oyster reefs (*Crassostrea virginica*) have been found in these areas; therefore it is possible that these areas are no longer able to support a *Vallisneria* population. Additionally, both in Montose and Point Clear, beds of the more halotolerant wigeon grass, *Ruppia maritima,* periodically occur in very sparse, small patches. The presence of oysters and *R. maritima* may indicate increases in salinity in recent time. The increase in salinity, if discernible, is not constant because there are no currently surviving oyster reefs above Weeks Bay along the eastern shore of Mobile Bay, although there was a reef just below Point Clear in 1970 (Tatum *et Alabama.* 1995) and local residents reported reef development in 1989-1990, but as of 1996 no oyster reefs were present. Therefore, another possible reason for the decline in *Vallisneria* beds along the eastern edge of the Bay may be substrate composition. A property owner in Montrose, Alabama, reported transplanting adult *Vallisneria* plants to his shallow shoreline area. Initially they grew quite well, but during the fall the plants became uprooted from the shifting soil caused by heavy storms. Although floating *Vallisneria* fruits have the capability to disperse to these areas quite easily, colonization would be difficult if the seedlings could not establish themselves in the shifting substrate and since 1996 the establishment of new beds near Montrose and Point Clear has not occurred.

McMillan (1991) examined the longevity of seagrass seeds and found that the seeds of many species could remain viable for 5 years or more, however, the duration of viability for *Vallisneria* seeds is unknown. Preliminary data generated for the current study reveals that *Vallisneria* fruits have the capability of floating an average of 33 days with some fruits floating up to 70 days (Table 1). Seed longevity would allow time for the fruits or individual seeds to be transported by water currents away from the original population and thus allow for effective seed dispersal and colonization. Intact fruits have been found washed up on the beach on Dauphin Island, Alabama, approximately 30 miles south of the nearest beds. These fruits were collected in waters with a salinity of 18ppt and germinated in the lab at both 0ppt and 12ppt (seedling survival was not monitored). Sediment cores from Meaher State Park, the site of a prolific *Vallisneria* bed, revealed an extensive seed bank with seed numbers reaching up to 25,000/m^{-2} (Figure 5). However, sediment cores from Montrose and Point Clear contained no *Vallisneria* seeds. Fruits and leaves were found washed up on two occasions at both sites in July 1996, showing that dispersal to these areas could occur but the seeds or seedlings do not seem to persist because no seedlings have been found. Research on *Myriophyllum spicatum* and *Potamogeton pectinatus* have shown that their seed germination and seedling survival rates are high under laboratory conditions but there is an apparent absence of seedling survival *in situ* (Patten, 1956; Amundsen 1978; R. J. Van Wijk, 1989).

Table 1. ***Vallisneria*** **fruit flotation. Forty fruits were collected on each date and monitored daily until 100% sank.**

Collection Date	Percent Floating	Days to 100% Sink
7/12/95	53.5	29
7/29/95	11.5	17
8/15/95	27.3	14
8/30/95	29.5	53
9/13/95	14.3	15
9/30/98	13.8	70
$\bar{x}$	22.7	33

