The Dissertation Committee for Kelly Marie Darnell Certifies that this is the approved version of the following dissertation:

UNDERSTANDING FACTORS THAT CONTROL SEAGRASS REPRODUCTIVE SUCCESS IN SUB-TROPICAL ECOSYSTEMS

Committee:

Kenneth H. Dunton, Supervisor

Edward Buskey

Benjamin Walther

Bryan Black

Norma Fowler

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by

Kelly Marie Darnell, B.A.; M.S.

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Dedication

For my parents and husband, whom I love very much.

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UNDERSTANDING FACTORS THAT CONTROL SEAGRASS REPRODUCTIVE SUCCESS IN SUB-TROPICAL ECOSYSTEMS

Kelly Marie Darnell, Ph.D.
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Supervisor: Kenneth H. Dunton

Seagrasses are submerged marine plants that provide essential ecosystem functions, but are declining in abundance worldwide. As angiosperms, seagrasses are capable of sexual reproduction, but also propagate asexually through clonal rhizome growth. Clonal growth was traditionally considered the primary means for seagrass propagation. Recent developments in genetic techniques and an increasing number of studies examining seagrass population genetics, however, indicate that sexual reproduction is important for bed establishment and maintenance. Few studies have investigated the reproductive biology and ecology of sub-tropical seagrass species, although this information is necessary for effective management and restoration. This work investigates the influence of pore-water nutrients on flowering, water flow on seed dispersal, consumption on seed survival, and describes the reproductive phenology in Texas for the two dominant seagrass species in the Gulf of Mexico: turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*). These species exhibit distinctive reproductive seasons that span summertime months, but reproductive output varies spatially and temporally. Results of an *in situ* nutrient enrichment experiment indicate that turtle grass produces fewer flowers (but more somatic tissue) when exposed to high pore-water ammonium than when exposed to low pore-water ammonium, suggesting that nutrient loading has the potential to reduce seagrass reproductive output. Seed consumption may also limit reproduction and recruitment in some areas, as laboratory feeding experiments show that several local crustaceans consume shoal grass and turtle grass seeds and seedlings, which do not survive consumption. Dispersal experiments indicate that seed movement along the substrate depends on local water flow conditions, is greater for turtle grass than shoal grass, and is related to seed morphology. Under normal water flow conditions in Texas, turtle grass secondary seedling dispersal is relatively minimal (< 2.1 m d^{-1}) compared to primary dispersal, which can be on the order of kilometers, and shoal grass secondary seed dispersal can be up to 1.1 m d^{-1}, but seeds are likely retained in the parent meadow. Results from this work can be used when developing seagrass management, conservation and restoration actions and provide necessary information concerning a life history stage whose importance was historically under-recognized.

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Introduction

Seagrasses are flowering marine angiosperms that occur in shallow estuarine and marine environments. By altering flow, facilitating particle settlement, stabilizing coastal sediments, and sequestering, cycling and exporting nutrients, seagrasses play an invaluable role in coastal systems (Orth et al. 2006). Additionally, seagrasses serve as habitat for ecologically and commercially important species, nursery grounds for developing juveniles, and a food source for vertebrate and invertebrate grazers (Waycott et al. 2009). Seagrasses support productive ecosystems, with annual productivities ranging from 300–1500 g C m^{-2} $year^{-1}$ (Mateo et al. 2006). Accordingly, Costanza et al. (1997) ranked seagrasses among the most economically valuable of all ecosystems.

Seagrass cover is declining worldwide and the rate of loss has accelerated in recent years, primarily due to human activities such as dredging, propeller scarring and coastal nutrient loading (Waycott et al. 2009). Whereas seagrasses generally recover following declines from natural impacts (Peterson et al. 2002, Walker et al. 2006), recovery from anthropogenic declines is rare without human intervention (Ralph et al. 2006). Consequently, conservation and restoration actions are necessary to mitigate seagrass losses (Kenworthy et al. 2006). For these actions to be successful an understanding of each species' reproductive and recruitment dynamics is essential for development of effective, life history-based management plans.

Seagrass expansion has been historically attributed to asexual rhizomatous growth, although seagrasses are also capable of sexual reproduction (Arber 1920). An increase in population genetic studies enabled by recent advances in genetic techniques, however, suggests that the importance of sexual reproduction for meadow establishment and maintenance has been under-recognized worldwide (Kendrick et al. 2012), including along the Texas coast (Travis and Sheridan 2006). Very little is known about seagrass reproductive biology and ecology as a result of the traditional vegetative-dominated view of seagrass expansion. For many species, this lack of knowledge has hindered management and conservation efforts (Kendrick et al. 2012).

Seagrasses share many reproductive characteristics with freshwater angiosperms, including spherical pollen, the absence of an endosperm in mature embryos and diaspore dispersal through flotation (Ackerman 2006). However, seagrasses have developed unique adaptations for sexual reproduction in a marine environment that are rare in their freshwater counterparts. These adaptations include reduced submarine flowers, dioecy (separate male and female plants), hydrophilous (submarine) and ephydrophilous (surface) pollination, and geocarpy (ripening of fruits underground) (Ackerman 2006). Reproductive strategies of the over 70 species of seagrasses that encompass 13 genera vary dramatically and are related to their polyphyletic evolutionary origin (den Hartog and Kuo 2006).

Five seagrass species occur along the Texas coast: turtle grass (*Thalassia testudinum,* family Hydrocharitaceae), shoal grass (*Halodule wrightii*, family Cymodoceaceae), manatee grass (*Syringodium filiforme,* family Cymodoceaceae), star grass (*Halophila engelmannii,* family Hydrocharitaceae), and widgeon grass (*Ruppia maritima,* family Ruppiaceae). Of these, turtle grass and shoal grass are the most common, followed by manatee grass and widgeon grass. Reproductive strategies differ among these species. Turtle grass is a dioecious plant that utilizes hydrophilous pollination and produces seeds that germinate within a buoyant fruit that detaches from the parent plant. Turtle grass thus has the potential for long-distance seed dispersal

(Kaldy and Dunton 1999). Shoal grass and manatee grass are also dioecious and utilize hydrophilous pollination. Seeds of these species are surrounded by a hard seed coat and likely remain near the parent plant after subterranean or benthic release, where they can lay dormant for extended periods of time forming a seed bank in the sediment (McMillan 1981, Orth et al. 2006a). Widgeon grass is monecious and pollination occurs at the water's surface (ephydrophilous pollination). Flowers of this species are produced on photosynthetic stems that sometimes detach from the plant and are transported with waves and currents before release of negatively buoyant seeds.

This work examines the reproductive biology and ecology of the dominant sub-tropical seagrass species in the northwest Gulf of Mexico, specifically focusing on: (1) spatial and temporal variability in seagrass reproductive phenology (timing) and output along the Texas and Mississippi coasts (2) the influence of pore-water nutrients on flowering, (3) the relationship between water flow and secondary seed dispersal along the substrate and (4) the consumptive effects of common crab species on seagrass seed survival. Results suggest that: (1) seagrasses in Texas and Mississippi exhibit distinctive reproductive seasons that span summertime months, but reproductive output varies spatially and temporally, (2) turtle grass produces fewer flowers (but more somatic tissue) when exposed to high pore-water ammonium than when exposed to low pore-water ammonium, (3) seed movement along the substrate depends on local water flow conditions, is greater for turtle grass than shoal grass, and is related to seed morphology, and (4) seed consumption by crustaceans may limit reproduction and recruitment in some areas, as seedlings do not survive consumption. This information extends our understanding of the knowledge of reproductive biology and ecology for these species and can be used to develop appropriate management and restoration strategies. Based on the results of this work, I recommend that:

- The high, localized seed densities for shoal grass and widgeon grass may make these species good candidates for restoration by seeding in Texas and Mississippi, as seeds of these species are likely more cost-effective and efficient to collect than seeds of other species. Future studies should investigate the restoration potential of shoal grass and widgeon grass seeds and work toward developing minimally invasive seed harvesting techniques that are not detrimental to donor beds.
- Managers should be aware that increases in nutrient loading, particularly in the form of submarine groundwater discharge, likely reduces turtle grass flowering. Efforts at collecting turtle grass seedlings should focus on low-nutrient areas and, if the promotion of turtle grass sexual reproduction is desired, pore-water nutrient levels should remain sub-optimally low.
- Seagrass restoration efforts by seeding in Texas should include placing seeds in or adjacent to the intended restoration site, as seeds disperse short distances (meters) along the substrate under normal hydrodynamic conditions in Texas coastal estuaries. Seed density should be high if rapid coverage is desired.
- Seedlings should be protected from consumers such as crabs or placed in areas with fewer consumers to optimize seedling recruitment in restoration efforts by seeding.

Chapter 1: Variability in Turtle Grass (*Thalassia testudinum*), Shoal Grass (*Halodule wrightii*) and Widgeon Grass (*Ruppia maritima*) Flowering, Fruiting and Seed Production in the Northwest Gulf of Mexico

Abstract

Seagrass cover is declining worldwide, largely due to direct and indirect human impacts. Successful management, conservation and restoration programs require a complete understanding of individual species' life histories. The historically undervalued importance of sexual reproduction in seagrass population dynamics prompted species-specific investigations of reproductive phenology and output. Here, I present the results of a study monitoring the reproductive phenology and output of three dominant seagrass species in the northwest Gulf of Mexico: turtle grass (*Thalassia testudinum*), shoal grass (*Halodule wrightii*) and widgeon grass (*Ruppia maritima*). I assessed flowering, fruit production, seed output, seed reserve density and plant biomass in several locations along the central Texas coast and in eastern Mississippi during the reproductive seasons of these three species. Results suggest that reproductive timing and output are heterogeneous over both small and large spatial scales and between years. The percent of flowering and fruit-bearing turtle grass shoots ranged from 0 to 15% and 0 to 10%, respectively and fruit densities ranged from 0 to 180 fruit m^{-2}. High density patches of shoal grass (611 seeds m^{-2}) and widgeon grass (306 seeds m^{-2}) seed reserves were found in some areas; densities also differed substantially among sites in Texas and Mississippi. The factors that influence reproductive timing and output for these species are not completely understood at this time, but are likely related to local environmental conditions and also under genetic control. Results from this study can serve to improve

the success of restoration efforts across the Gulf Coast by identifying areas with high reproductive output and/or seed densities.

Introduction

Seagrasses are submerged marine flowering plants (class Monocotyledonae) that form expansive meadows in coastal systems worldwide. Their importance for supporting food webs, cycling nutrients, sequestering carbon and serving as essential habitat has been well established (Larkum et al. 2006). Global seagrass cover has recently been estimated at 177,000 km^3 (Spalding et al. 2003) and is declining. The rate of loss has accelerated in recent years. As a result, several of the 70 seagrass species are at risk, and two qualify as threatened (Short et al. 2011). Both natural and anthropogenic factors contribute to seagrass loss, but the magnitude of anthropogenic impacts dramatically outweighs natural disturbances (Ralph et al. 2006). Natural perturbations that contribute to seagrass decline include overgrazing (Valentine and Duffy 2006), uprooting from storm activity (Waycott et al. 2009) and death from the seagrass wasting disease pathogen, *Labyrinthula* sp. (Short et al. 1987). Although natural factors can temporarily reduce seagrass cover, recovery generally occurs (Morris and Virnstein 2004, Walker et al. 2006). Seagrass declines from anthropogenic factors such as mechanical damage, sediment loading, nutrient loading and eutrophication, however, show little recovery without human intervention (Ralph et al. 2006).

Seagrasses have been managed since the early 20th century (Green and Short 2003). Current management plans are often multifaceted and incorporate a variety of measures, including protection (Boesch et al. 2003), reduction of major threats (Bjork et al. 2008), transplantation of adult shoots (Fonseca et al. 1998), increased public outreach and education (Schwenning 2001), monitoring (Koch 2001), and to a lesser degree, restoration by seeds (Orth et al. 2006). Some regional efforts have been successful at improving water quality and mitigating seagrass decline, but many of the restoration efforts using transplantation of adult ramets have been ineffective over a large scale (Fonseca et al. 1998). The use of seeds holds promise for restoring seagrass cover, as large-scale seed harvesting and planting efforts have been successful with eelgrass (*Zostera marina*) in the Delmarva Coastal Bays, U.S.A. (Orth et al. 2006a). Orth et al. (2006, 2006a) argue that restoration efforts should involve seed spreading or planting, because, for some species, large numbers of easily-harvestable seeds are produced, harvesting seeds from a donor bed is likely less harmful than removing adult ramets, and harvesting seeds may be more time and cost-effective than harvesting adult plants. Additionally, the increased genetic diversity provided by seeds enhances resilience over genetically homogenous beds (Hughes et al. 2004). Before restoration efforts using seeds can begin in earnest, however, the reproductive phenology for each species needs to be understood and source populations for seeds need to be identified. Furthermore, the recently recognized importance of seeds for establishing new seagrass patches reinforces the need to understand the spatial and temporal dynamics of seagrass reproduction (Kendrick et al. 2012).

Expansion of seagrass meadows occurs through propagation of belowground rhizomes and sexual reproduction resulting in the output of seeds. Ramet expansion from clonal rhizome extension was traditionally considered the primary means of seagrass propagation (Arber 1920). Combined with the fact that seagrass flowers are reduced and relatively inconspicuous, this assumption resulted in a historical predominance of literature examining clonal growth and relatively few studies examining sexual reproduction. Recent developments in genetic techniques and the increasing number of studies examining seagrass population genetics, however, indicate that sexual reproduction does occur and that seeds are able to disperse long distances (Waycott et al.

2006, Kendrick et al. 2012). The historically under-recognized importance of sexual reproduction for seagrass populations has resulted in an incomplete understanding of many species' reproductive characteristics.

Seven seagrass species are found in the Gulf of Mexico, including one species that is listed as threatened and designated as critical habitat under the Endangered Species Act (Johnson's seagrass, *Halophila johnsonii*, Federal Register 1998, 2000), and another that is considered near threatened according to criteria for the International Union for the Conservation of Nature (IUCN) (star grass, *Halophila engelmanni*, Short et al. 2011). Five species occur along the Texas coast, where much of this study was conducted: turtle grass (*Thalassia testudinum*), shoal grass (*Halodule wrightii*), manatee grass (*Syringodium filiforme*), widgeon grass (*Ruppia maritima*), and star grass (*Halophila engelmanni*). Of these species, shoal grass and turtle grass are the most common (Onuf et al. 2003) and widgeon grass is often found in low salinity areas. The coast has about 1,000 km^2 of seagrass cover, the vast majority of which (> 90%) is located south of the San Antonio Bay system (Onuf et al. 2003).

Seagrasses in Texas are faced with the same anthropogenic stressors as elsewhere, including mechanical damage (mainly from propeller scarring and dredging), sediment loading, nutrient loading and eutrophication (Pulich 1999). As a result, seagrass cover has declined statewide and has become fragmented in many areas (Pulich and Onuf 2007). This decline spurred the development of the Seagrass Conservation Plan for Texas in 1999 that outlined resource management issues and strategies to protect and preserve Texas seagrasses. The Conservation Plan recommended that the status and trends of seagrasses should be assessed coast-wide (Dunton 1999), and as a result, a Texas Statewide Seagrass Monitoring Program was established in 2011 that incorporates a 3-

tiered coast-wide sampling design (Wilson et al. 2013, www.texasseagrass.org). Additionally, on 1 September 2013, uprooting of seagrasses by boat propellers in Texas bays became illegal, a violation classified as a Class C Misdemeanor (Texas Parks and Wildlife Department 2013). The decline of, and recent conservation efforts for, seagrasses in Texas (Pulich 1999) and the potential use of seeds for restoration (Orth et al. 2006) prompts investigation of the reproductive characteristics and phenology of species in this area.

The two most common seagrass species in Texas, turtle grass and shoal grass, form separate monospecific meadows in this region, but also co-occur in mixed beds. Under optimum light and substrate conditions, turtle grass is the competitively dominant species, whereas shoal grass is an early colonizing, pioneer species (Zieman 1982). In Texas, both species exhibit seasonal patterns in biomass, with a peak in summer and a dieback in the late fall and winter (Dunton 1990, Kaldy and Dunton 2000).

Morphologically, turtle grass is a relatively large species, with long (up to 80-cm), strap-like leaves between 0.2–2 cm wide (van Tussenbroek et al. 2010). Along the central Texas coast, light limitation restricts turtle grass to shallow waters (< 2 m deep), although this species can reach depths of 10–15 m in clear water (van Tussenbroek et al. 2010). In Texas, the turtle grass reproductive season spans late spring and summer months (Kaldy and Dunton 1999). Inflorescences are produced at the base of the shoot; females generally produce 1 flower, whereas males can produce multiple flowers. Fruits can dehisce (open) while still attached to the parent plant, releasing seedlings near the vicinity of the parent. In this case, germinated seedlings are the dispersal units. More commonly, though, fruits detach from the parent plant, are buoyant, and can be transported by currents up to 360 km before dehiscence and seedling settlement on the substrate (van

Dijk et al. 2009). When this occurs, the dispersal unit is initially the fruit, but becomes the seedling after its release from the fruit. Turtle grass seeds have no distinct period of dormancy; seedlings germinate within the fruit and are metabolically active when released. One study has examined turtle grass reproductive timing and output along the southern part of the Texas coast (Kaldy and Dunton 2000). However, turtle grass reproductive dynamics along the central part of the coast have not been examined.

Shoal grass is a morphologically smaller species (leaves: 2–5 mm wide × 3–30 cm long) than turtle grass, but is similarly constrained by light to depths < 2 m in Texas. Flower production and fruit and seed development in Texas occur in spring and early summer (McMillan 1976). After fertilization of the flowers, 1–2 fruits, each containing one seed, are produced by the female plant. Seeds are released from the fruit at or below the sediment surface adjacent to the parent plant and are surrounded by a hard seed coat, which enables an extended period of dormancy (up to 4 years) and forms a seed reserve in the sediment (McMillan 1981). In this species, seeds are the dispersal units. In several observational studies, McMillan reported the incidence of shoal grass flowering (1976) and seed germination (1983) and quantified seed reserves (1981, 1985) along the Texas coast. However, since McMillan's efforts, to my knowledge, no studies exist examining shoal grass reproductive dynamics.