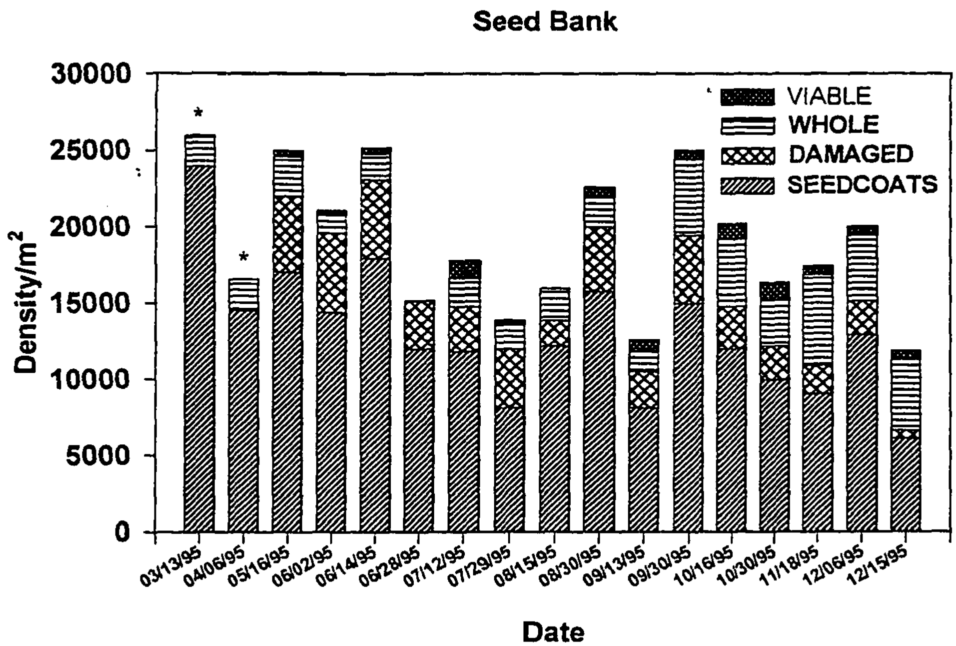

Figure 5. The seed bank of *Vallisneria* seeds/m^2 at Meaher State park. Seedcoats, empty but intact seedcoats; Damaged seedcoats and whole seeds that are physically damaged and would not germinate; Whole-intact seeds; Viable-whole seeds that germinate after stimulation. Sediment cores were taken with a cylinder tube (d=3.5cm, h=10cm).

There is no permanent prevailing current in Mobile Bay, but water does tend to be more saline on the eastern shore (personal communication Will Schroeder, 1996). This implies that the gulf water moves in and up the eastern shore while the fresher water is pushed down the western shore which might explain why few fruits/seeds are dispersed to the eastern shore. This is not always the case, however, since most of the currents in Mobile Bay are wind and tide driven, giving no real prevailing current (personal communication Will Schroeder, 1996).

It is not known if *Vallisneria* seeds over-winter in the sediment or germinate immediately after release from the fruit. Over-wintering would probably be the best strategy for survival because a seed produced in the late summer may not have time to germinate, grow, and produce adequate biomass to survive or make winter buds before the onset of winter. Ferasol *et Alabama* (1995) found that cold treating *Vallisneria* seeds did not stimulate or enhance germination. In Mobile Bay, winter bud production is not observed, so the seeds could germinate at any point and may survive the winter in areas that are not exposed at low tide, but this is not known.

Very few studies have examined the effects of salinity on freshwater emergent or submerged plant seed germination. Delesalle and Blum, (1994) examined the effect(s) of salinity on seed germination for a freshwater emergent, *Sagittaria latifolia* (arrowleaf) and compared maternal families of *Sagittaria* to see whether different families react differently to the same salinity. They found that in all cases salinity decreased germination, delayed emergence, and decreased survival and growth rates of all the families. McKee and Mendelssohn, (1989) found that mature *Sagittaria lancifolia* plants could survive up to 9ppt. but started showing signs of tissue damage at 4.8ppt. *Sagittaria lancifolia* is usually found along the shore of a body of freshwater or in a freshwater marsh. In Mobile Bay, it is located near prolific *Vallisneria* beds (field observations 1995-1996). Since the two species are found in the same general areas, increased salinity may have a similar affect on *Vallisneria*, but there is no data to support this assumption.

MATERIALS AND METHODS

Seed germination

Two hundred ripe fruits of *Vallisneria americana* were collected in the field and returned to the lab on June 5, 1996. Each fruit was measured for length, weight, and the number of seeds. Preliminary tests used 200 intact fruits in different salinity treatments to test the effects of salinity on germination rates. Thirty-five percent of the fruits ruptured and the seeds germinated while sixty-five percent of the fruits did not dehisce after one and one half years and those fruits contained seeds that had germinated within the fruit and had perished without fruit rupture. Additionally, leaving the fruit intact may have buffered seeds from any salinity effects; therefore, the data from this first year could not be analyzed.

For the second year of experimental studies. two hundred fruits were cut open and the seeds removed. Seeds were then rinsed thoroughly to remove the gelatinous matrix. The seeds from each of the two hundred fruits were kept separate in petri dishes and were divided into 4 pre-treatment groups (50 dishes each). Three standard seed pre-treatments were used consisting of: acid-shock, seed coat abrasion, cold-shock, along with a control group of seeds (no pre-treatment). See a description of the pre-treatments below.

* Acid shocking: seeds dipped into concentrated H_2SO_4 for two seconds then rinsed gently with distilled water (John Burns, personal communication for *Vallisneria* seeds from southern Louisiana).

* Abrasion: seeds gently rubbed between two pieces of fine grade (400 grit) sand paper for 10 seconds.

* Cold shock: submerged seeds placed into a refrigerator for two weeks at 4°C then removed to an environmental chamber at 26°C.

* Control: seeds removed from the fruit and submerged in the petri dishes at 26°C.

Following pre-treatment, the seeds were exposed to one of five salinity levels: 0, 2, 4, 6 or 12ppt. (10 dishes each). For clarification of this experimental setup, see Table 2. The number of seeds per petri dish varied depending on how many seeds were in the individual fruit.

The water in each petri dish was replaced weekly with distilled water at the appropriate salinity enriched with the following nutrients (after Smart and Barko, 1985): $CaCl*2H_20$ @ 91.7mg/L, $MgSO_4*7H_20$ @ 69.0mg/L, $NaHCO_3$ @ 58.4mg/L, $KHCO_3$ @ 15.4mg/L and Instant Ocean to the desired salinity.

The salinity treatments began on June 25,1996. Experimental light and temperature conditions were maintained in a walk-in environmental chamber. The temperature was set at 26°C to match the peak summer temperature of the sediment-water interface at the fruit collection site, and light was provided by using Phillips F40 AGRO 40-watt fluorescent bulbs located approximately 30cm above the petri dishes. LiCor light intensity measurements were 64μmol/m^2/s PAR(comparable to measured field conditions at a water column depth of 1m). The lights were placed on a timer giving 13.5 hours of daylight in a 24 hour period, similar to natural cycles during the summer months.

The petri dishes were monitored daily for one month for percent germination and the seedlings produced were used in the following experiment.

Table 2. Design of germination experiment.

Pre-treatment	Salinity Treatment (ppt.)				
	0	2	4	6	12
Acid-shock	Seeds of 10 fruits in	Seeds of 10 fruits in	Seeds of 10 fruits in	Seeds of 10 fruits in	Seeds of 10 fruits in
Abraded	"	"	"	"	"
Cold-shock	"	"	"	"	"
Control	"	"	"	"	"

Seedling survival and growth

Laboratory experiments

As they germinated, the first thirty seedlings in each petri dish were planted in small cups containing sediment from the seed collection site. Five seedlings per cup were placed in an aquarium with water at the same salinity as that used for germination, and under the same lighting and temperature conditions. An average height of the five seedlings in each cup was measured weekly for two months by submerging a small ruler in each cup. Water was replaced weekly with enriched water as above. After fifty-six days, the seedlings were removed from the sediment and an exact height measurement from base to the tip of the tallest leaf was recorded.

Field experiments

The germination of *Vallisneria* seeds was tested under natural conditions in Mobile Bay, Alabama. Three sites were selected for field experiments: Montrose, Alabama. Point Clear, Alabama, and Meaher State Park, Alabama (where the fruits were collected). Ten fruits were planted just below the sediment surface in each of four plastic trays per site. Each tray was covered by clear 1/4 in. mesh and placed in the field at a mean depth of 1m. just off shore at each site. The seeds were not removed from the fruit or pre-treated in hopes of duplicating exactly what happens in the field. They were checked semi-monthly for four months and salinity, germination rate, and seedling survival was monitored.

To test for seedling survival, one hundred lab-grown *Vallisneria* seedlings, approximately 4-5cm tall, were planted in 4 trays (25 ea.). Seedlings were grown at 4ppt to help prevent salinity shock when transplanted to the field. Two of the trays were placed just off shore at a mean depth of 1m., on the eastern shore of Mobile Bay, at Montrose and at Point Clear on July 25, 1996 and monitored for seedling survivAlabama On the date of planting the salinity at Montrose and Point Clear was 4.4ppt and 10ppt respectively. To help prevent the seedlings from being uprooted they were planted into a nylon bag of site sediment. Small holes were made in the bag and the seedlings were inserted into the sediment. Seedlings were not placed at the control site (Meaher State Park) because previous studies indicated that the trays were quickly colonized by epiphytes and *Rangia* clams (see results). The experiment was duplicated on October 29, 1996.

Data analysis

Data analysis was conducted with SAS statistical software. In the germination experiment, the independent variables were salinity and seed pre-treatment, and the dependent variable was percent germination. A one-way ANOVA was performed between germination percent and seed pre-treatment, and between germination percent and salinity. To test for interaction between seed pre-treatment and salinity, a two-way ANOVA was run. One-way ANOVAs were also used to test the dependent variables of growth rate and survival, with the independent variable of salinity. All ANOVAs were run with general linear models procedure followed by Student-Newman-Keuls test to look for significant differences between means.