Here, we present a descriptive study on the reproductive phenology and output of the dominant seagrass species in the northwest Gulf of Mexico, turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*). Specifically, I monitored flowering, fruit production, seed output, seed reserve density and plant biomass in several locations along the central Texas coast during the seagrass reproductive season. I also investigated reproductive phenology and output of seagrasses in a separate system in eastern

Mississippi where widgeon grass (*Ruppia maritima*) is abundant. This information is necessary for the development of appropriate management plans and can be used to aid future restoration efforts.

Materials and Methods

Study sites

Reproductive monitoring was conducted at several sites in Corpus Christi (East Flats), Aransas (Traylor Island, Mud Island) and Redfish (Hog Island) Bays along the Texas Coastal Bend (Figure 1.1). East Flats is a shallow (< 2 m) embayment within the Nueces Estuary and has continuous monospecific and mixed turtle grass and shoal grass meadows that are relatively well protected from waves and adverse sea conditions by barrier islands and shoals. Several previous studies have examined turtle grass and shoal grass biology and physiology in this area (Dunton 1990, 1994, Czerny and Dunton 1995, Lee and Dunton 2000). Seagrass reproductive phenology and output were monitored at four sites within East Flats: a monospecific shoal grass bed (SG), a mixed shoal grass-turtle grass bed (MX), and two monospecific turtle grass beds, separated from one another by approximately 200 m (TG1, TG2) (Figure 1.2a). Sites in Redfish Bay (Hog Island, HI) and Aransas Bay (Traylor Island, TI, and Mud Island, MI) are in the Mission-Aransas Estuary and within the boundaries of the Mission-Aransas National Estuarine Research Reserve (MANERR). Monitoring stations in the MANERR coincide with locations of a seagrass monitoring program that was established in August 2011, which created permanent triplicate transects perpendicular to the shore at each of the sites to evaluate seagrass condition based on landcape-scale dynamics (Tier 3 sites, www.texasseagrass.org, Figure 1.2b,c,d). Permanent transects at Hog Island are relatively well protected, receive little wave action, and their sediments are primarily composed of shell hash (personal observation). Transects at Traylor and Mud Islands are more exposed than those at Hog Island, and their sediments are composed of mud, silt and clay (Evans et al. 2012). Traylor Island, Hog Island and Mud Island have monospecific and mixed meadows of turtle grass, shoal grass and manatee grass.

Corpus Christi, Aransas and Redfish Bays are bar-built estuaries with mainly wind-driven tides; the lunar tidal range is generally less than 15 cm (Evans et al. 2012). Seasonal high tides occur during the spring and fall and seasonal low tides occur during the winter and summer (Evans et al. 2012). Seasonal water temperatures range from 10 to 30°C (Dunton 1990).

Reproductive monitoring was also conducted in the Grand Bay National Estuarine Research Reserve (GNDNERR) in Grand Bay, MS to compare seagrass reproductive phenology between separate systems in the Gulf of Mexico (Figure 1.3). Seagrass cover in the GNDNERR is dominated by shoal grass and widgeon grass (*Ruppia maritima*) at depths < 2 m. Monitoring was conducted along triplicate transects established in 2003 at three sites (Grand Bay, Jose Bay, and Middle Bay) as part of a seagrass biological monitoring program (Figure 1.3). The Grand Bay Estuary is characterized as a retrograding delta with relatively restricted freshwater inflow and sediment loading. Unlike salinities along much of the MS coast, salinities in the Grant Bay Estuary are regularly above 30 ppt (MS DNR 1998) Seasonal water temperatures in this Estuary range from 2 to 36 °C (MS DNR 1998).

Samples were also collected in conjunction with the Texas Statewide Seagrass Monitoring Program (www.texasseagrass.org) to assess shoal grass dormant seed densities over a larger spatial scale. Sampling sites spanned most of the Texas coast that has seagrass cover, with the northernmost sites in the MANERR and the southernmost sites in Lower Laguna Madre.

Reproductive Monitoring

East Flats, Corpus Christi Bay

Seagrass reproductive phenology and output were assessed approximately every two weeks in one monospecific shoal grass bed (SG), two monospecific turtle grass beds (TG1, TG2), and one mixed shoal grass-turtle grass bed (MX) in East Flats, Corpus Christi Bay during the 2011 (12 May–4 August), 2012 (4 April–8 August) and 2013 (6 May–23 August) reproductive seasons (Figure 1.1, 1.2a). During each monitoring event, the number of seagrass shoots, flowers, fruits and seeds were counted in six replicate 0.1 m^2 quadrats in each of the two monospecific turtle grass beds and the mixed seagrass bed. I did not attempt to count reproductive tissues in the shoal grass bed because shoal grass flowers, fruits and seeds cannot readily be felt by hand, unlike with turtle grass.

Four replicate round cores (9.5 cm wide × 10 cm deep) were collected randomly at each of the sites and sieved *in situ* with a 500-µm mesh sieve to retain above and belowground plant material and intact (~2 mm) and broken shoal grass seeds, but remove sediment. Care was taken not to break flowers, fruits or seeds. Cores were placed in bags and returned to the University of Texas Marine Science Institute (UTMSI) where they were kept frozen until processing.

A variety of data were obtained from each core, including the number of shoots of each species and the number of reproductive shoots of each species. If a shoot was reproductive, the number of flowers or fruits were counted, shoot sex was determined (if possible), and, if present, seeds were counted. Aboveground (leaf), belowground (roots and rhizomes) and reproductive (flower, fruit and/or seed) biomass was separated, leaves were wiped free of epiphytes and tissues were dried to a constant weight at 60°C, after which biomass was weighed to the nearest 0.0001 g. The root:shoot ratio (dried belowground biomass:aboveground biomass) was determined and reproductive allocation (RA) was calculated for reproductive shoots in 2012 and 2013 as the proportion of aboveground shoot biomass that was allocated to reproductive (flower) tissue (Bazzaz et al. 2000, Kaldy and Dunton 2000). Seed-containing turtle grass fruits from 2011 (n=32) and 2012 (n=1) were combined to determine average number of seeds per fruit. Intact dormant shoal grass seeds were also counted in each core and tested for viability by placing the seed in seawater. A seed was considered viable if it immediately sank, and unviable if it remained floating (Marion and Orth 2010). Environmental parameters (water temperature, salinity and pH) were measured with a YSI 600XL data sonde at the top of the seagrass canopy in the two turtle grass meadows during the time of monitoring in 2012 and 2013.

Mission-Aransas and Grand Bay National Estuarine Research Reserves

Seagrass reproductive characteristics were assessed at three sites in the MANERR (Traylor Island, Mud Island and Hog Island, Tier 3 sites under the Statewide seagrass monitoring program, www.texasseagrass.org) in 2011, 2012 and 2013 during the turtle grass and shoal grass reproductive seasons. Sites were monitored once in 2011 at the time of MANERR transect establishment (mid-August). In 2012 and 2013, sites were monitored monthly from 15 May–22 September 2012 and 3 June–5 August 2013.

Monitoring was conducted at randomly selected shallow (< 1m), intermediate (~1m) and deep (> 1m) stations along each transect for a total of 9 monitoring stations per site (Figure 1.2). If turtle grass was present at the station, seagrass shoots, fruits and seeds were counted in six replicate 0.1 m^2 quadrats. One core (9.5 cm wide × 10 cm deep) was collected at each station, sieved, and returned to UTMSI for processing (as described above). Environmental parameters (water temperature, salinity and pH) were measured with a YSI 600XL data sonde at the time of monitoring.

Seagrass reproductive phenology and output were assessed in the GNDNERR at shallow (< 1m), intermediate (~ 1m) and deep (> 1m) stations along each of the triplicate transects in Grand Bay, Jose Bay, and Middle Bay during 2011, 2012 and 2013 (Figure 1.3a,b,c). Sites were visited twice per year during the reproductive season: 3 and 25 August 2011, 23 April and 11 June 2012, and 31 April and 17 June 2013. Turtle grass was not present at any of the stations, so quadrats were not used. One core was collected at each station and returned frozen to UTMSI for processing, as described above.

Texas statewide shoal grass seed reserves

One core (9.5 cm wide × 10 cm deep) was collected in conjunction with the Texas statewide seagrass monitoring program at most of the monitoring sites during late summer and early fall 2012 (n=563). Cores were sieved *in situ* over a 500-µm mesh sieve, seagrass tissue was discarded and intact and broken dormant seeds were retained. Cores were returned to UTMSI and frozen until intact dormant seeds were counted. Previous experiments indicated that freezing and thawing did not break shoal grass or widgeon grass seeds. Seagrass species presence data for each site were obtained from the statewide monitoring program to compare seed densities to aboveground plant densities of each species.

Statistical analyses

For samples collected in East Flats, I measured the number of seagrass shoots per sample, the number of fruiting shoots per sample, the number of flowering shoots per sample, the proportion of the shoots that were reproductive in each sample and the aboveground and belowground biomass per sample. Shoot number, aboveground biomass and belowground biomass per sample were analyzed using generalized linear models with a Poisson distribution and log link function. Similarly, the frequency of reproductive shoots per sample (number of reproductive plants/total number of plants in a core) was analyzed with a generalized linear model with a Poisson distribution and a log link function. The root-to-shoot ratio was converted to the proportion of aboveground biomass to total biomass for analysis. These data were analyzed using a generalized linear model with a negative binomial distribution and the log link function. After analyses, values were back-transformed to the root-to-shoot ratio and this metric was reported. Turtle grass reproductive allocation, turtle grass fruit density, and shoal grass seed densities were analyzed with generalized linear models with a Poisson distribution and a log link function. In all analyses with data collected from East Flats, site and year were the predictor variables and were fixed effects.

For each seagrass species sampled in the Mission-Aransas and Grand Bay NERRs, I measured shoot density, aboveground biomass, belowground biomass, the root-to-shoot ratio and, only for shoal grass and widgeon grass, the number of dormant seeds. All of the analyses of NERR core data had the same predictor variables: site, transect nested with site, year, and depth. Depth was a categorical variable of three levels: shallow, intermediate, and deep. Each transect had the same three depths (shallow, intermediate, and deep). Year was also treated as a categorical variable. Depth, site, and year are factorial with respect to each other. Each transect was re-sampled in each year. Each sample (core), the lowest level of the design, was therefore nested with transect-year combination; core locations were not re-used. Site, depth, and year were considered to be fixed effects and transect nested within site was a random effect. Each analysis initially included the interactions terms depth x site, depth x year, and site x year; these were dropped from the final model if non-significant. For those models that did not include transect x depth or transect x year terms, the variation associated with these terms was pooled with the residual variation.

For samples collected in the Mission-Aransas NERR, turtle grass and shoal grass aboveground biomass, belowground biomass and shoot density and shoal grass dormant seed density were analyzed using generalized linear models with a Poisson distribution and the log link function. Turtle grass and shoal grass root-to-shoot ratio data were converted to the proportion of aboveground biomass to total biomass for analysis. These data were analyzed using generalized linear models with a binomial distribution and the logit link function. After analyses, values were back-transformed to the root-to-shoot ratio and this metric was reported.

For samples collected in the Grand Bay NERR, shoal grass and widgeon grass aboveground biomass, belowground biomass, shoot density and widgeon grass dormant seed density were analyzed using generalized linear models with a Poisson distribution and a log link function. Shoal grass and widgeon grass root-to-shoot ratio data were converted to the proportion of aboveground biomass to total biomass for analysis. These data were analyzed using generalized linear models with a negative binomial distribution and the log link function. After analyses, values were back-transformed to the root-to-shoot ratio and this metric was reported.

A chi-squared test was used to test if the presence or absence of shoal grass seeds was related to the presence or absence of shoal grass plants for statewide dormant seed reserve densities. Because of the low number of sites with widgeon grass, assumptions were not met for a chi-squared test, so a Fisher's Exact test was used to see if the presence or absence of widgeon grass seeds was related to the presence or absence of widgeon grass plants. Residuals were normally distributed for both analyses.

Data with residuals that were normally distributed are presented as the mean ± standard error (mean ± S.E.). Data that were transformed to obtain normality of the residuals or analyzed with generalized linear models are reported as the back-transformed mean and lower and upper confidance intervals (mean, lower confidance interval–upper confidance interval).

Results

East Flats, Corpus Christi Bay

Turtle grass and shoal grass were the dominant seagrass species in East Flats. Aside from a few *Halophila engelmannii* shoots collected in one of the monospecific turtle grass beds in 2013, all samples contained either turtle grass or shoal grass or a mixture of both species. Most of the turtle grass shoots sampled with quadrats were not reproductive, with the exception of one flower-bearing shoot at one of the monospecific turtlegrass sites (TG1) and one fruit-bearing shoot at the second monospecific turtlegrass site (TG2) on 21 June 2013.

Flowering turtle grass shoots were collected in cores 12 May–20 June 2011, 4 April–5 June 2012 and 6 May–21 June 2013. Sex was determined for 27 of the 34 flowering shoots collected over the study. Of these, 3 shoots were female and 24 were male. Each female shoot bore one flower, whereas male shoots had an average of 2.2 ± 0.16 flowers shoot^{-1} (mean ± S.E.). All flower-bearing shoots from TG2 ($n = 16$) were male. Of the flowering shoots in TG1, 8 were male and 3 were female and the majority of fruits in East Flats were collected from this site (86%). Fruit-bearing turtle grass shoots were collected 26 May–1 August 2011 and 15 May–5 June 2012. Each of these shoots had 1 fruit. In 2013, only one fruit-bearing shoot was collected on 5 July, and seeds within the fruit had already been released. Turtle grass fruits contained 1.7 ± 0.15 seeds fruit^{-1} and 4 of the fruits in TG1 contained 1 aborted seed. The average percent of flowering turtle grass shoots at sites in East Flats ranged from 0.7–2.3% and the average percent of fruiting shoots ranged was 0.2% at both of the monospecific turtle grass sites (Table 1.1). The proportion of flowering shoots did not differ among years ($p=0.91$), but was higher in TG1 than TG2 ($p<0.0001$, Table 1.1). Average allocation of biomass to reproductive structures for flowering shoots ranged from ~34–37% and did not vary between sites ($p=0.70$) or over years ($p=0.27$). Turtle grass fruit density peaked at 181 fruits m^{-2}, but on average was less than 5 fruits m^{-2} (Table 1.1). Neither fruit density nor the proportion of fruiting shoots differed between sites (fruit density: $p=0.69$; proportion fruiting: $p=0.98$) or year (fruit density: $p=0.14$, proportion fruiting: $p=0.16$).

One fruit-bearing shoal grass shoot was collected on 6 May 2012 in the mixed bed (MX). This shoot bore 2 fruits, each containing 1 seed. Aside from this shoot, all other collected shoal grass plants were non-reproductive. Dormant shoal grass seed densities ranged from 0–282 seeds m^{-2} (0–2 seeds $core^{-1}$), and most cores did not contain any seeds. Average dormant shoal grass seed densities were below 30 seeds m^{-2} (Table 1.2). Seed density did not vary by site ($p=0.28$) or year ($p=0.72$) and although many of the cores did not contain any intact seeds, nearly every core contained broken pieces of seeds. One widgeon grass seed was found in the shoal grass bed (SG) on 21 May 2011. Intact dormant seeds of all species were viable.

The monospecific turtle grass beds (TG1, TG2) had the highest biomass and the shoal grass bed had the lowest (Table 1.3). Biomass differed among years and the mixed bed had the highest root:shoot ratio (Table 1.3). The shoal grass bed had the highest shoot density, followed by the mixed bed and the monospecific turtle grass beds (Table 1.3). Water temperature, salinity and pH were within normal ranges for East Flats during the summer months (Table 1.4, Dunton 1990).

Mission-Aransas and Grand Bay NERRs

Mission-Aransas NERR

Monospecific and mixed shoal grass, turtle grass and manatee grass (*Syringodium filiforme*) beds were present at sites in the MANERR, although manatee grass was relatively sparse. Most turtle grass shoots sampled in quadrats were not reproductive. However, on 3 June 2013, one flowering shoot was sampled at Hog Island and another was sampled at Traylor Island.

Flowering turtle grass shoots were collected in cores on 5 May and 18 June 2012 and 3 June 2013. Of the 13 collected flowering shoots, sex could be determined visually for five of the shoots. Two were identified as male and three as female, and all but two were collected from Hog Island. Each female shoot had one flower and each of the two male shoots had four flowers. Overall, the percent of flowering shoots at each site was low (Table 1.5). At each site, average reproductive allocation ranged from 0 to 18.9%. Two fruit-bearing turtle grass shoots were collected during the study on 28 June 2012 and 5 August 2013. Both shoots were collected from Hog Island, had one fruit each, and each fruit contained one seed.

Fruit-bearing shoal grass shoots were collected at Traylor Island on 15 May 2012. Of the 7 collected reproductive shoots, three had two attached fruits and four had one attached fruit. Each fruit contained one seed. On average, 0.51 ± 0.37% of shoal grass shoots at Traylor Island contained one or more fruits, and average fruit density was 28.2 ± 10.7 fruits m^{-2}. Although no reproductive manatee grass shoots were collected, two cores from Traylor Island contained detached manatee grass flowers and fruits (15 May 2012). One core contained 4 flowers and the other contained 1 flower and 4 fruits, each with 1 seed. Manatee grass fruits and seeds were found in a mixed turtle grass, shoal grass and manatee grass bed.

Shoal grass was the only species present in sediment seed reserves. Seed density at Traylor Island was as high as 3,950 seeds m^{-2} (28 seeds $core^{-1}$), but average seed densities by site ranged from 0 to 618 seeds m^{-2} (Table 1.2). Seed densities differed between sites and years, but did not vary by water depth (site: $p=0.003$, year: $p<0.0001$, depth: $p=0.34$). Traylor Island had more seeds than both Mud Island and Hog Island; seed densities did not differ between Mud and Hog Islands. Overall seed densities were highest in 2011, followed by 2012, then 2013. Approximately half of the cores (51%)

contained broken seed pieces. Only 1 dormant seed was found at Hog Island and all seeds were viable.