RESULTS

Seed Germination

Laboratory experiments

There was no evidence of interaction between seed pre-treatment and salinity level (Table 3, p=.0001), so analysis of seed pre-treatment effects was performed with all salinity levels combined and analysis of salinity effects was performed with all pre-treatments combined.

Seeds pre-treated with abrasion had a significantly higher percentage germination, with a mean value of 30.23% (minimum value of 0.8% and maximum value of 78.4%), than the control and other pre-treatment groups which had a mean percent germination of less than 1.25% (Figure 6)(Table3, p=.0001). The cold shock and acid shock groups were not significantly different from the control group which had no pre-treatment and yielded the lowest mean percentage germination at 0.85% (minimum value of 0 and a maximum value of 8.9%).

The five salinity levels tested had no apparent effect on percentage germination with all pre-treatments combined (Table 3, p=.7214). Figure 7 shows percentage germination for all five salinity levels. There was a slight decrease in germination at 12ppt but it was not significant. All the germination values were low, including the control group (0ppt).

Table 3. ANOVA Results of hypothesis tested.

Variables	P value
Two way Anova: Test for interaction between seed pre-treatment and salinity level	p=.0001
One way Anova: Germination and seed pre-treatment	p=.0001
One way Anova: Germination and salinity	p=.7214

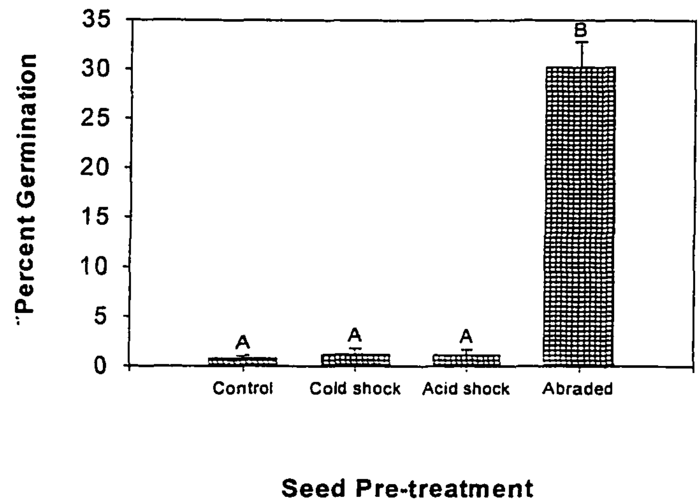

Figure 6. The results of seed pre-treatment on percentage germination with all salinities combined. (Values with the same letter are not significantly different)

Table 4. ANOVA results of germination and seed pre-treatment.

ANOVA Results					
Source	DF	Sum of Squares	Mean Square	F Value	Pr $_{c}$ F
Model	3	3.18448353	1.06149451	111.90	0.0001
Error	196	1.85932191	0.00948634		
Corr. Total	199	5.04380543			

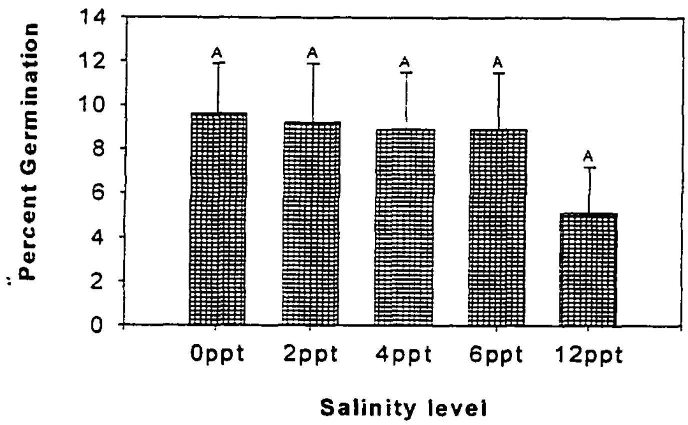

Figure 7. The result of the salinity effects on percent germination with all pre-treatments combined (p=0.7214).

Table 5. ANOVA results of germination and salinity.