Turtle grass aboveground and belowground biomass differed among sites, depths and years, and was highest at Hog Island (Table 1.6). Shoot density was not different among sites, although it approached significance (Table 1.6). Shoal grass shoot density and belowground biomass were highest at Traylor Island, but did not differ across depths (Table 1.6). Aboveground biomass, however, was similar among sites, depths and years (Table 1.6). Water temperature, salinity and pH were within normal ranges for the Mission-Aransas NERR during the summer months (Table 1.4, cdmo.baruch.sc.edu).

Grand Bay NERR

Sites in the Grand Bay NERR were composed of monospecific and mixed shoal grass and widgeon grass beds. All sampled shoal grass shoots were non-reproductive. Flowering widgeon grass shoots were collected in April, June and August, and the average percent of flowering shoots ranged from 0 to 4% per site (Table 1.7). No reproductive widgeon grass shoots were found in Grand Bay and Jose Bay in 2011 and Middle Bay in 2012 and 2013.

Shoal grass biomass was highest at Grand Bay and differed among years (Table 1.8). Shoot density also differed among years, but all biomass parameters were similar across depths (Table 1.8). Widgeon grass belowground biomass was highest in Grand Bay (Table 1.8). All other parameters were similar among bays, and aboveground biomass and the root:shoot ratio differed across years (Table 1.8).

Dormant shoal grass seeds were found in only 3 cores, with a range of 0–282 seeds m^{-2} (0–2 seeds $core^{-1}$). Widgeon grass seeds were found in higher abundance than shoal grass seeds and ranged from 0–2,963 seeds m^{-2} (0–20 seeds core^{-1}), although average seed densities at each site were much lower (Table 1.2). Widgeon grass dormant seed density did not vary with water depth (p=85) or by site (0.13). Dormant seeds of both species were viable, and most cores contained broken seed pieces.

Texas statewide shoal grass seed reserves

Of the 563 sites sampled along the Texas coast, 191 (~33%) contained dormant shoal grass seeds. Seed densities ranged from 0–4,515 seeds m^{-2} (32 seeds core^{-1}). The majority of seeds in the MANERR were collected west of and adjacent to Traylor Island (Figure 1.4a) and the majority of seeds in Corpus Christi Bay were collected north of the Corpus Christi Ship Channel near Redfish Bay and in the southeast portion of the Bay between dredge spoil islands and Mustang Island (Figure 1.4b). In Upper Laguna Madre, most seeds were found north of the entrance to Baffin Bay (Figure 1.4c) and the few seeds found in Lower Laguna Madre were south of Port Mansfield (Figure 1.4d). The presence of dormant shoal grass seeds at a site was significantly related to the presence of shoal grass plants at that site ($p < 0.0001$). Of all the intact shoal grass seeds collected, only 1 seed was unviable. Broken pieces of shoal grass seeds were found at the majority of sites (n=436).

Cores at 80 of the sites (~14%) contained dormant widgeon grass seeds. Widgeon grass seed densities ranged from 0–4,092 seeds m^{-2} (29 seeds core^{-1}), but the majority of cores with widgeon grass seeds (78%) had fewer than 3 seeds. Widgeon grass seeds were dispersed throughout Corpus Christi Bay and Upper and Lower Laguna Madre (Figure 1.5) and were mostly found with shoal grass seeds. The presence of widgeon grass seeds at a site was not significantly related to the presence of widgeon grass plants at that site ($p = 0.10$).

Discussion

Results of this monitoring study suggest that reproductive timing and output of the dominant seagrass species in the northwest Gulf of Mexico are spatially and temporally variable. Turtle grass (*Thalassia testudinum*) flowering intensity, fruit production, and plant biomass differed greatly among sites along the central Texas coast. Some locally high densities of shoal grass (*Halodule wrightii*) and widgeon grass (*Ruppia maritima*) seed reserves throughout Texas and eastern Mississippi suggest that these species may be good candidates for restoration. The results of this study highlight the need to investigate factors contributing to this variation for these species whose reproductive potential has been historically undervalued. Studies examining reproductive phenology and effort provide life history information necessary for the development of appropriate management and conservation plans.

Turtle grass reproductive phenology and output

Turtle grass reproductive phenology and output have been examined in several systems throughout the Caribbean and Gulf of Mexico. The average densities of flowering shoots in monospecific turtle grass beds at East Flats, Texas were within the average ranges reported by Gallegos et al. 1992 in the Mexican Caribbean (3–12%), Durako and Moffler (1985) in Tampa bay, Florida (10-21%) and Kaldy and Dunton (2000) in Lower Laguna Madre, Texas (13–30%). As has been found in previous studies

(Duarko and Moffler 1987, van Tussenbroek 1994), turtle grass flowering intensity showed considerable variation among my sites. Whereas flowering shoot densities in East Flats were within ranges in other areas, the highest average annual density in the MANERR was very low (< 1%) despite being < 20 km from East Flats. Plasticity in reproductive output is common among species such as turtle grass that are exposed to a range of environmental conditions and different levels of environmental stress (Mooney et al. 1991). van Tussenbroek (1994) observed high spatial and annual variability in turtle grass flowering in the Mexican Caribbean and suggested that environmental differences, such as nutrient input or wave action, could contribute to this variability. Hog Island receives relatively little wave action compared to my other sites in the MANERR (pers. obs.), but the nutrient regime of Hog Island is unknown. We recommend that studies assess the effects environmental factors such as these on reproductive output (see Chapter 2).

Reproductive allocation (RA) was similar between sites and years at East Flats. RA was lower in the MANERR than at East Flats, and was comparable with results of Kaldy and Dunton (2000) in Lower Laguna Madre (16%). Terrestrial plants display similar biomass investments in reproductive structures. For example, RA in the perennial *Plantago major* can range from 10 to nearly 80% (Reekie 1998). Although estimates based on biomass allocation likely underestimate total energy investment, the calculation of RA is nevertheless useful to estimate energy investment for comparisons over space and time (Bazzaz et al. 2000).

Although the sexual identity of a turtle grass plant is easy to identify when a plant is fruiting (and is thus female), identifying shoot sex based on floral morphology can be difficult. However, we were able to determine the sex of 80% of the flowers collected in

East Flats. Of these, 100% of the shoots from one of the two sites (TG2) were male. The male-skewed ratio suggested that we may have concentrated sampling on an individual genet, as turtle grass is dioecious and all shoots in a clone are the same sex (van Tussenbroek et al. 2010). Both male and female flowers were collected from the other monospecific turtle grass site (TG1), confirming that we collected at least two separate genets there. Most of the fruiting shoots in East Flats (86%) were collected from this site. The average proportion of fruiting shoots in East Flats was comparable to results of Kaldy and Dunton (2000) (< 10% fruiting) in Lower Laguna Madre. Fruit densities in East Flats (0–181 fruits m^{-2}), however, spanned a larger range than has been reported previously (up to 70 fruits m^{-2}, Kaldy and Dunton 2000), highlighting the variability present in this system. Compared to East Flats, the percent of fruiting shoots at Hog Island in the MANERR was very low, with only 1 fruit collected per year.

Fruit densities reported for seagrass species that are closely related to turtle grass are similar to densities from East Flats, and higher than densities at Hog Island. For example, *Thalassia hemprichii*, a 'twin species' to turtle grass that inhabits the western Pacific and West Indian Ocean, produces 128–134 fruits m^{-2} in the Philippines (Rollon et al. 2001). Turtle grass fruits can contain up to 9 seeds per fruit, but the average number is usually much lower (den Hartog 1970). Fruits in East Flats had 2 seeds, on average, whereas both fruits collected in the MANERR contained 1 seed. These numbers are similar to those reported in Florida (Orpurt and Boral 1964) and south Texas (Kaldy and Dunton 2000). Interestingly, we collected 4 fruits in East Flats that contained aborted seeds. The incidence of aborted seagrass seeds has only been rarely reported (Campey et al. 2002), but deserves further attention, as it has potential implications for pollen limitation, resource limitation or sibling competition (Stephenson 1981).

Turtle grass reproduction is highly seasonal. Although flower primordia have been reported in Florida in winter months (Moffler et al. 1981), the bulk of seagrass reproduction occurs during late spring and summer (Duarko and Moffler 1987, van Tussenbroek 1994). In this study, flowers were present from early April to late June. Fertilization likely began in early May, as fruits were collected from late May to August. I observed one dehisced fruit in July, suggesting that fruit and seed maturation begin in the early part of the summer.

Variability in dormant seed reserves

Fruit-bearing shoal grass shoots were collected in East Flats and Traylor Island in May 2012, but overall densities remained low (0–0.51%). Although I documented fruiting shoots, I did not collect any flowering shoots. A small number of studies have documented shoal grass flowering and fruiting. McMillan (1976) observed flower and fruit development in Redfish Bay, TX between April and August and described the number of flowering shoots as ‘abundant.’ Johnson and Williams (1982) collected flowering and fruiting shoots in St. Croix, U.S. Virgin Islands between March and May and reported high, but variable, densities of reproductive structures (mean ± S.D.: 779 ± 780). Additionally, McGovern and Blackenhorn (2007) collected fruit-bearing shoots from June to September in Mississippi Sound near Mobile Bay, AL, and like this study, did not collect flowering shoots. Shoal grass flowers are highly reduced and difficult to see. As a result, their presence is not often documented (B. van Tussenbroek, pers. comm.). I took great care to inspect each shoot for the presence of flowers and am confident that, with the exception of fruit-bearing shoots, all shoal grass shoots were non-reproductive.

Shoal grass seeds are released at the base of the shoot near or below the sediment surface and can remain dormant for up to 4 years (McMillan 1991). McMillan (1981) reported an average dormant seed density of 260 seeds m^{-2} (no S.E. provided) and a range of 26–3,120 seeds m^{-2} in Redfish Bay, TX. In this study, dormant seed densities were relatively low in East Flats (< 30 seeds m^{-2}), and did not vary between sites or years. However, in the MANERR, seed densities were much higher and spatially and temporally heterogenous. The highest seed densities in the MANERR were collected from Traylor Island (> 200 seeds m^{-2}), where the majority of fruiting shoots were also collected. Dormant shoal grass seeds were also collected in the GNDNERR, but at relatively low densities (< 20 seeds m^{-2}).

In addition to being collected in monospecific shoal grass beds and mixed shoal grass-turtle grass beds, dormant seeds were also collected in monospecific turtle grass beds. McMillan (1981) similarly collected dormant seeds from monospecific turtle grass meadows in Redfish Bay, Texas. The ubiquitous distribution of dormant seeds among substrates suggests that (1) reproductive shoal grass plants were once present in all of these locations, and/or (2) seeds dispersed to these locations. Since dispersal of shoal grass seeds under normal water flow conditions occurs over relatively short distances (on the order of meters), one likely source of seeds are the nearby shoal grass plants, which were noted in the immediate vicinity. Although it is possible that shoal grass plants were once present at these sites, as shoal grass is a pioneer species and often colonizes an area before turtle grass (Zieman 1982), my tests for seed viability indicate that seeds were produced within the last four years, the duration that shoal grass seeds remain viable (McMillan 1991). Turtle grass beds have been persistent at my sites for several decades

(Pulich and Onuf 2007), so it is more probable that shoal grass seeds were transported to these turtle grass beds.

The presence of dormant shoal grass seeds in cores collected along the entire coast showed a positive relationship with the presence of shoal grass plants, further indicating that shoal grass seed dispersal distance is limited and seeds are likely retained in the vicinity of the parent meadow. Although the statewide dormant seed sampling can provide information on the location of individual seed species and microscale (core-size) seed density, these data must be interpreted with caution, as only one core was collected at each site. The small-scale (on the order of meters) variability in shoal grass seed densities observed in this monitoring along the central Texas coast highlights the need for taking replicate cores or subsampling from a larger area to accurately quantify seeds. General observations from this statewide sampling, however, indicate that dormant shoal grass seeds are present, but spatially variable along the entire Texas coast.

Widgeon grass plants are present along the central Texas coast (Dunton 1990), but were not present at my sampling sites. Dormant widgeon grass seeds were rarely found in cores in East Flats and the MANERR, and although present in cores collected statewide, were generally in low numbers. Widgeon grass, however, is a dominant species in the GNDNERR and was present at the majority of my sampling sites. Flowering shoots were collected during all sampling months (April, June and August) and seeds were by far more abundant than shoal grass seeds. Widgeon grass is a cosmopolitan species, and is found worldwide in fresh, brackish and saline environments (McGovern 2009).

Unlike turtle grass and shoal grass, widgeon grass produces photosynthetic stems on which flowers and fruits are produced. These stems can detach from the plant and disperse long distances with waves and currents (Ailstock and Shafer 2004). Additionally, plant material (including seeds) is consumed by waterfowl, which can result in biotic seed dispersal (Figuerola et al. 2002). I found no relationship between the presence of widgeon grass plants and the presence of widgeon grass seeds in statewide Texas cores, which is possibly a result of the dispersal potential of these seeds. Widgeon grass plants are highly fecund relative to other seagrass species, likely due to the high pollination success of the hermaphroditic flowers and this species' reliance on sexual reproduction (see below; Kantrud 1991). Bonis et al. (1995), for example, reported that although dormant seed densities are highly variable on a microscale, densities can be as high as 73,000 seeds m^{-2} in the Mediterranean. Seeds can remain viable in the sediment for up to 3 years (Kantrud 1991). Although this species can overwinter, shoots in many areas die-off in the late fall and seeds recolonize meadows in the spring (Dunton 1990, Kahn and Durako 2006).

McGovern (2009) examined widgeon grass reproductive dynamics over the reproductive season (May–September 2006) in western Alabama and at locations near my study sites in the GNDNERR. Although average peak seed densities were similar between this study and the Alabama sites in McGovern (2009) and sampling methods were similar (i.e. core sampling), the average seed densities reported at sites in the GNDNERR (3,480 and 10,154) are one to two orders of magnitude higher than my recorded densities. This further demonstrates the small-scale variability in seed densities and emphasizes the need to understand factors that regulate seed production and dispersal.

The majority of cores collected in this study contained seed coat pieces. McMillan (1981) also reported that cores in Texas contained seed halves. Consumption of seagrass reproductive structures, including seeds, has been described for several temperate seagrass species (Fishman and Orth 1996, Orth et al. 2002). The most common consumers are decapod crustaceans such as crabs (Holbrook et al. 2000*,* Fishman and Orth 1996) and shrimp (Nakaoka 2002). It is possible that the high number of broken seed pieces was due to consumption by animals within or atop the sediment (see Chapter 4). Alternatively, these seed pieces could be portions of the seed coat that remained after seed germination.

Seagrass biomass allocation patterns

The variability in turtle grass and shoal grass shoot densities and biomass between sites and years has been previously demonstrated for these species during spring and summer in Texas (Pulich 1985, Dunton 1990, Lee and Dunton 1996). Temperature and irradiance are major factors controlling seagrass growth, and in Texas, biomass peaks in late summer after sustained high temperatures and long photoperiods (Phillips et al. 1981, Dunton 1990). Lee and Dunton (1996) examined turtle grass production and biomass in East Flats and reported increases in biomass throughout the spring and early summer leading to peak aboveground biomass (355 g dry wt m^{-2}), belowground biomass (~400 g dry wt m^{-2}) and shoot density (531 shoots m^{-2}) in September. Although not calculated by Lee and Dunton (1996) the root:shoot ratios (RSR) were consistently >1 throughout the study, reflecting greater belowground than aboveground biomass and high productivity. Root:shoot ratios can be used to estimate seagrass condition, and those plants with a RSR above 2 demonstrate high productivity, whereas those with a RSR <1 exhibit low productivity (Dunton et al. 2010).

In my study, turtle grass aboveground biomass was variable between sites and years. Peak aboveground biomass in East Flats was lower than reported by Lee and Dunton (1996), but peak belowground biomass was higher and RSRs at turtle grass-dominated sites were commonly > 4, indicating meadows were highly productive. Shoot densities were higher than those reported in Lee and Dunton (1996), but within the ranges reported in other areas (Gallegos et al. 1992). Although turtle grass biomass at Hog Island (Redfish Bay) in the MANERR was marginally lower than estimates of Kopecky and Dunton (2006) for this area, my shoot densities and RSRs were higher, indicating that more, but smaller, shoots were present at my site, and these shoots had a greater proportion of belowground biomass. It is interesting that both aboveground and belowground biomass and RSR were highest at TG1 in East Flats, where the highest proportion of flowering shoots was recorded. Also, the highest turtle grass biomass in the MANERR was documented at the only site where fruits were collected (Hog Island). As with terrestrial plants, the dynamics of seagrass reproductive phenology and output undoubtedly involve the interplay of genetic control, environmental factors and plant condition (Bazzaz et al. 2000), and the degree of influence of individual factors warrants further study.

Shoal grass was the dominant seagrass species at Traylor Island and had higher aboveground and belowground biomass than turtle grass at this site. Aboveground and belowground biomass and shoot densities differed among sites but were within ranges reported for central (Dunton 1990) and south (Pulich 1985) Texas. Among sites in the MANERR, Traylor Island had the highest shoot densities, belowground biomass and dormant seed densities, and was the only site where reproductive plants were collected.

Root:shoot ratios were representative of shoal grass throughout Texas (Dunton 1990) and the consistently high RSRs (> 2) suggest that meadows displayed high productivity.

Widgeon grass and shoal grass were the dominant species in the GNDNERR. Compared to the MANERR, shoal grass at my sites in Mississippi had less aboveground and belowground biomass, lower RSRs and exhibited more spatial and temporal variability than those plants in the MANERR. We observed no reproductive shoal grass plants in the MANERR and dormant seed densities were low. Widgeon grass had lower RSRs than shoal grass and were commonly less than one. Unlike turtle grass and shoal grass, widgeon grass has reduced belowground structures, reflecting its annual plant-like growth strategy with shoot senescence in the winter and recolonization by seeds in the spring. To compensate for reduced root and rhizome structure, widgeon grass preferentially uptakes dissolved inorganic nitrogen through the leaves rather than the roots (Stevenson 1988) and is found in low-energy environments (van Tussenbroek et al. 2010). McGovern (2009) reported peak widgeon grass biomass in early summer and shoot senescence toward the end of the season. Biomass was highest at my sites in August 2011, suggesting that our collections were made before the annual shoot senescence in fall. My biomass measurements are similar to reports from this area (McGovern 2009) and other areas in the northern Gulf of Mexico (Harrison 1982, Cho and Poirrier 2005). Species composition and biomass differences between the MANERR and GNDNERR can most likely be attribute to environmental characteristics between the sites.

Implications for restoration

Widespread declines in seagrass cover primarily from direct and indirect human impacts have amounted to a loss of nearly 20% of seagrasses over the past several decades (Green and Short 2003). Whereas restoration attempts by transplanting adult shoots have mostly been unsuccessful over large scales (Fonseca et at. 1998), programs that include the harvest and sowing of seeds have resulted in sustained increases in seagrass cover (Orth et al. 2006a). Restoration using seeds is common in terrestrial and wetland systems worldwide (van der Valk et al. 1992, Gustafson et al. 2005). However, despite the ubiquitous loss of seagrass cover across latitudes, seagrass seed-based restoration has only been attempted with temperate species (e.g. *Zostera marina*), although it has been considered with species such as widgeon grass in Maryland (Ailstock and Shalfer 2004). The first steps to seed-based restoration include assessing the restoration potential of individual species and understanding the species-specific reproductive phenologies and patterns in reproductive output throughout the species' range. Here, I have attempted to describe patterns and variability in reproduction for the dominant seagrass species in the northwest Gulf of Mexico: turtle grass, shoal grass and widgeon grass. Results suggest that species in this region have discernible reproductive seasons, but reproductive output is spatially and temporally variable. Turtle grass fruit production and development occurs over the span of several months (May–August), and therefore effective seed harvest may be difficult. The high, localized densities of shoal grass and widgeon grass seeds, on the other hand, may make these species more cost-effective and efficient to collect. This study was only a first step at describing the reproductive phenology and output of the dominant seagrass species in the northwest Gulf of Mexico. I recommend that future studies investigate the restoration potential of shoal grass and widgeon grass seeds and work toward developing minimally invasive seed harvesting techniques. Future work should also investigate the biological and ecological factors influencing each species’ reproductive phenology and output.

Table 1.1. Turtle grass (*Thalassia testudinum)* reproductive characteristics at sites in East Flats, Corpus Christi Bay, Texas over the 2011, 2012 and 2013 seagrass reproductive seasons. Values are presented as the back-transformed mean and back-transformed lower and upper 95% confidance intervals.

Parameter	Site	Mean (lower–upper 95% CI)
Flowering Shoots (%)	Turtle grass 1	2.3 (0.1–7.3)
	Turtle grass 2	0.7 (0.2–2.3)
Fruiting Shoots (%)	Turtle grass 1	0.2 (0.06–0.9)
	Turtle grass 2	0.2 (0.06–0.9)
Fruit Density (m^{-2})	Turtle grass 1	4.7 (1.3–17.1)
	Turtle grass 2	3.9 (1.0–14.6)
Reproductive Allocation (% of aboveground biomass)	Turtle grass 1	37.0 (23.2–59.0)
	Turtle grass 2	33.5 (24.8–45.3)

Table 1.2. Density of dormant shoal grass (*Halodule wrightii*) and widgeon grass (*Ruppia maritima*) seeds at sites in East Flats and the Mission-Aransas National Estuarine Research Reserve in Texas, and sites in the Grand Bay National Estuarine Research Reserve in Mississippi in 2011, 2012 and 2013. Values are presented as the back-transformed mean and back-transformed lower and upper 95% confidance intervals.

Site	Dormant seeds (seeds m^{-2})
East Flats, Texas	Shoal grass
Turtle grass 1	11.1 (4.9–25.3)
Turtle grass 2	20.5 (10.9–38.8)
Mixed	26.3 (14.5–46.3)
Shoal grass	16.4 (8.5–31.6)
Mission-Aransas NERR, Texas	Shoal grass
Hog Island	0
Mud Island	60.4 (21.3–171.4)
Traylor Island	617.8 (435.1–877.1)
Grand Bay NERR, Mississippi	Widgeon grass
Grand Bay	190.8 (85.6–425.1)
Middle Bay	260.0 (123.0–549.2)
Jose Bay	73.2 (25.2–212.2)

Table 1.3. Seagrass biomass parameters during the 2011, 2012 and 2013 seagrass reproductive seasons at four sites in East Flats, Corpus Christi Bay, Texas: two monospecific turtle grass (*Thalassia testudinum*) beds (Turtle grass 1, Turtle grass 2), a mixed turtle grass-shoal grass (*Halodule wrightii*) bed (Mixed), and a monospecific shoal grass bed (Shoal grass). Data are presented as the back-transformed means and back-transformed lower and upper 95% confidance intervals. Aboveground and belowground biomass and shoot density were analyzed using generalized linear models with a Poisson distribution and log link function. The root:shoot ratio was analyzed using a generalized linear model with a negative binomial distribution and the log link function. Letters in parentheses denote significant differences between sites determined by a Tukey HSD post hoc comparison. Data were obtained from core samples.

Parameter	Factor	p	Site	Mean (lower–upper 95% CI)
Aboveground Biomass	Site	<0.0001	Turtle grass 1	622.1 (562.5–687.9) (A)
(g dry weight m^{-2})	Year	0.02	Turtle grass 2	354.1 (310.0–404.5) (B)
			Mixed	282.5 (246.5–323.8) (B)
			Shoal grass	119.8 (96.6–148.5) (C)
Belowground Biomass	Site	<0.0001	Turtle grass 1	622 (616–628) (A)
(g dry weight m^{-2})	Year	<0.0001	Turtle grass 2	354 (350–358) (B)
			Mixed	283 (279–286) (C)
			Shoal grass	120 (117–122) (D)
Root:Shoot Ratio	Site	<0.0001	Turtle grass 1	4.8 (4.2–5.5) (B)
	Year	0.09	Turtle grass 2	5.1 (4.5–5.8) (B)
			Mixed	10.5 (9.5–11.6) (A)
			Shoal grass	3.7 (3.2–4.2) (C)
Shoot Density	Site	<0.0001	Turtle grass 1	1667 (1657–1675) (C)
(shoots m^{-2})	Year	<0.0001	Turtle grass 2	910 (903–916) (D)
			Mixed	3508 (3496–3520) (B)
			Shoal grass	5843 (5827–5860) (A)

Table 1.4. Environmental parameters (water temperature (°C), salinity, pH) measured with a YSI 600XL data sonde at the time of seagrass monitoring at two stations (Turtle grass 1, Turtle grass 2) in East Flats (Corpus Christi Bay), and sites within the Mission-Aransas National Estuarine Research Reserve during 2012 and 2013: Traylor Island (Aransas Bay), Mud Island (Aransas Bay), and Hog Island (Redfish Bay), Texas.

Site	Year	Temperature	Salinity	pH
East Flats				
Turtle grass 1	2012	27.6 ± 0.5	35.1 ± 1.8	8.06 ± 0.06
	2013	28.7 ± 0.5	41.2 ± 2.1	8.05 ± 0.09
Turtle grass 2	2012	28.1 ± 1.0	34.5 ± 1.9	8.15 ± 0.02
	2013	29.0 ± 0.2	41.5 ± 4.1	8.22 ± 0.02
Mission-Aransas NERR				
Traylor Island	2012	28.5	34.1	7.84
	2013	27.8 ± 0.2	36.3 ± 3.4	7.9 ± 0.2
Mud Island	2012	28.7	35	8.09
	2013	29.2 ± 1.1	38.2 ± 2.7	8.14 ± 0.06
Hog Island	2012	30.7	35.3	–
	2013	28.2 ± 0.2	38.7 ± 2.2	8.02 ± 0.15

Table 1.5. Turtle grass (*Thalassia testudinum)* reproductive characteristics at sites in the Mission-Aransas National Estuarine Research Reserve, Texas over the 2011, 2012 and 2013 seagrass reproductive seasons. Values are presented as mean ± S.E.. Dashes (–) represent no data.

Parameter	Site	2011	2012	2013
Flowering Shoots (%)	Hog Island	0	0.04 ± 0.01	0.025 ± 0.02
	Mud Island	0	0.005 ± 3.0	0
	Traylor Island	0	0	0.04 ± 0.04
Fruiting Shoots (%)	Hog Island	0	0.003 ± 0.003	0.003 ± 0.003
	Mud Island	0	0	0
	Traylor Island	0	0	0
Fruit Density (m^{-2})	Hog Island	0	0.003 ± 0.003	0.003 ± 0.003
	Mud Island	0	0	0
	Traylor Island	0	0	0
Reproductive Allocation (% of aboveground biomass)	Hog Island	0	18.9 ± 7.2	10.5 ± 2.6
	Mud Island	0	–	0
	Traylor Island	0	0	–

Table 1.6. Turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrighii*) biomass parameters during the 2011, 2012 and 2013 seagrass reproductive seasons at three sites in the Mission-Aransas National Estuarine Research Reserve, Texas: Traylor Island, Mud Island and Hog Island. Data are presented as the back-transformed means and back-transformed lower and upper 95% confidance intervals. Turtle grass and shoal grass aboveground biomass, belowground biomass and shoot density were analyzed using generalized linear models with a Poisson distribution and the log link function. Turtle grass and shoal grass root:shoot ratios were analyzed using generalized linear models with a binomial distribution and the logit link function. Letters in parentheses denote significant differences between sites determined by a Tukey HSD post hoc comparison.

Parameter	Factor	p	Site	Mean (lower–upper 95% CI)
Turtle grass (*Thalassia testudinum*)				
Aboveground Biomass	Site	0.01	Traylor Island	11.4 (3.9–34.0) (B)
(g dry weight m^{-2})	Depth	<0.0001	Mud Island	42.1 (18.2–97.6) (AB)
	Year	<0.0001	Hog Island	121.9 (56.6–262.6) (A)
Belowground Biomass	Site	0.008	Traylor Island	33.9 (11.8–97.5) (B)
(g dry weight m^{-2})	Depth	<0.0001	Mud Island	173.2 (79.4–377.6) (A)
	Year	<0.0001	Hog Island	429.9 (207.4–891.2) (A)
Root:Shoot Ratio	Site	0.45	Traylor Island	2.6 (1.5–4.5)
	Depth	0.0006	Mud Island	2.6 (1.9–3.6)
	Year	0.0023	Hog Island	3.2 (2.4–4.4)
Shoot Density	Site	0.05	Traylor Island	115 (30–444)
(shoots m^{-2})	Depth	<0.0001	Mud Island	544 (172–1715)
	Year	0.002	Hog Island	1128 (367–3460)
Shoal grass (*Halodule wrightii*)				
Aboveground Biomass	Site	0.68	Traylor Island	12.3 (0.01–118)
(g dry weight m^{-2})	Depth	0.2	Mud Island	0.2 (0.0–2.4)
	Year	0.12	Hog Island	0.04 (0.0–2.6)
Belowground Biomass	Site	0.0003	Traylor Island	144.1 (112.8–182.2) (A)
(g dry weight m^{-2})	Depth	0.47	Mud Island	19.4 (10.3–36.5) (B)
	Year	<0.0001	Hog Island	4.1 (1.1–16.0) (B)
Root:Shoot Ratio	Site	0.84	Traylor Island	2.7 (1.5–4.9)
	Depth	0.96	Mud Island	2.0 (0.9–4.4)
	Year	0.55	Hog Island	1.8 (0.08–40.6)
Shoot Density	Site	0.001	Traylor Island	3842 (2841–5195) (A)
(shoots m^{-2})	Depth	0.22	Mud Island	729 (374–1421) (B)
			Hog Island	139 (30–631) (B)

Table 1.7. The percent of flowering widgeon grass (*Ruppia maritima*) shoots at sites in the Grand Bay National Estuarine Research Reserve, Mississippi, during the 2011, 2012 and 2013 seagrass reproductive seasons. Data are represented as mean ± SE % over the sampling season for each year. Samples were collected in August 2011 and in April and June in 2012 and 2013. Widgeon grass was not present in samples from Middle Bay in 2012, as denoted by the dash (–).

Parameter	Site	2011	2012	2013
Flowering Shoots (%)	Grand Bay	0	4.0 ± 3.7	0.8 ± 0.5
	Jose Bay	0	0.7 ± 0.7	1.0 ± 1.0
	Middle Bay	2.4 ± 1.6	–	0

Table 1.8. Shoal grass (*Halodule wrighii*) and widgeon grass (*Ruppia maritima*) biomass parameters during the 2011, 2012 and 2013 seagrass reproductive seasons at three sites in the Grand Bay National Estuarine Research Reserve, Mississippi: Grand Bay, Jose Bay and Middle Bay. Data are presented as the back-transformed means and back-transformed lower and upper 95% confidance intervals. Shoal grass aboveground and belowground biomass, root:shoot ratio and shoot number and widgeon grass aboveground and belowground biomass and root:shoot ratio were analyzed using generalized linear models with a Poisson distribution and the log link function. Widgeongrass shoot number was analyzed using a generalized linear model with a negative binomial distribution and the log link function. Letters in parentheses denote significant differences between sites determined by a Tukey HSD post hoc comparison.

Parameter	Factor	p	Site	Mean (lower–upper 95% CI)
Shoal grass (*Halodule wrightii*)				
Aboveground Biomass	Site	0.02	Grand Bay	6.9 (3.7–12.5) (A)
(g dry weight m^{-2})	Depth	0.37	Jose Bay	5.6 (3.0–10.6) (A)
	Year	<0.0001	Middle Bay	1.0 (0.4–3.0) (B)
Belowground Biomass	Site	0.01	Grand Bay	15.0 (8.9–25.0) (A)
(g dry weight m^{-2})	Depth	0.68	Jose Bay	9.5 (5.3–17.0) (B)
	Year	<0.0001	Middle Bay	2.4 (0.9–6.5) (C)
Root:Shoot Ratio	Site	0.02	Grand Bay	1.7 (1.0–3.0) (A)
	Depth	0.59	Jose Bay	0.9 (0.5–1.8) (AB)
	Year	0.17	Middle Bay	0.3 (0.1–0.8) (B)
Shoot Density	Site	0.05	Grand Bay	1388 (889–2169)
(shoots m^{-2})	Depth	0.77	Jose Bay	1100 (669–1810)
	Year	0.007	Middle Bay	432 (195–957)
Widgeon grass (*Ruppia maritimia*)				
Aboveground Biomass	Site	0.56	Grand Bay	18.9 (10.9–32.7)
(g dry weight m^{-2})	Depth	0.99	Jose Bay	13.6 (7.2–25.8)
	Year	0.0002	Middle Bay	19.0 (10.8–33.2)
Belowground Biomass	Site	0.04	Grand Bay	9.4 (5.4–16.1) (A)
(g dry weight m^{-2})	Depth	0.86	Jose Bay	3.6 (1.6–8.1) (AB)
	Year	0.45	Middle Bay	2.2 (0.8–6.1) (B)
Root:Shoot Ratio	Site	0.07	Grand Bay	0.4 (0.2–0.9)
	Depth	0.92	Jose Bay	0.1 (0.04–0.4)
	Year	0.02	Middle Bay	0.07 (0.02–0.29)
Shoot Density	Site	0.04	Grand Bay	1348 (747–2432) (A)
(shoots m^{-2})	Depth	0.94	Jose Bay	760 (423–1363) (AB)
	Year	0.85	Middle Bay	414 (220–781) (B)

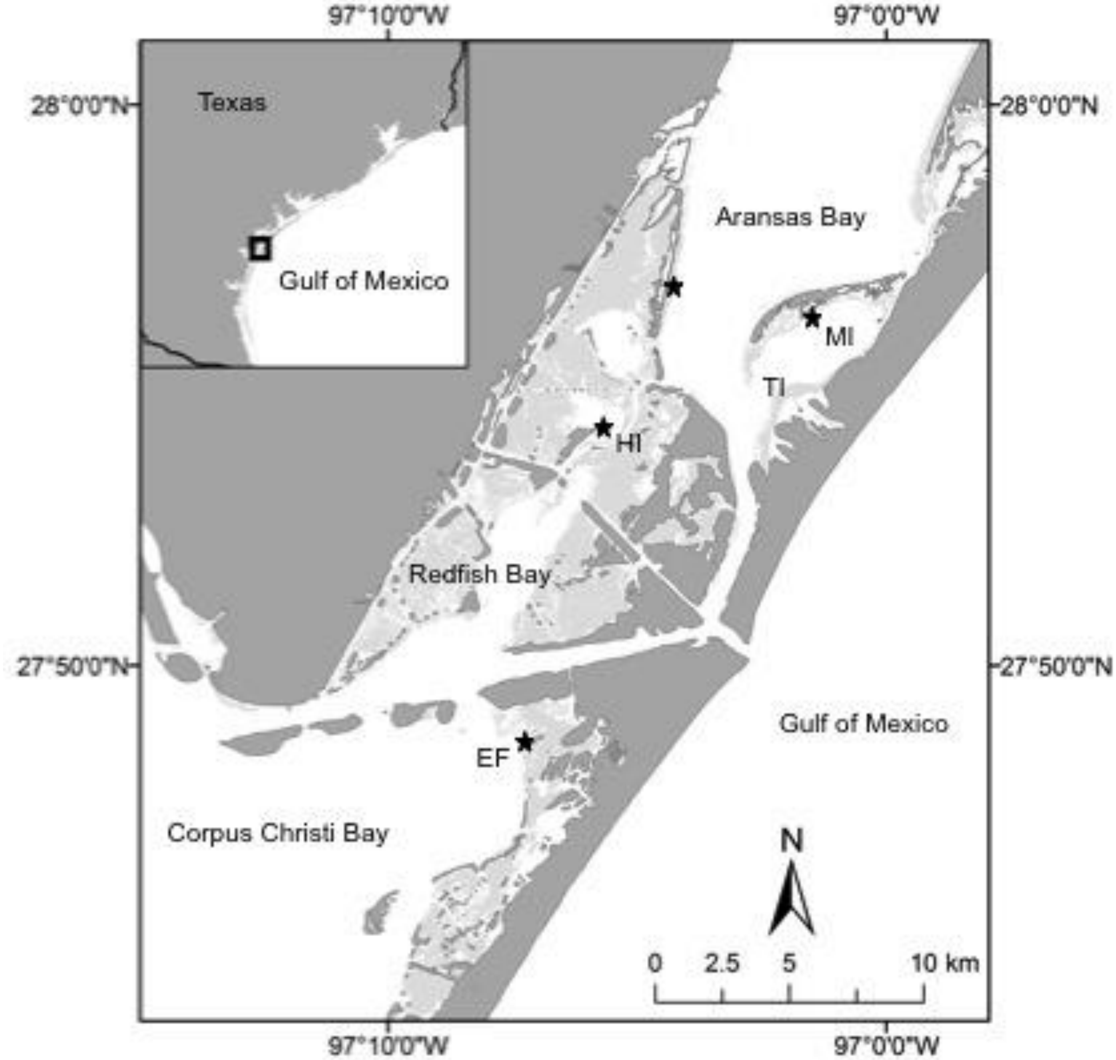

Figure 1.1. Seagrass reproductive monitoring sites in East Flats (EF), Corpus Christi Bay, and three sites within the Mission-Aransas National Estuarine Research Reserve: Traylor Island (TI), Mud Island (MI) and Hog Island (HI), Texas. Sites were monitoring during the 2011, 2012 and 2013 seagrass reproductive seasons.