ANOVA Results					
Source	DF	Sum of Squares	Mean Square	F Value	$Pr_c F$
Model	4	0.05320077	0.01330019	0.52	0.7214
Error	195	4.99060467	0.02559284		
Corr. Total	199	5.04380543			

Field experiments

No seedlings were produced from the fruits planted in trays at the collection site Meaher Park, or at Montrose and Point Clear. The trays at Meaher Park site were quickly colonized by *Rangia* clams and after four months a tray sediment sample revealed no seeds remaining. The trays at Montrose and Point Clear also revealed no seeds after four months and when the mesh was removed from the trays, juvenile blue crabs were found. They appeared to have settled into the trays as small juveniles and had grown up in the trays.

Seedling survival and growth

Laboratory experiments

Figure 8 shows the results throughout the 56 day survival experiment. Higher salinity levels significantly affected seedling survival and growth. There was no significant difference in the percentage of seedlings surviving up to day 14, all ranged between 64.40% to 76.47% survival, except for the 12ppt seedlings that had a 91.57% survivAlabama The 12ppt seedlings had a significantly higher percent survival up to day 28, but declined quickly thereafter with almost no growth throughout the entire experiment (Figure 9). There was a suspended algae problem in the 0ppt and 2ppt aquariums which may have had an adverse effect on seedling growth and survivAlabama The water was exchanged weekly, but the algae quickly returned to compete with the seedlings for the available light and nutrients. There was no significant difference in plant survival between the 0, 2, and 4ppt plants over the 56 day experiment. On day 56 the highest percent survival between these three levels was 63.13%, at 4ppt followed by 55.82% at 2ppt and 50.60% at 0ppt.

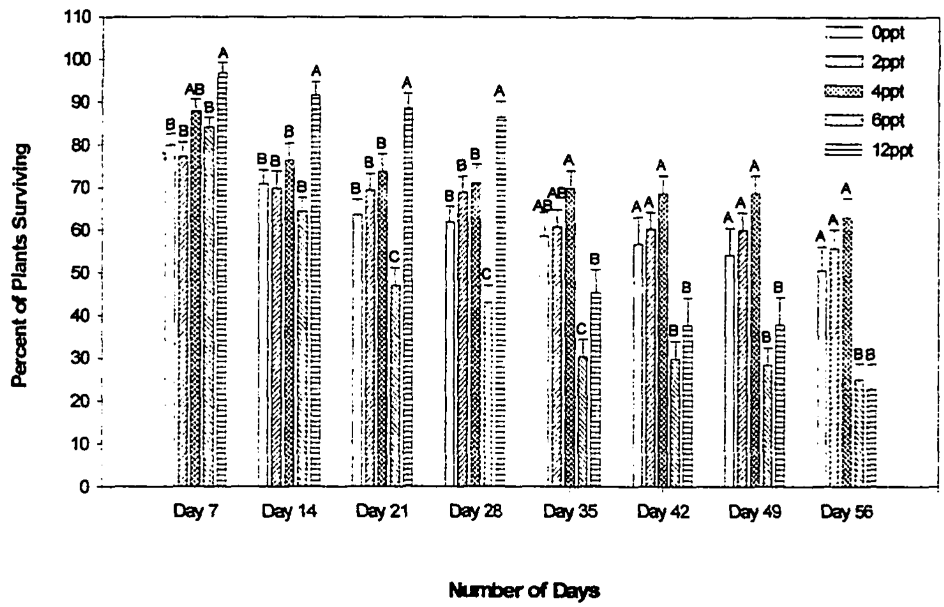

Figure 8. The percentage of plants surviving over the 56 day experiment. One way ANOVAs were run between the five salinity levels for each week. Bars with the same letter are not significantly different.