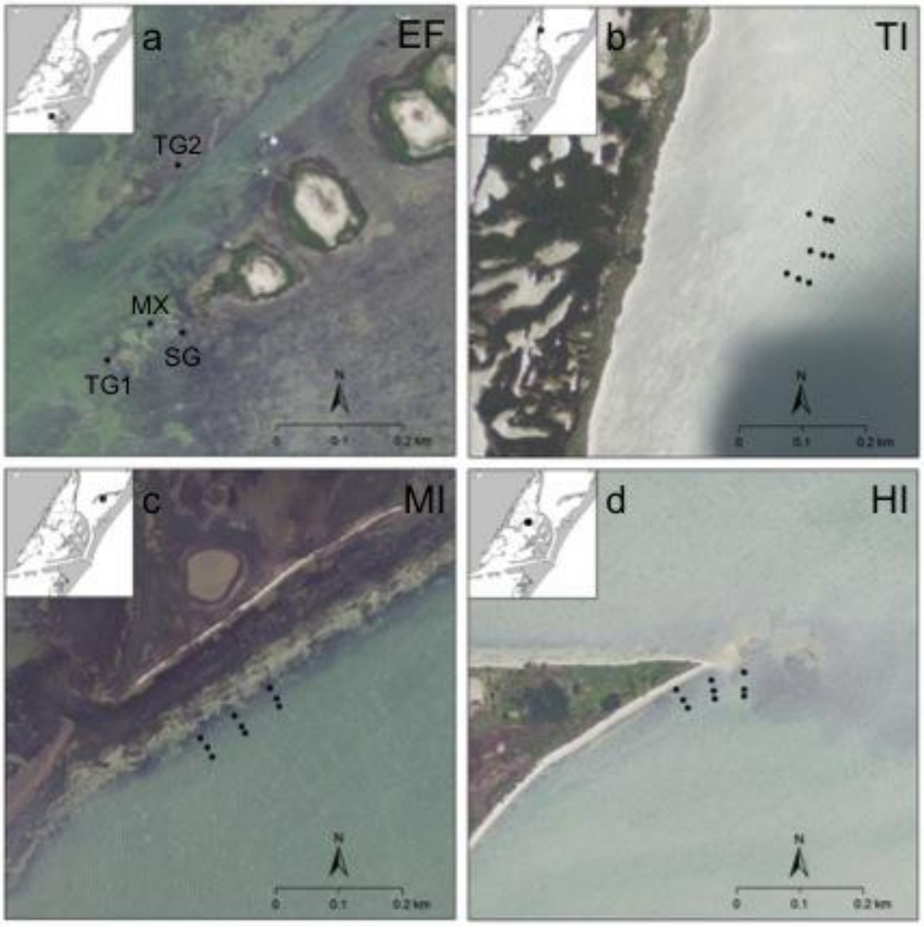

Figure 1.2. Orthoimagery of seagrass monitoring sites in Texas. In East Flats, Corpus Christi Bay, monitoring occurred at random locations in each of four sites (Turtle grass 1(TG1), Turtle grass 2 (TG2), Mixed (MX) and Shoal grass (SG)) every other week during the seagrass reproductive season (a). Seagrasses were monitoring at three sites in the Mission-Aransas National Estuarine Research Reserve (Traylor Island (b), Mud Island (c) and Hog Island (d)) once in 2011 and monthly throughout the seagrass reproductive seasons in 2012 and 2013. Samples were collected at three depths along triplicate transects at each of these sites (b,c,d). Orthoimagery was obtained from the USGS National Geospatial Program.

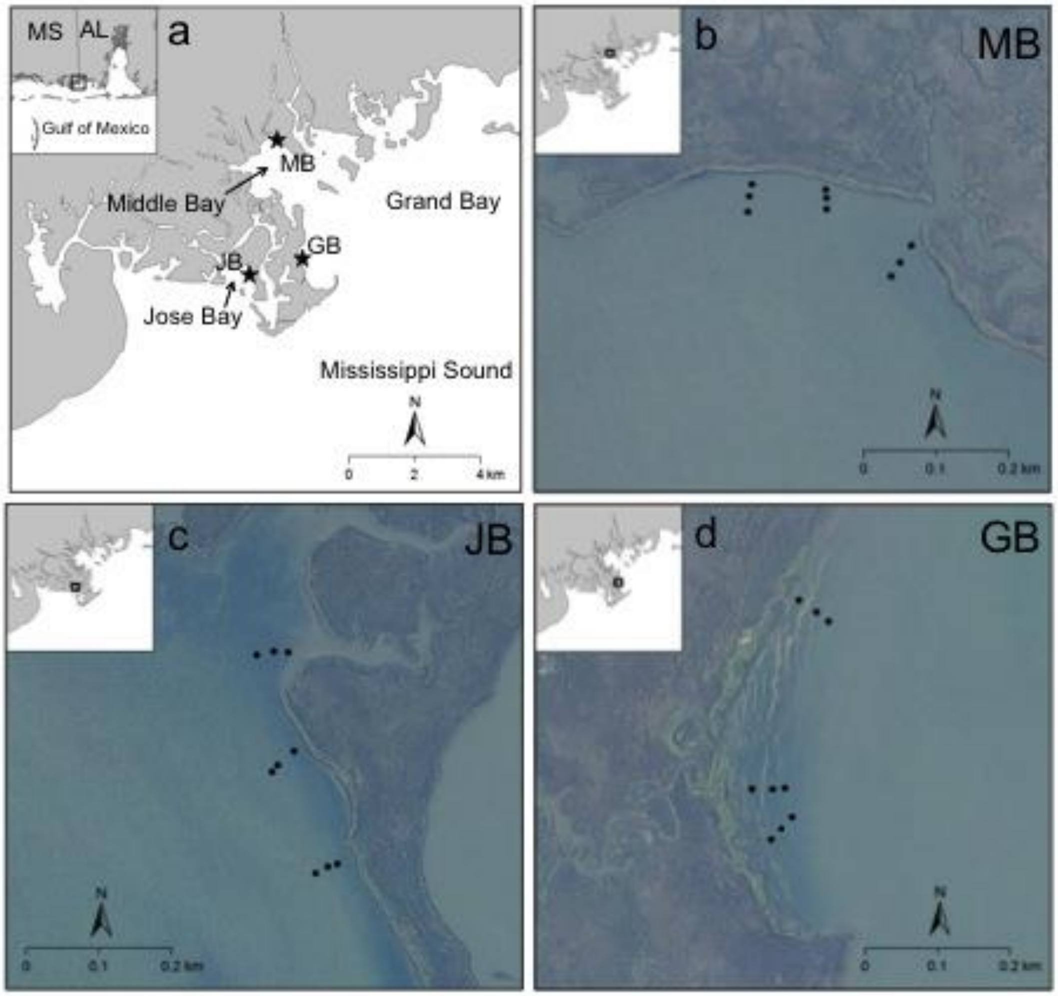

Figure 1.3. Orthoimagery of seagrass monitoring sites in the Grand Bay National Estuarine Research Reserve, Mississippi: Middle Bay (MB), Grand Bay (GB) and Jose Bay (JB). Sites were monitored in August 2011 and in April and June 2012 and 2013. Orthoimagery (b, c, d) was obtained from the USGS National Geospatial Program.

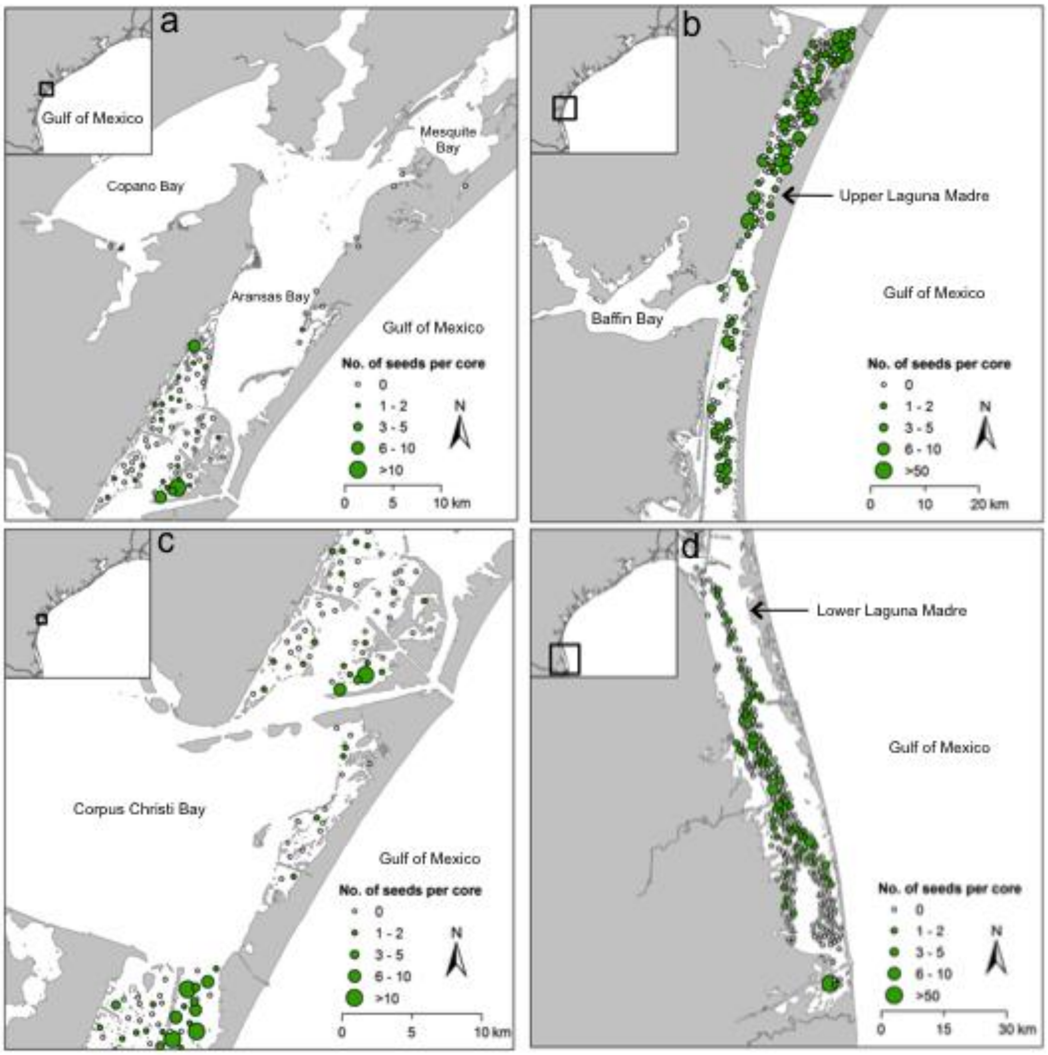

Figure 1.4. Number of dormant shoal grass (*Halodule wrightii*) seeds in sediment cores collected along the Texas coast as part of the statewide seagrass monitoring program. Cores were collected in Aransas Bay, Copano Bay and Mesquite Bay, which are part of the the Mission-Aransas National Estuarine Research Reserve (MANERR) (a), Upper Laguna Madre (b), Corpus Christi Bay (c) and Lower Laguna Madre (d). In addition to displaying seed densities in the MANERR, panel 'a' also displays seed densities from the 'Corpus Christi Bay' stations. Seed density is reported as the number of seeds per core (9.5 cm wide × 10 cm deep). One core was collected at each site.

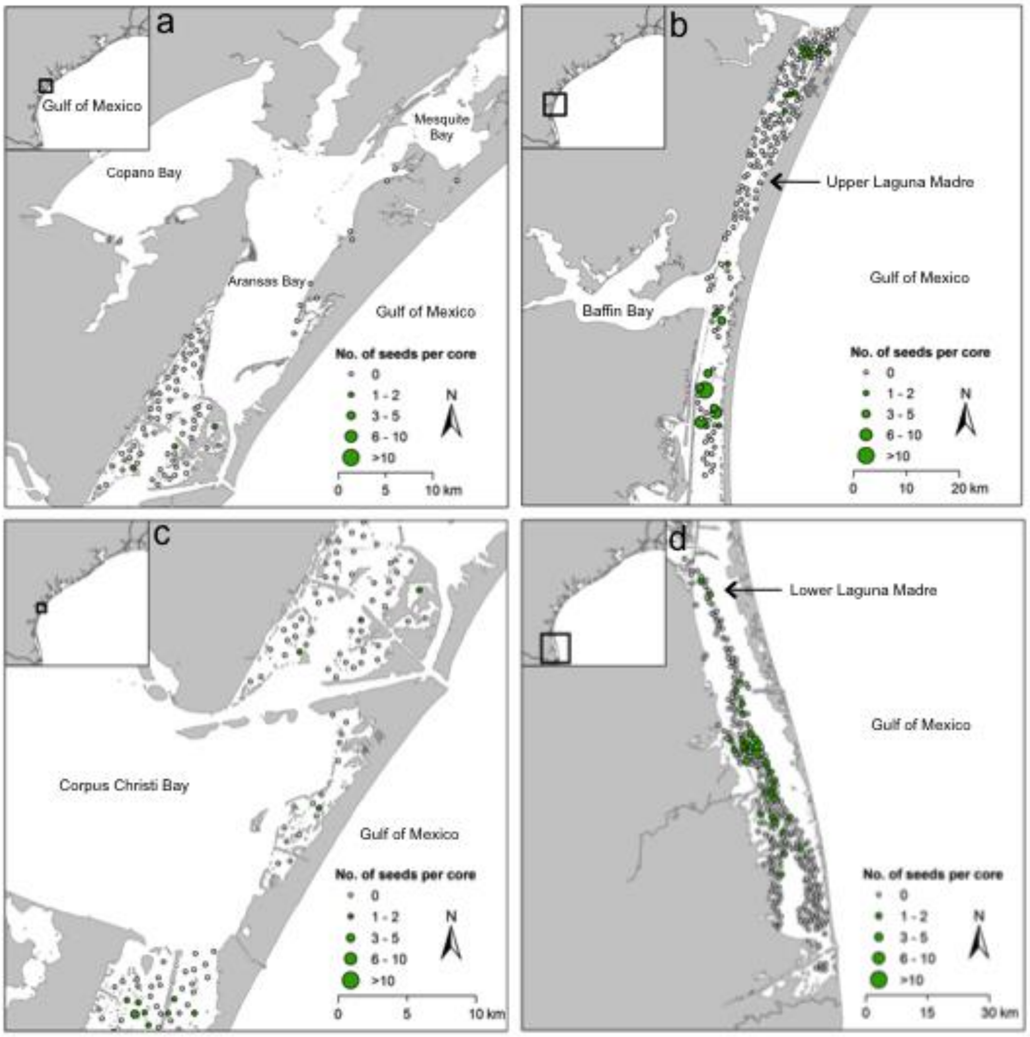

Figure 1.5. Number of dormant widgeon grass (*Ruppia maritima*) seeds in sediment cores collected along the Texas coast as part of the statewide seagrass monitoring program. Cores were collected in Aransas Bay, Copano Bay and Mesquite Bay, which are part of the Mission-Aransas National Estuarine Research Reserve (MANERR) (a), Upper Laguna Madre (b), Corpus Christi Bay (c) and Lower Laguna Madre (d). In addition to displaying seed densities in the MANERR, panel ‘a’ also displays seed densities from the ‘Corpus Christi Bay’ stations.Seed density is reported as the number of seeds per core (9.5 cm wide × 10 cm deep). One core was collected at each site.

Chapter 2: Plasticity in Turtle Grass (*Thalassia testudinum*) Flower Production in Response to Pore-water Availability of Nitrogen

Abstract

I used a manipulative field-based experiment to assess the effect of pore-water nutrients on turtle grass (*Thalassia testudinum*) flower production. Experiments were conducted within monospecific turtle grass beds in Lower Laguna Madre, Texas, a region with consistently low water column (< 2 μM and < 0.2 μM dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP), respectively) and pore-water nutrient levels (< 50 μM and < 16 μM DIN and DIP, respectively). Two months before the onset of the 2012 reproductive season I enriched 50 turtle grass plots with Osmocote Smart-Release fertilizer (19-6-12) buried at the rhizome layer. Each enriched plot was paired with an unenriched plot and adjacent underground rhizomes were severed between the two to prevent translocation of nutrients. This procedure resulted in increased pore-water ammonium concentrations of 679 ± 188 μM in enriched plots, compared to 204 ± 34 μM in unenriched plots. After the onset of the reproductive season (May), I examined turtle grass shoot morphology, elemental composition and reproductive status. Unenriched plots had a higher proportion of reproductive shoots (0.12) than enriched (0.06) plots, but shoots from enriched plots had more leaves that were longer and wider than the leaves of their unenriched counterparts. Enriched shoots assimilated the additional available nitrogen into leaf tissue, but did not assimilate additional phosphorus. My results suggest that turtle grass exhibits plasticity in reproduction as a response to nutrient availability, whereby under low pore-water nutrient conditions, some resources are diverted to sexual reproduction from somatic growth.

Introduction

Sexual reproduction is energetically costly and, as a result, reproductive output often varies over a range of spatial and temporal scales (Obeso 2002). For many plants, resource allocation to reproduction and somatic growth is related to environmental conditions such as temperature, photoperiod, water availability and nutrients (Wada and Takeno 2010). This plasticity in reproduction is common and especially pronounced when resources are limiting (Harper 1977).

Nutrients such as nitrogen and phosphorus are essential for plant growth and are heterogeneous within the soil over fine spatial scales (Larcher 1975). Their availability can influence plant morphology, production, and ecological interactions such as herbivory and competition (Larcher 1975; Gao et al. 2013). In many species of terrestrial herbaceous plants, nutrient availability also influences resource allocation to reproductive modes (Doust and Doust 1988). Whereas some plants induce sexual reproduction under high nutrient conditions (Campbell and Halama 1993), others do so when exposed to stressfully low nutrient levels (stress-induced flowering; Wada et al. 2010). For example, two eudicots, *Pharbitis nil* and *Perilla frutescens* var. *crispa*, flower in response to poor nutrition (Wada and Takeno 2010) while flowering in *Arabidopsis thaliana* is triggered by low nitrate (NO_3^-) conditions (Marin et al. 2011). Although the influence of nutrients on terrestrial plant reproductive allocation has been widely studied, the effect of nutrient availability on reproductive allocation in their marine counterparts (seagrasses) is unknown.

Seagrasses are a widespread group of over 70 species of submerged marine plants that, unlike most terrestrial plants, can assimilate inorganic nutrients through both above-ground (leaf) and below-ground (root) tissues. The complex mechanisms of uptake and assimilation of pore-water and water column nutrients by seagrasses have been studied at length (Duarte 1990; Lee and Dunton 1999), and as a result, it is well known that nutrient availability affects most aspects of seagrass biology, physiology and ecology (Armitage et al. 2005). Seagrass growth is often nutrient-limited, as studies enriching pore-water and/or water column nutrients have shown marked increases in growth and changes in morphology (Duarte 1990; Ferdie and Fourqurean 2004). The exact nutrient limiting seagrass growth is often species-, location- and/or time-dependent (Fourqurean et al. 1992). However, the most common nutrients limiting seagrass growth and production are nitrogen and phosphorus (Duarte 1990). Worldwide increases in anthropogenic nutrient loading and eutrophication are exposing seagrasses to higher than normal nutrient levels (Nixon 1995), and though it is expected that this increased nutrient loading will alter seagrass growth, productivity and ecology (Burkholder et al. 2007), the effects on seagrass reproduction remain unclear.

Seagrasses are angiosperms and have the ability to reproduce sexually and also propagate clonally through lateral rhizome growth. Historically, sexual reproduction was considered rare for seagrass genera (den Hartog 1970) and this expected rarity led to a dominance of literature examining clonal growth (Hemminga and Duarte 2000). However, recent research indicates that sexual reproduction is important for both seagrass bed establishment and maintenance (van Dijk et al. 2009). The limited historical research on seagrass reproduction leaves many questions unanswered, especially questions about environmental factors that influence reproductive output and the relative importance of vegetative versus sexual reproduction.

Turtle grass (*Thalassia testudinum* Bank ex König) is a dominant seagrass species throughout the Gulf of Mexico and Caribbean and usually grows in areas with low water column ($PO_4^{3-} < 1$ μM and $NH_4^+ + NO_3^- < 3$ μM; van Tussenbroek et al. 2006) and pore-water (NH_4^+ between 2–200 μM, Lee and Dunton 1999) nutrients. Nutrient uptake occurs through both the leaves and roots (Lee and Dunton 1999) and nutrient addition experiments have resulted in increased somatic growth and production (Lee and Dunton 1999a). As with all seagrass species, turtle grass expands clonally by horizontal rhizome extension and also reproduces sexually. Horizontal propagation is relatively slow, with rhizome extension only 19–35 cm $year^{-1}$ $apex^{-1}$ (Gallegos et al. 1992; van Tussenbroek 1998). Turtle grass plants are dioecious (each clone is individually male or female), but clones of both sexes often grow intermixed (van Tussenbroek et al. 2006). The turtle grass reproductive season varies along the species range, but is generally in the summer (van Tussenbroek et al. 2006). During the reproductive season, inflorescences are produced near the sediment at the base of the shoot, and upon successful pollination of the female inflorescence, a fruit is produced. Fruits contain one to six seeds, but most commonly have two (Kaldy and Dunton 2000; van Tussenbroek et al. 2010). To my knowledge, only two studies have investigated the relationship between nutrients and seagrass reproduction, and these have focused on *Zostera marina* (Short 1983) and *Ruppia drepanensis* (Santamaria et al. 1995).

Here, I present measurements directed at determining how nutrient availability influences turtle grass flowering and reproductive output. I determined the influence of pore-water and assimilated nutrients on (1) turtle grass flower production and (2) turtle grass somatic (leaf) growth. I hypothesized that (1) turtle grass demonstrates reproductive plasticity in response to pore-water nutrient availability, and (2) flower production and somatic growth are inversely related. I conducted a nutrient enrichment experiment in turtle grass-dominated beds in south Texas during Spring 2012 and evaluated the reproductive status and somatic growth of turtle grass relative to pore-water ammonium levels and leaf elemental composition (carbon, nitrogen and phosphorus levels and molar ratios).

Materials and methods

Study sites

Nutrient enrichment experiments were conducted in Lower Laguna Madre (LLM), Texas (Figure 2.1). This area is characterized by consistently low dissolved inorganic-N as reflected in both pore-waters (~30 µM pore-water ammonium, Lee and Dunton 1999a) and water-column (~1 µM water-column NH_4^+ NO_3^-, and NO_2^-, Lee and Dunton 1999a) nutrients and has been the site of several prior studies investigating seagrass biology (Lee and Dunton 1999, 1999a). Five sites, each 150 m × 150 m, were selected, of similar depth (122 ± 5.33 cm) and occupied by monotypic turtle grass meadows (Figure 2.1). Within each of the five sites (29, 33, 39, 42, 45), ten stations were randomly chosen (n= 50 stations total) with at least 15 m between stations to avoid re-sampling individual genets (van Dijk and van Tussenbroek 2010; Figure 2.1). Each station contained paired unenriched and enriched plots (see below).

Environmental parameters (water temperature, salinity, pH) were measured at the beginning (29 March 2012), middle (26 April 2012) and end (22 May 2012) of the experiment with a YSI 600XL data sonde. Measurements were taken at the top of the seagrass canopy in each of the five sites at the center of the ten stations.

Nutrient enrichment experimental design

Approximately 2 months before onset of the reproductive season in LLM (K. Darnell, pers. obs), two adjacent 0.25 m^2 plots were established at each station. One plot served as a control (unenriched plot) and the other plot was enriched with Osmocote Smart-Release® Plant Fertilizer (N-P-K 19:6:12; enriched plot). In the enriched plots, 30.375 g of fertilizer pellets were wrapped in cheesecloth to obtain the manufacturer's suggested application dosage and buried ~10 cm below the sediment surface at the rhizome layer in the center of the plot (Lee and Dunton 1999a). Cheesecloth bags were approximately 8 cm tall × 8 cm wide. Based on the manufacturer's values, the amount of N and P applied to each 0.25 m^2 enriched plot was 5.77 g N (ammoniacal nitrogen and nitrate) and 1.8 g P (phosphate). Unlike aboveground fertilizer application, belowground fertilizer application eliminates potential confounding effects of increased leaf epiphyte cover (Lee and Dunton 1999a). A third plot was added to a random subset of stations (n = 2 stations per site) to test effects of cheesecloth bag burial (empty bag control). In these plots, an empty cheesecloth bag was buried belowground at the rhizome layer. Rhizomes were severed along the perimeter of each plot to a depth of ~30 cm to avoid potential translocation of nutrients through the rhizomes (Lee and Dunton 1999a).

The experiment was initiated on 29 March 2012. At the time of plot establishment, two replicate sediment samples were collected from the center of each plot at a subset of stations with an 80 mL syringe for analysis of sediment pore-water NH_4^+. Sediment pore-water was obtained by centrifugation (5000 xg for 20 minutes) and NH_4^+ content was analyzed using standard colorimetric techniques following Parsons et al. (1984). Three intact turtle grass shoots consisting of all aboveground and belowground tissue were also collected from the center of each plot for assessment of seagrass reproductive status, morphology, and leaf nutrient content (see below).

The experiment concluded on 22 May 2012, 54 d after experimental plots were established. At the conclusion of the experiment, two replicate sediment samples were again collected from the center of each plot at a subset of stations and analyzed for sediment pore-water NH_4^+ content. Fertilizer bags were also collected from each enriched plot and re-weighed, and daily nitrogen delivery was estimated assuming uniform delivery over the duration of the experiment. Five intact turtle grass shoots were collected from the center each plot for assessment of seagrass reproductive status, morphology, age, and leaf nutrient content (see below).

Seagrass reproductive status, morphology and age

Each shoot collected at the beginning and end of the experiment was assessed for reproductive status by inspecting it for the presence of reproductive tissues (inflorescences or fruits), and the proportion of reproductive shoots per plot was calculated. For March and May, longest leaf area (cm^2) was calculated for each shoot by multiplying the length (cm) and width (cm) of the longest leaf on that shoot. For May samples only, the aboveground dry weight (g) of each shoot was determined by scraping the leaves free of epiphytes and drying at 60 °C to a constant weight. Dry weights of epiphytes on leaf tissue were obtained for a subset of these shoots. Also for May samples only, shoot age was estimated by counting the number of leaf scars on the vertical rhizome and dividing by the annual leaf production rate for turtle grass in this area (13 leaves $year^{-1}$, Kaldy et al. (1999)).

Leaf tissue nutrient analyses

Leaf tissue carbon, nitrogen and phosphorus were measured in one randomly chosen shoot per plot in both March and May. Shoots were rinsed, wiped free of epiphytes and dried at 60°C to a constant weight before being ground to a fine powder using a Wig-L-Bug® grinding mill. Ground tissue was sent to the University of California at Davis Stable Isotope Facility for analysis of total carbon and total nitrogen using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples were analyzed for total phosphorus at the University of Texas Marine Science Institute on a Shimadzu UV-2401 PC UV-VIS Recording Spectrophotometer following a modified protocol from Chapman and Pratt (1961). Carbon, nitrogen and phosphorus data were used to calculate %C, %N and %P and molar C:N:P ratios.

Statistical analyses

In each of two samples per plot in March and May, we measured pore-water NH_4^+. On each of three shoots per plot (March) or five shoots per plot (May) we measured leaf area and recorded whether or not it was reproductive. On one shoot per plot, in both March and again in May, we measured a set of leaf nutrient variables (proportion C, proportion N and proportion P). In May only, on each of five shoots per plot, we measured its dry mass, its age, and the dry mass of its epiphytes.

We analyzed pore-water NH_4^+, proportion C, proportion N, proportion P and epiphyte biomass using analysis of variance (ANOVA) with site and treatment as fixed factors and station nested within site as a random factor. Residuals were normally distributed. To obtain normally distributed residuals of some of the other variables, we transformed the measured values before analysis with ANOVA. We analyzed the square-root of leaf area, the square-root of dry mass, the square-root of shoot age, and the log10 of C:N, of C:P, and of N:P. Reproductive status of each shoot (the proportion of shoots that were reproductive) was analyzed with a generalized linear model with a binomial distribution and a logit link function.

Each analysis of a leaf nutrient variable had the same predictor variables: site, station nested with site, and treatment. Treatment is factorial with respect to site. There was one sample per treatment-station combination (i.e., per plot). Site and treatment were considered to be fixed effects. Station nested within site was considered to be a random effect. Each analysis initially included the interaction term site x treatment; it was dropped from the final model if non-significant. The analyses of pore-water NH_4^+, leaf area, shoot dry mass, shoot age, epiphyte biomass, and whether or not a shoot was reproductive each had the same predictor variables: site, station nested with site, treatment, and plot. Treatment is factorial with respect to site. Site and treatment were considered to be fixed effects. Station nested within site was considered to be a random effect, as was plot.

Data with residuals that were normally distributed are presented as the mean ± standard error (mean ± S.E.). Data that were transformed to obtain normality of the residuals are reported as the back-transformed mean and back-transformed lower and upper 95% confidance intervals.

Results

In March, prior to the initiation of the experiment, enriched and unenriched plots did not differ for any of the variables measured (Table 2.1). In May, there were no differences between unenriched and control (empty bag control) plots, indicating that cheesecloth bag burial did not influence results with respect to disturbance of belowground tissues (Table 2.2).

Environmental parameters

Water temperature increased throughout the experiment from 25.3 ± 0.4 °C (mean ± S.E.) in March to 25.6 ± 0.3 in April, and 27.0 ± 0.6 °C in May (Table 2.3). All sites remained saline throughout the experiment (March: 32.8 ± 0.5, April: 30.1 ± 0.2, May: 34.6 ± 0.6), and pH displayed low spatial and temporal variability (March: 8.00 ± 0.06, April: 8.33 ± 0.11, May: 7.91 ± 0.02) (Table 2.3).

Pore-water ammonium

At the beginning of the experiment, prior to nutrient enrichment, sediment pore-water NH_4^+ levels were relatively low in both the unenriched (198.9 ± 33.9 μM) and enriched (154.0 ± 17.8 μM) plots ($F_{1,37.58}$, p = 0.06; Figure 2.2). Fertilizer bag weight decreased by 62.3 ± 1.5% over the course of the experiment, delivering 0.34 ± 0.01 g fertilizer day^{-1} to the enriched plots. As a result, pore-water NH_4^+ levels were elevated in the enriched plots (679.9 ± 188.1 μM) compared to the unenriched plots (203.6 ± 34.7 μM; $F_{1, 28.11}$, $p < 0.0001$) by the conclusion of the experiment in late May (Figure 2.2).

Shoot reproductive status, morphology and age

All shoots were non-reproductive in March, confirming that my experiment began before the onset of the turtle grass reproductive season in this area. In May, 89 out of 472 shoots (18.9 %) were reproductive. Of the reproductive shoots, 62 had developing inflorescences and 27 bore fruit. Unenriched plots had 2x the proportion of shoots that were reproductive (0.12 (0.07–0.18)) (back-transformed mean (back-transformed lower and upper 95% confidance intervals)) than enriched plots (0.06 (0.03–0.10)) ($F_{1,425}$, p = 0.0002) (Figure 2.3a). Shoots in enriched plots, however, had more aboveground biomass ($F_{1,433.5}$, $p < 0.0001$, Figure 2.3b) and a greater leaf area ($F_{1,431.2}$, $p < 0.0001$, Figure 2.3c) compared to unenriched shoots. Epiphyte dry weight, however, was similar for shoots from unenriched and enriched plots ($F_{1, 82.33}$, p = 0.21). Estimated shoot ages ranged from one to ten years with 97% of shoots between one and six years. Age did not differ significantly between unenriched and enriched shoots ($F_{1, 435.9}$, p = 0.51, Figure 2.4a) and most (96%) of the reproductive shoots were between ages two and six years old (Figure 2.4b).

Leaf tissue carbon, nitrogen and phosphorus content

Leaf tissue carbon (%C), nitrogen (%N), phosphorus (%P) and molar C:N:P ratios did not differ between unenriched and enriched plots at the beginning of the experiment (March, Table 2.1) and all nutrient data were within the reported range for turtle grass (Duarte 1990). At the conclusion of the experiment in May, shoots collected from the enriched plots had significantly higher leaf tissue %C ($F_{1, 43.95}$, p = 0.008) and %N ($F_{1,42.93}$, $p < 0.0001$) than shoots from unenriched plots (Table 2.4). The %C and %N in enriched shoots were greater at 37.78 ± 0.17% and 2.90 ± 0.05%, respectively, compared to unenriched shoots at 36.84 ± 0.33% and 2.45 ± 0.05%. Phosphorus content, however, was similar in both treatments ($F_{1,38.88}$, p = 0.8618), at 0.18 ± 0.01% in both enriched and unenriched shoots (Table 2.4). Enriched shoots also had lower molar C:N (15.38 (14.82–

15.95, $F_{1,43.06}$, $p < 0.0001$) and higher molar N:P (36.44 (34.14–38.89), $F_{1,38.61}$, $p < 0.0001$) than unenriched shoots (C:N: 18.14 (17.42–18.90); N:P: 29.67 (27.54–31.97), reflecting the enhanced leaf tissue nitrogen in enriched plots. Molar C:P did not differ between treatments ($F_{1,38.5}$, $p = 0.7145$) and was 558.77 (518.62–602.03) and 547.67 (502.36–597.06) for enriched and unenriched shoots, respectively (Table 2.4).

Discussion

My results demonstrate that turtle grass produces more flowers under low nutrient conditions (pore-water NH_4^+ 203.6 ± 34.7 μM), than under high nutrient conditions (pore-water NH_4^+ 679.9 ± 188.1 μM) (Figure 2.5). When exposed to high nutrients, turtle grass produces fewer flowers and increases somatic growth of aboveground leaf tissue with more leaves that are longer and wider than their unenriched conspecifics. Although observational studies have suggested a connection between nutrients and seagrass reproduction (e.g. Short 1983), this is the first direct experimental evidence of plasticity in flower production in response to *in situ* pore-water nutrient concentrations. These results suggest that the global increase in anthropogenic nutrient loading to coastal systems (Nixon 1995), particularly in the form of groundwater nutrient delivery, is likely to reduce turtle grass flower production and could have dramatic population-level consequences such as reducing genetic diversity for this foundation species and other closely related *Thalassia* species.

Stress-induced flowering

Stress-induced flowering as a result of sub-optimal conditions is a common resource allocation strategy for terrestrial and aquatic angiosperms because of the plants' inability to physically escape poor surroundings (Wada and Takeno 2010). Common stressors that induce flower production include high or low light, drought, mechanical stimulation, low nitrogen and poor nutrition (Wada and Takeno 2010). Inducing sexual reproduction under sub-optimal conditions increases (1) the likelihood that recruitment of offspring to the population will occur in the next, possibly more favorable season, and (2) the potential for offspring to escape (disperse) from the stressful conditions near the parent (Williams 1975). Fruits and seeds of turtle grass have the capability to disperse long distances from the parent plant and, therefore, from local environmental conditions. Buoyant fruits detach from the parent plant where they float to the surface and are transported by currents up to 360 km (van Dijk et al. 2009). Taking into account local current and wave conditions in Lower Laguna Madre, Texas where my experiment was conducted, Kaldy and Dunton (1999) estimated that turtle grass fruits disperse up to 15 km from the parent plant before the negatively buoyant seeds are released and settle to the substrate.