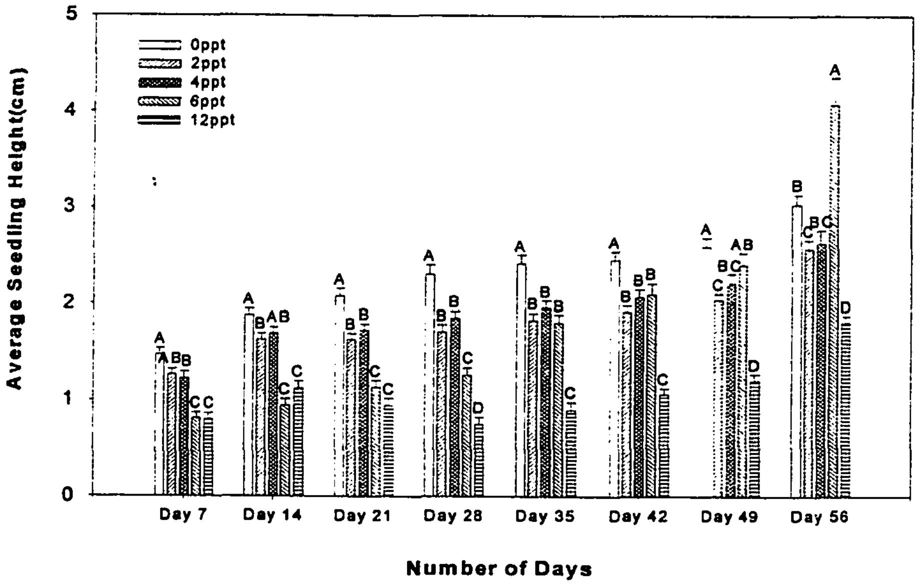

Figure 9. Average plant height over the 56 day experiment (values were taken every 7 days). One way ANOVAs were run between the five salinity levels for each week. Bars with the same letter are not significantly different.

Seedling growth was highest at 0ppt throughout the majority of the experiment and the general trend was slower growth at higher salinity (Figure 9). At the end of the experiment the 0ppt seedlings had an average height of 3.03cm. The height of 2ppt and 4ppt seedlings remained similar throughout the experiment and were never significantly different, being 2.56cm and 2.62cm respectively, at the end of the experiment. At day 42 the remaining 25.08% seedlings from the 6ppt group showed an increase in height and by day 56 were the tallest seedlings; However, at this time the smaller seedlings of this group were dying off (Figure 8) leaving only the tall seedlings. These remaining 6ppt seedlings generally seemed unhealthy with long, thin, yellowing leaves.

Field seedling experiment

Fifteen days after the planting date, on July 25,1996, the plants were checked and of the 50 initial plants at each site, only 8 were present at Montrose and 2 at Point Clear. The salinity levels had risen to 8.9 ppt at Montrose and 13.6 ppt at Point Clear. The plants were checked 15 days later and none were present at either site. The duplicate experiment on October 29, 1996 was initiated with salinity levels at Montrose and Point Clear at 5.9 ppt and 15.9 ppt respectively and all perished within 15 days.

DISCUSSION

The low salinity levels tested did not significantly affect germination. As mentioned earlier, some fruits were collected that had washed onto the beach of Dauphin Island Alabama, some 30 miles south of any known *Vallisneria* bed. These fruits had been floating in 18ppt sea water for several days before they were washed onto the beach, returned to the lab, and seeds removed from the fruits were placed into freshwater. The seeds from these fruits were scarified with tweezers and had a germination percent of 85%. The floating fruits were intact, and thus may have protected the seeds from any effects of salinity.

Our experiment also revealed that *Vallisneria* seedlings had low survival and growth rates with increased salinity levels. The experimental design kept the seedlings at constant salinity, but in the field salinity levels are constantly changing; so this would have a more pronounced effect on seedling growth and survivAlabama The field studies at Montrose and Point Clear showed that recolonizing these areas with seeds and seedlings may not be possible. It would also be very difficult, or impossible, to enhance recolonization to these areas with transplanted adult plants because these areas appear to have become too saline, thus inhibiting *Vallisneria* growth. Adult plants were previously transplanted in both areas but they did not persist.

In the first year of preliminary experiments, intact fruits were used in the petri dishes instead of seed extraction and seed pre-treatment. This was done to allow the seeds to mature completely to reach their highest germination potential, but problems arose when the seeds germinated within the fruits and the seedlings perished without the fruit ever rupturing. Also, the timing of germination was a problem because the seeds were germinating slowly over 1 ½ years after the experiment was initiated so some type of germination stimulus needed to be used. In nature the seeds would mature inside the fruit and the fruit would eventually break down releasing the mature seeds, but fruits kept in a sterile petri dish at a constant temperature with no biological or physical means of fruit decay will remain essentially intact.

The problem with seed extraction from the fruit was selecting fruits with mature seeds. Immature seeds do not germinate and those seeds that do result in seedlings that do not persist as well as seedlings produced from mature seeds (personal experience and work by Ferasol *et Alabama.* 1995). Ferasol *et Alabama,* (1995) found that mature dark seeds of *Vallisneria* underwent greater germination (57%) when compared to the immature light colored seeds (30%). They used seed coat scarification with a needle to stimulate germination and found that it increased germination from 30% (no scarification) to 90%. However, scarifying each seed is impractical when using large numbers of seeds. Abrading the seeds in mass with sand paper is physically similar to using a needle. with much less accuracy, and still increased mean germination from 0.85% to 30.23%.