Long-range propagule dissemination under low nutrient conditions, which results from stress-induced flowering, seems appropriate for species that have large ranges in distribution. Aquatic vascular plants often display broader distribution ranges than terrestrial plants (Santamaria 2002), but the influence of nutrients on flower induction in freshwater plants remains unclear. Although Rogers et al. (1992) reported that increased N-availability promoted seed pod production in the freshwater angiosperm *Vallisneria americana*, Lokker (2000) more recently found no effect of surrounding nutrient conditions on flower production. In seagrasses, Short (1983) reported that flowering in eelgrass (*Zostera marina*), a widely distributed species with a potential for long distance seed dispersal (up to 150 km, Harwell and Orth 2002) similar to turtle grass, was inversely correlated with pore-water NH_4^+ concentration. Short (1983) sampled eelgrass in in Izembek Lagoon, AK along a nutrient gradient, but other factors such as water depth and light intensity confounded his results. My experimental study with turtle grass provides evidence that the pattern observed by Short (1983) may indeed be a direct effect of pore-water NH_4^+ concentration. Similar to *Z. marina*, *Ruppia* spp. are among the most geographically widespread seagrasses and can be dispersed long distances by waterfowl (Figuerola et al. 2003). Santamaria et al. (1995) reported that *Ruppia drepanensis* stimulates flowering and produces more flowers in nutrient-poor sediments than nutrient-replete sediments, indicating that the pattern I observed with turtle grass may be common among many seagrass genera.

Although turtle grass seeds have the potential for long distance dispersal by current-mediated transport of buoyant fruits, it has been reported that seeds are sometimes released while the fruit it still attached to the parent plant (van Tussenbroek et al 2010). This strategy would dramatically reduce seed dispersal distance and likely eliminate escape from local sub-optimal conditions. Turtle grass seedlings, however, rely on internal nutrient stores for two to six months following release from the fruit (Kaldy and Dunton 1999). Such a strategy increases the likelihood of propagule survival under variable nutrient conditions, which are highly dynamic within seagrass meadows and would likely change over the course of two to six months (Lee and Dunton 1999a). Seagrasses rely heavily on pore-water nutrients, which are spatially and temporally variable and depend on several processes including organic matter remineralization, detrital flux, diffusion to the water column, precipitation and adsorption (Hemminga and Duarte 2000). A more complete understanding of the factors that influence seagrass propagule dissemination will benefit from studies that address Allee effects, connectivity among patches, and propagule retention in the water column.

Stress-induced flowering has been reported for several seagrass species as a response to sub-optimal conditions. For example, *Cymodocea nodosa* in the northwest Mediterranean increases flowering frequency when disturbed by subaqueous dune migration (Marba and Duarte 1995), and in a recent meta-analysis, Cabaco and Santos (2012) reported that in 72% of cases, seagrass reproductive effort increased with disturbances such as mechanical damage, hydrodynamic stress, and effects associated with eutrophication. For my study species, turtle grass, Gallegos et al. (1992) found increased flowering in the Mexican Caribbean in response to disturbance by Hurricane Gilbert. My results and those of Gallegos et al. (1992) of increased reproduction under sub-optimal conditions are supported by reports of smaller turtle grass genets (indicating more sexual reproduction) in oligotrophic areas compared to larger genets in eutrophic areas (van Dijk and van Tussenbroek 2010).

Environmental parameters such as nutrient availability can also influence the timing of plant reproduction (Lacey 1986). It is possible that the sub-optimally low nutrient levels in the unenriched plots stimulated the turtle grass plants to flower earlier, or, alternatively, that the elevated nutrient levels in the enriched plots delayed flowering. I did not track plant flowering throughout the entire reproductive season and am not able to make conclusions based on the influence of nutrients on reproductive timing, but recommend that future studies examine the influence of nutrient supply on the timing of seagrass flowering.

Nitrogen limitation

Turtle grass generally grows in areas with low ambient pore-water NH_4^+ levels and receives most of its nitrogen from these belowground pools (Fourqurean et al. 1992). Pore-water NH_4^+ levels from my study are within the range reported in turtle grass meadows and the levels in my enriched treatment (679.9 ± 188.1 μM) are considered high for turtle grass beds (Fourqurean et al. 1992a, Lee and Dunton 1999). The tissue levels of N and P in unenriched turtle grass from my study are similar to levels reported previously for this non-eutrophic area. Lee and Dunton (1999a) and Kaldy and Dunton (2000) conducted studies near my experimental plots and reported ranges for nitrogen of 1.75–2.0% and 1.7–2.7%, respectively, and a more recent monitoring study by Dunton (unpublished data) found a range of phosphorus in turtle grass leaf tissue near my study sites of 0.06–0.3%. Although the ambient pore-water NH_4^+ levels at the beginning of my experiment and in the unenriched plots at the end of the experiment are higher than the worldwide average for seagrass beds (mean: 86 μM NH_4^+, Hemminga 1998), it appears that turtle grass in this study was nitrogen-limited. Duarte (1990) suggested that plants with less than 1.8% N and a C:N ratio of 19.75:1 are nitrogen limited. Despite a leaf tissue %N of 2.4% and a C:N ratio of 18:1, turtle grass in this study took up and assimilated the excess nitrogen in the enriched treatment and increased somatic growth. Similarly, Lee and Dunton (1999a) reported that turtle grass from Lower Laguna Madre, Texas is N-limited, despite tissue N content above the reported threshold. Duarte (1990) also suggested those plants with less than 0.20 %P and a greater C:P ratio than 474:1 are phosphorus-limited. Turtle grass shoots from my site were below the %P (0.18%) and above the C:P (575:1) thresholds as suggested by Duarte (1990), but did not assimilate the excess P that was likely provided in the enriched treatment. Inorganic phosphorus readily binds to carbonate sediments, which can induce P-limitation (Short 1987). For example, Fourqurean et al. (1992a) reported that turtle grass and *Halodule wrightii* in carbonate sediments within Florida Bay, Florida are phosphorus-limited when pore-water soluble reactive phosphorus levels are < 2 μM. However, the sandy sediments at my sites make it unlikely that the excess P was unavailable to the plants, further supporting that the lack of assimilation was physiologically rather than environmentally dictated. These data highlight the need to assess nutrient limitation based on experimental evidence rather than nutrient content and molar ratios alone.

Seagrass nutrient requirements are species-specific. In Florida Bay, Fourqurean et al. (1992a) found that *shoal grass* has a 2.6 and nearly 5-fold higher N- and P- demand than turtle grass. As a result, reproductive responses to nutrient conditions are likely species-specific, and different nutrient levels or thresholds may be necessary for species to exhibit reproductive plasticity. My *in situ* experiment addressed reproductive output in turtle grass under natural and nutrient amended conditions equivalent to a three-fold increase in pore-water ammonium. Consequently, I am unable to determine if a threshold nutrient level or ratio exists whereby the plant switches resource allocation from reproductive tissues to somatic growth. Such information would provide a much more precise physiological understanding of plant reproductive strategies collected under an experimental gradient of pore-water NH_4^+ levels.

Coastal nutrient loading

The worldwide increase in coastal nutrient loading has been implicated in seagrass die-offs in many areas (Burkholder et al. 2007). Often, nutrient loading occurs by surface runoff, which increases water column nutrients and has several detrimental effects on seagrasses including epiphyte accumulation and light limitation (Burkholder et al. 2007). To avoid the confounding factors that accompany water column fertilization (i.e. epiphyte accumulation and light limitation) and test only the effect of nutrients on turtle grass reproduction, I injected fertilizer directly into the sediment for uptake by belowground tissues. Although results from this study may not be directly applicable to areas with water column nutrient loading (because of the confounding factors mentioned above), I can nevertheless conclude that turtle grass reproduces less under elevated pore-water NH_4^+ conditions. However, my results are directly applicable to areas that receive submarine groundwater discharge (SGD). Reports of nutrient loading via SGD are becoming increasingly common and can represent a substantial source of nutrients at a local scale (Moore 1996). For example, in Nueces Bay along the cental Texas coast, the average normalized SGD seepage rate is 0.4 cm day $^{-1}$ (Breier and Edmonds 2007), and along the 780 km Texas coastline, SGD can be up to 335,000 m^3 d^{-1}, although it is spatially and temporally variable (Chowdhurry et al. 2004). Several studies have reported that seagrasses readily assimilate nutrients from SGD and these nutrients regulate seagrass distribution and increase growth (Carruthers et al. 2005; Mutchler et al. 2007). Turtle grass is frequently found in low nutrient areas that periodically receive nutrient-rich SGD and is known to assimilate wastewater nitrogen from submarine spring water (Carruthers et al. 2005). Mutchler et al. (2007) and Peterson et al. (2012) reported that turtle grass assimilated terrestrial-derived groundwater nutrients along the Yucatan Peninsula and in Jamaica, respectively. Additionally, Kamermans et al. (2002) concluded that nutrient-rich groundwater intrusion influenced diversity and abundance of *Thalassia hemprichii*, a closely related and morphologically similar ‘twin species’ to turtle grass. *Thalassia hemprichii* is geographically widespread and found throughout the western

Pacific and West Indian Ocean. Both *Thalassia* species support diverse assemblages of fauna and micro- and macroalgae and likely cover hundreds of thousands of square kilometers worldwide (van Tussenbroek et al. 2006). Although studies examining sexual reproduction in T. *hemprichii* are limited, its importance for meadow establishment and maintenance is recognized (Rollon et al. 2001), and a potential reduction in flowering of *Thalassia* spp. with increased nutrients could have substantial worldwide implications. Given the results of this study, it is possible that increases in nutrient-rich SGD could reduce flowering in *Thalassia* species. We suggest that future studies should investigate the influence of SGD pulse frequency and timing on seagrass flowering.

Shoot age and flowering

Plant age is often highly correlated with reproductive output (Larcher 1975). I estimated the age of each shoot collected in May (n = 472) and the similarity of shoot ages between enriched and unenriched plots indicates that age did not confound my results. Several descriptive studies have reported the occurrence and frequency of reproductive turtle grass shoots in a given area. The percent of reproductive shoots from my unmanipulated, unenriched plots (23.5%) was higher the the percentage reported by Gallegos et al. (1992) in the Mexican Caribbean (6.2%), but between estimates from Durako and Moffler (1985) in Florida Bay (17.8%) and Kaldy et al. (1999) near my study site in Lower Laguna Madre (~35%). Most of the reproductive shoots in this study (96%) were between ages 2 and 6, although a few shoots were ages 1, 7 and 10. These data are similar to those of Witz and Dawes (1995) who reported that turtle grass flowering in Tampa Bay mostly begins after the 1st or 2nd year of life, and van Tussenbroek (1994) who reported that turtle grass in Puerto Morelos Reef Lagoon, Mexico begins flowering between the 2nd and 5th year.

Although several studies have investigated the effects of nutrient enrichment on turtle grass, this is the first to examine impacts on flowering. Turtle grass exhibits plasticity in reproduction as a response to nutrient availability, whereby under low pore-water nutrient conditions resources are diverted to sexual reproduction rather than somatic growth. Anthropogenic nutrient loading, particularly in the form of groundwater nutrient delivery, could decrease overall turtle grass flowering and potentially reduce genetic diversity of this species. This work could be extended through future studies focusing on the influence of disturbances associated with coastal nutrient runoff into the water column such as epiphyte accumulation and light limitation on seagrass flowering, including investigations that focus on the existence of nutrient thresholds that are linked to flowering frequency.

Table 2.1 Results of linear mixed models comparing shoots from unenriched and enriched turtle grass (*Thalassia testudinum*) plots in in Lower Laguna Madre, Texas in March, prior to fertilization. Values are reported as mean ± SE if residuals were normal. If residuals were transformed to achieve normality, the back-transformed mean and back-transformed lower and upper 95% confidance intervals are reported. None of the measured parameters were significantly different (p>0.05) between unenriched and enriched plots.

	March			
	Unenriched	**Enriched**	**F**	***P***
Pore-water NH_4^+ (µM)	160.8 (124.8–207.3)	124.2 (96.3–164.0)	1, 37.58	0.0578
Proportion of Reproductive Shoots	0	0	-	-
Longest Leaf Area (cm^2)	6.2 (5.9–6.6)	6.3 (6.0_6.7)	1, 244.7	0.6058
Leaf %C	35.8 (35.4–36.1)	35.8 (35.5–36.2)	1, 48.16	0.8263
Leaf %N	2.6 ± 0.0	2.6 ± 0.0	1, 43.46	0.4548
Leaf %P	0.17 (0.16–0.19)	0.18 (0.16–0.20)	1, 43.34	0.5620
Leaf molar C:N	16.4 (15.9–16.9)	16.2 (15.6–16.8)	1, 43.56	0.5988
Leaf molar C:P	536.5 (492.9–583.9)	511.7 (463.3–565.1)	1, 42.54	0.4503
Leaf molar N:P	33.2 ± 1.2	32.7 ± 1.2	1, 44.55	0.7056

Table 2.2. Results of linear mixed models comparing shoots from unenriched (unmanipulated) and control (bag only) turtle grass (*Thalassia testudinum*) plots in Lower Laguna Madre, Texas in May. Values are reported as mean ± SE if residuals were normal. If residuals were transformed to achieve normality, the back-transformed mean and back-transformed lower and upper 95% confidance intervals are reported. None of the measured parameters were significantly different ($p>0.05$) between unenriched (unmanipulated) and control (bag only) plots.

	May			
	Unenriched	**Control**	**F**	***P***
Pore-water NH_4^+ (μM)	165.8 (121.5–226.2)	126.0 (86.1–184.2)	1, 27.02	0.1859
Proportion of Reproductive Shoots	0.11 (0.01–0.28)	0.13 (0–0.35)	1, 77.11	0.6791
Longest Leaf Area (cm^2)	8.1 (6.7–9.7)	8.4 (7.2–9.6)	1, 76.30	0.4355
Aboveground Dry Wt. (g)	0.06 (0.05–0.07)	0.06 (0.05–0.07)	1, 76.82	0.5677
Leaf %C	37.0 ± 0.5	36.2 ± 0.4	1, 7.00	0.3487
Leaf %N	2.4 ± 0.1	2.2 ± 0.1	1, 7.14	0.0824
Leaf %P	0.21 (0.16–0.25)	0.18 (0.14–0.23)	1, 7.46	0.2422
Leaf molar C:N	18.4 ± 0.5	19.5 ± 1.0	1, 6.97	0.2095
Leaf molar C:P	499.4 ± 45.4	547.0 ± 57.1	1, 7.27	0.5347
Leaf molar N:P	27.1 ± 2.3	27.8 ± 2.2	1, 7.46	0.8436

Table 2.3. Temperature (°C), salinity and pH at the turtle grass (*Thalassia testudinum*) canopy measured at the initiation (29 Mar 2012), middle (26 Apr 2012) and end (23 May 2012) of the nutrient enrichment experiment in Lower Laguna Madre, Texas. Environmental parameters were measured with a YSI 600XL data sonde at the center of the 10 stations at each site.

		Site				
Date	**Parameter**	**29**	**33**	**39**	**42**	**45**
29 Mar 2012	Temperature (°C)	24.2	26.4	25.6	24.8	25.7
	Salinity	31.6	34.6	32.1	32.9	33.0
	pH	7.95	8.15	8.09	8.02	7.8
26 Apr 2012	Temperature (°C)	26.5	25.8	25.6	25.0	24.9
	Salinity	30.5	29.6	30.4	30.0	30.1
	pH	8.6	8.35	8.44	8.31	7.95
23 May 2012	Temperature (°C)	27.9	26.0	26.1	26.1	28.8
	Salinity	33.8	33.7	33.7	34.9	37.0
	pH	7.95	7.9	7.86	7.94	7.91

Table 2.4. Results of linear mixed models comparing elemental composition of shoots from unenriched and enriched turtle grass (*Thalassia testudinum*) plots in Lower Laguna Madre, Texas in May, at the conclusion of the experiment. Values are means ± S.E if residuals were normally distributed. If data were transformed to obtain normally distributed residuals, the back-transformed mean and back-transformed lower and upper 95% confidance intervals are reported.