Through lab experiments and communication with others working with *Vallisneria,* two other methods of seed pre-treatment were discovered. John Burns achieved up to 70% germination by acid-shocking *Vallisneria* seeds (personal communication), while Ferasol *et Alabama,* (1995) reported that cold treating the seeds enhanced germination (no percentage given). These two methods were also used to determine the best method for stimulating *Vallisneria* seeds to germinate, however, it was determined that they only slightly enhanced germination. In nature the seed coat would break down through biotic (bacteria, fungi) and chemical action as well as physical action (by rubbing against sand grains or from manipulation from other organisms). For experimental purposes, seed coat abrasion was the best method for stimulating *Vallisneria* seeds to germinate when using a large number of seeds.

Using seeds and seedlings of *Vallisneria* to propagate even a freshwater colony might be difficult. *Vallisneria* beds were examined throughout upper Mobile Bay in the freshwater areas for many years and no seedlings have ever been seen. Studies on the bay-wide seedbank reservoir (6 sediment cores bimonthly for 2 years) revealed no seedlings either.

Many aquatic species, like *Vallisneria,* produce small seeds that are resistant to environmental stress, but contain relatively small energy reserves (Madsen, 1991). Because of this limitation , developing seedlings are often susceptible to unfavorable conditions (varying water levels, high turbidity, water currents, herbivory) during the initial establishment period, when seedlings are fragile and the population is sparse (Titus and Hoover, 1991). Several studies on *Myriophyllum spicatum* (Ministry of Environment, 1981; Grace and Wetzel, 1978; Patten, 1955) have indicated that it produces many viable seeds, however, some authors (Patten, 1956; Amundsen, 1978) contend that this is not important in the spread of this species because there is an apparent absence of seedling survival *in situ;* the same trend has been shown for *Potamogeton pectinatus* (Van Wijk, 1989). From the present work on *Vallisneria* in Mobile Bay and those on *Myriophyllum spicatum* and *Potamogeton pectinatus,* a common trend of low seedling survival rates *in* *situ* can be noted but further investigation is necessary to confirm this. New beds must arise from seeds or winter buds that have been uprooted, even though very few seedlings have been seen in the field in Mobile Bay. The seeds, however, seem to be germinating in the field because sediment samples reveal that the majority of the propagules consist of empty seedcoats that show no signs of damage. The fate of seedlings, however, is unknown.

Colonization of an area by *Vallisneria* would be most successful using adult plants (Smart *et Alabama.* 1996). Transplanting winter buds has been successful with the northern ecotype of *Vallisneria* (Korschgen and Green, 1988) but the southern ecotype does not appear to produce winter buds (Smart *et Alabama,* 1996; personal observations). The results of our present experiment indicate that the use of *Vallisneria* seeds and seedlings for the colonization of an area, whether it be freshwater or in water with low salinity, is impractical and unsuccessful. Transplantation of mature plants seems to be the most logical method for the establishment of *Vallisneria* beds in Mobile Bay.

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APPENDIX

APPENDIX

The numerical values for figures 8 and 9 by day and salinity level.

Salinity Level						
Day		0ppt	2ppt	4ppt	6ppt	12ppt
7	% Survival	80.00	77.37	87.84	84.00	96.84
	Height (cm)	1.47	1.27	1.22	0.81	0.80
14	% Survival	70.90	69.85	76.47	64.40	91.57
	Height (cm)	1.88	1.63	1.69	0.94	1.12
21	% Survival	63.63	69.95	73.72	47.11	88.42
	Height (cm)	2.08	1.62	1.73	1.13	0.94
28	% Survival	61.81	68.95	70.98	43.05	86.31
	Height (cm)	2.30	1.71	1.85	1.26	0.74
35	% Survival	58.78	60.89	69.80	30.16	45.26
	Height (cm)	2.41	1.83	1.96	1.80	0.90
42	% Survival	56.66	60.29	68.62	29.83	37.89
	Height (cm)	2.45	1.92	2.06	2.10	1.06
49	% Survival	54.24	60.00	68.62	28.47	37.89
	Height (cm)	2.59	2.04	2.21	2.40	1.20
56	% Survival	50.60	55.82	63.13	25.08	23.15
	Height (cm)	3.03	2.56	2.62	4.07	1.80

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