	May			
	Unenriched	**Enriched**	**F**	***P***
Leaf %C	36.84 ± 0.33	37.78 ± 0.17	1, 43.95	0.008
Leaf %N	2.45 ± 0.05	2.90 ± 0.05	1, 42.93	< 0.0001
Leaf %P	0.18 ± 0.01	0.18 ± 0.01	1, 38.88	0.8618
Leaf molar C:N	18.14 (17.42–18.90)	15.38 (14.82–15.95)	1, 43.06	< 0.0001
Leaf molar C:P	547.67 (502.36–597.06)	558.77 (518.62–602.03)	1, 38.50	0.7145
Leaf molar N:P	29.67 (27.54–31.97)	36.44 (34.14–38.89)	1, 38.61	< 0.0001

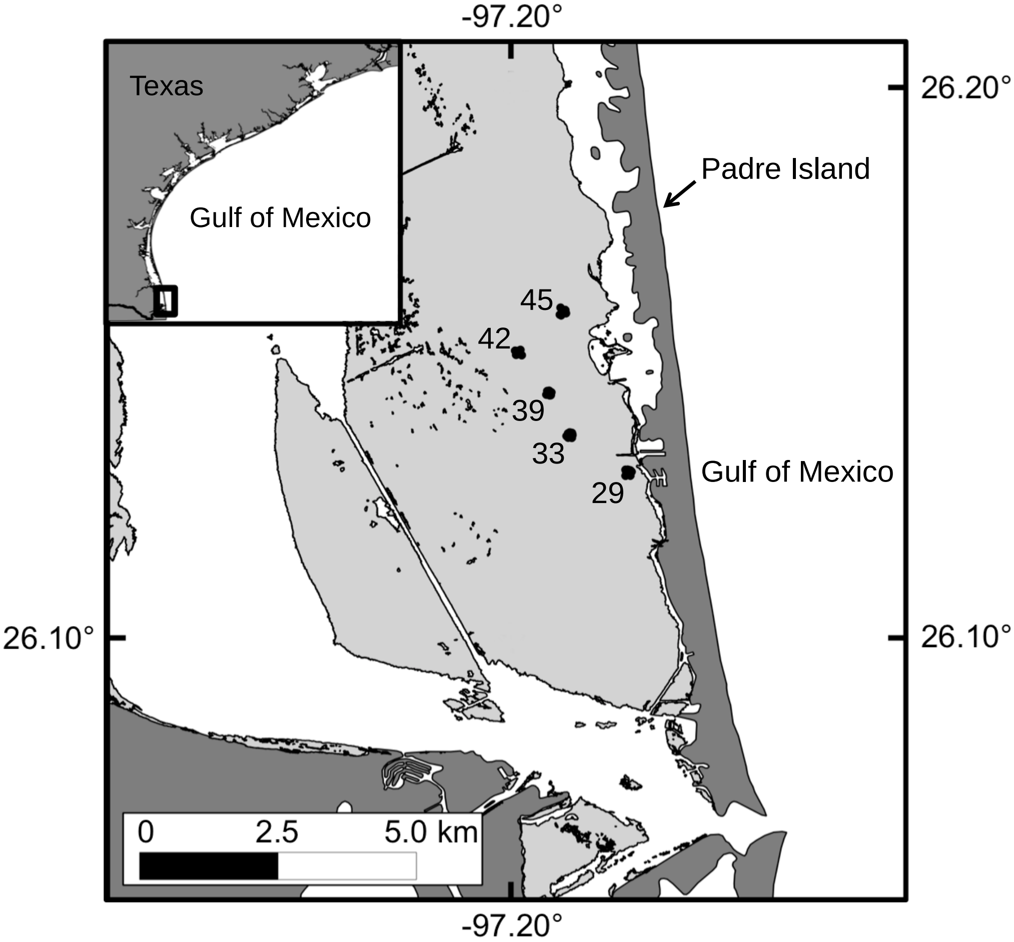

Figure 2.1. Location of nutrient enrichment experiment sites in Lower Laguna Madre, Texas. Dark gray represents land and light gray represents continuous seagrass cover extent obtained from the NOAA Coastal Services Center Benthic Habitat Mapping 2004/2007 Benthic Data Set. Each of the 5 sites (45, 42, 39, 33, and 29) included 10 randomly placed stations.

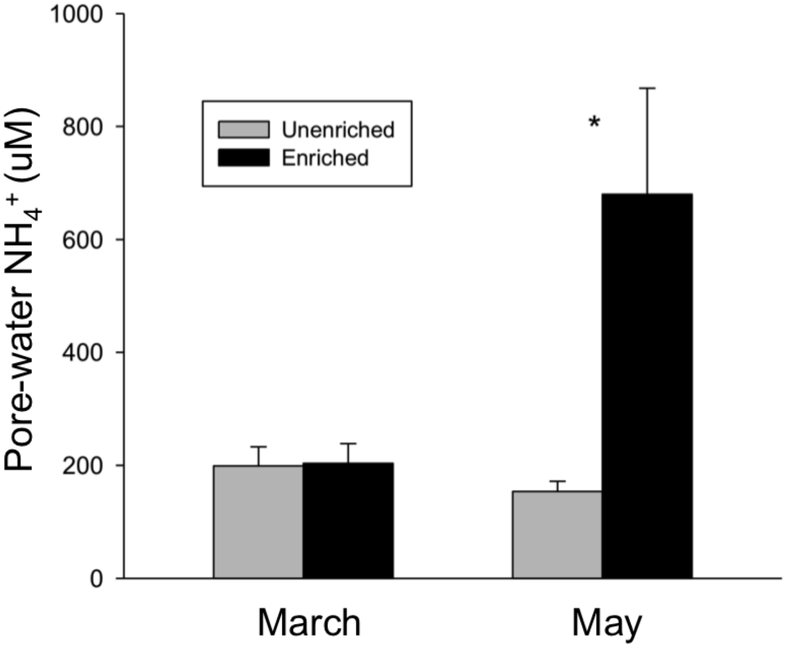

Figure 2.2. Mean (± S.E.) pore-water NH_4^+ concentrations of plots unenriched and enriched with nutrients where turtle grass (*Thalassia testudinum*) was collected at the beginning (March) and end (May) of the experiment. * denotes significant differences.

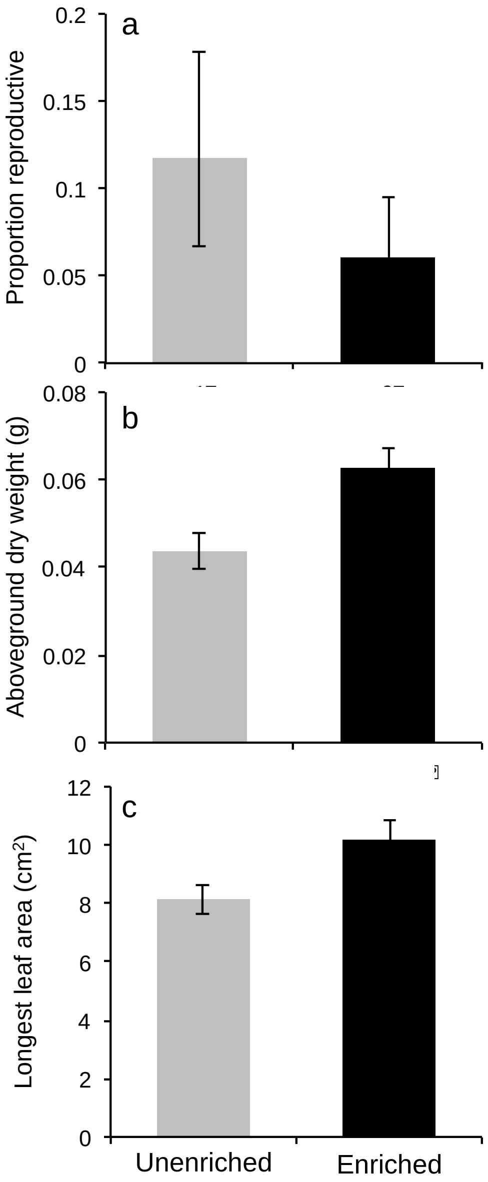

Figure 2.3. Proportion of reproductive shoots (a), aboveground dry weight (b) and longest leaf area (c) of turtle grass (*Thalassia testudinum*) from plots unenriched and enriched with nutrients at the end of the experiment in May. Back-transformed means and back-transformed upper and lower 95% confidance intervals are presented. The proportion of reproductive plants, aboveground dry weight and longest leaf area were all significantly different ($p < 0.05$) between the unenriched and enriched plots.

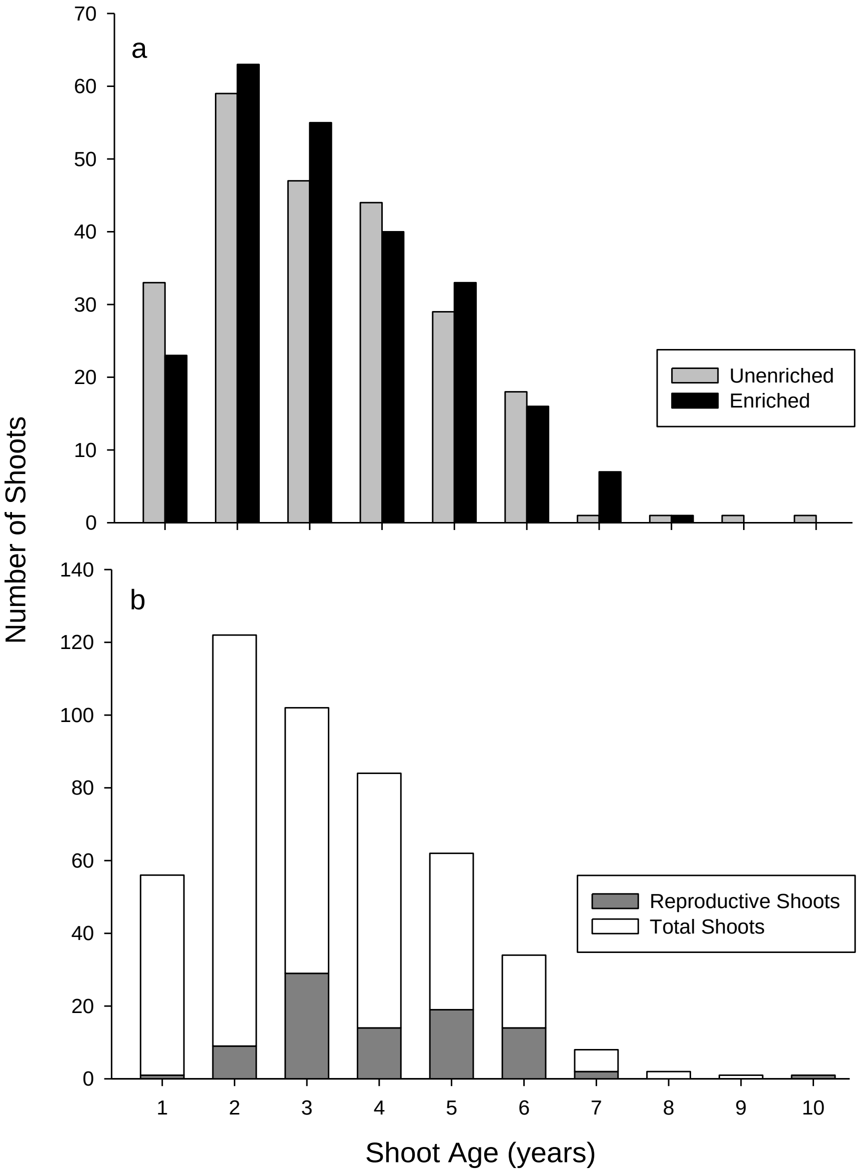

Figure 2.4. Number of turtle grass (*Thalassia testudinum*) shoots of each age from plots unenriched and enriched with nutrients in May (a) and the number of reproductive shoots by age out of the total number of combined unenriched and enriched turtle grass shoots in May (b).

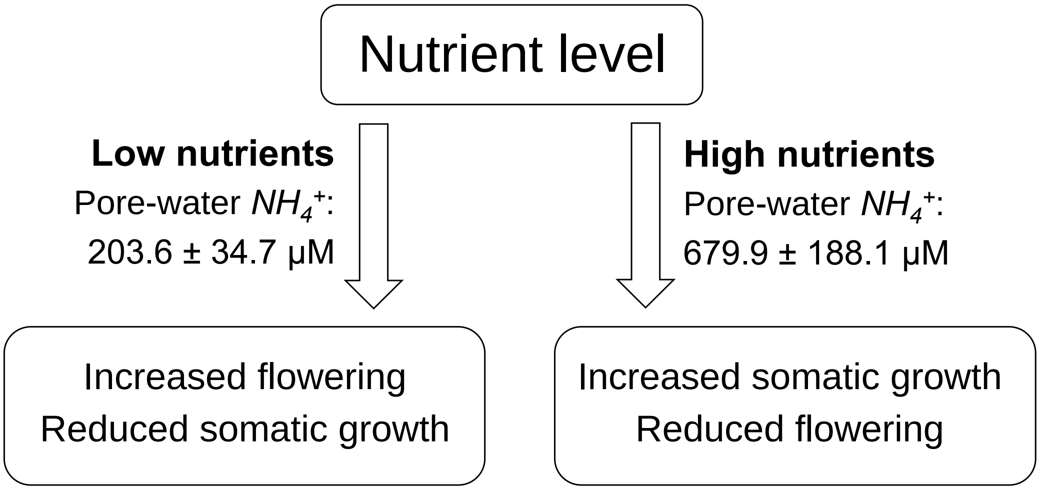

Figure 2.5. A simple conceptual model that depicts the effects of pore-water ammonium levels on flowering and somatic (leaf) growth in turtle grass (*Thalassia testudinum*) for the western Gulf of Mexico.

Chapter 3: Secondary Seed and Seedling Dispersal of Two Sub-tropical Seagrass Species with Differing Reproductive Adaptations, and Implications for Restoration

Abstract

I quantified the effects of water flow on secondary seed and seedling dispersal for two seagrass species with different reproductive adaptations: turtle grass (*Thalassia testudinum*) whose large seeds (15.1 ± 0.8 mm tall) have the potential for long distance dispersal by current-mediated transport of buoyant fruits, and shoal grass (*Halodule wrightii*), whose small (2.1 ± 0.1 mm), dormant seeds create a persistent seed bank and are likely retained near the parent plant. Results from *in situ* dispersal experiments in Aransas Bay, Texas, indicate that under normal flow conditions (mean water velocity < 5 cm s^{-1}) movement of turtle grass seedlings is greater over bare sand than in seagrass and that seedlings have the potential to move up to 2.1 m d^{-1}. Fine hairs on the base of turtle grass seedlings trap sand grains, which likely leads to final seed establishment after a few days and a potential maximum secondary dispersal distance of < 20 m. This distance is minimal compared to this species' potential primary seed dispersal distance, which can be hundreds of kilometers. Results from *in situ* experiments with shoal grass seeds indicate that secondary dispersal is greater in seagrass beds than bare sand, as seeds in the unvegetated areas were often trapped in troughs and ripples in the sediment. Under normal flow conditions, shoal grass seeds have the potential to move up to 1.1 m d^{-1} and a maximum potential secondary dispersal distance of < 10 m. Seed dispersal experiments indicate that secondary dispersal is species-specific, related to seed morphology and tightly coupled to each species' reproductive syndrome. Secondary seed dispersal has the potential to shape plant population structure, aid in colonization of unvegetated habitats, and should be taken into consideration in restoration projects using seeds.

Introduction

Seed dispersal represents a critical life history stage for many plant species. Dispersal from the parent plant can decrease sibling competition, enable seed escape from mortality near the parent, aid in recolonizing disturbed habitats, and increase the likelihood of the seed finding a suitable substrate in which to grow (Willson and Traveset 2000). The 'seed shadow', or spatial distribution of dispersed seeds around the parent plant, is often described by (1) the number or density of seeds at different distances from the parent plant, and (2) the direction of seed dispersal (Janzen 1971, Willson and Traveset 2000). For many terrestrial plants, the seed shadow is frequently the most dense within a few meters of the parent plant, decreasing exponentially with increasing distance from the parent (Willson and Traveset 2000). Many factors influence the shape of the seed shadow, including plant height (Greene and Johnson 1996), seed morphology (Bakker et al. 1996), habitat patchiness (Hoppes 1988), wind speed and direction (Howe and Smallwood 1982), and biotic dispersal agent behavior (Bakker et al. 1996).

The distance a seed disperses from the parent plant is tightly coupled to the species' reproductive syndrome (Bakker et al. 1996). Most plant species are specialized either for efficient seed dispersal or for building a persistent localized seed bank (Bakker et al. 1996). Seed bank-forming seeds often remain dormant in the sediment until a trigger (genetic or environmental) stimulates germination (Amen 1968). By having a distinct period of dormancy, these seeds are not only dispersed in space, but also dispersed in time (Bakker et al. 1996). Many species that produce dormant seeds are early successional and occur in slightly disturbed environments, where the seed bank facilitates population persistence following a disturbance that damages the adult population (Bakker et al. 1996). Dispersal from the parent plant may not be as critical for species with a dormant seed bank as it is for a species without one, although dispersal generally still enhances the likelihood that seeds will reach a suitable growing substrate and potentially colonize a new, suitable habitat (Comins et al. 1980).

Seed dispersal can be mediated by abiotic or biotic factors. Seed morphology frequently indicates the general dispersal mechanism. For example, wind-dispersed propagules often have discernible wings or plumes to facilitate lift, whereas species that rely on biotic ingestion and excretion by animals are more palatable than their wind-dispersed counterparts (Howe and Smallwood 1982). The two phases of seed dispersal, primary and secondary dispersal, jointly encompass all seed movement after release from the parent plant. Primary dispersal includes initial seed deposition on the substrate, and secondary dispersal involves all subsequent seed movement (Watkinson 1978). Primary dispersal of wind-dispersed seeds, for example, involves the airborne transport of seeds from the parent plant to the ground, and secondary dispersal encompasses all subsequent seed movement along the ground and in it (Greene and Johnson 1996). Several studies have highlighted the importance of characterizing secondary dispersal because of its ability to substantially alter the seed shadows resulting from primary dispersal and because it may be more important than primary dispersal in shaping plant population structure and demography (Harper 1977, Chambers and MacMahon 1994).

Although a large body of work exists on secondary seed dispersal in terrestrial plants, relatively little is known of secondary seed dispersal adaptations or the resulting seed shadows in seagrasses. Seagrasses are submerged marine angiosperms capable of sexual reproduction and asexual clonal growth by subsurface rhizome elongation. Interestingly, the relatively few (~70) species of seagrasses display a remarkably wide variety of reproductive adaptations. Species have unique characteristics of reproductive timing, effort, mode (surface or submarine flowering and pollination), and reproductive structure morphology (Ackerman 2006). For example, some species (e.g. *Thalassia testudinum*, *Zostera marina*) are reproductive in the spring and summer, and others (e.g. *Posidonia* and *Amphibolis*) flower in the fall and winter (Ackerman 2006). While some species produce many seeds per fruit (e.g. *Halophila decipiens*, 30 seeds fruit^{-1},van Tussenbroek et al. 2010), others produce a single seed within a fruit (e.g. *Syringodium filiforme*, McMillan 1981).

Because of the variation in seagrass reproductive adaptations, it is also likely that these species exhibit substantial variation in seed dispersal distances (Bakker et al. 1996). Historically, studies examining seagrass expansion have focused on clonal growth, as it was considered the dominant form of propagation of aquatic plants (Arber 1920). As a result, seagrass reproductive biology and ecology are not fully understood. Recent studies have reported that over large spatial scales (kilometers), genotypic diversity for individual species is high, suggesting that (1) the role of sexual reproduction in seagrasses was historically undervalued and, (2) seed dispersal likely contributes to observed genetic diversity (Kendrick et al. 2012). Therefore, studies focused on seagrass reproductive biology, and particularly seed dispersal, are necessary to fully understand seagrass propagation and life history dynamics.

Seagrass seeds, like their terrestrial counterparts, are dispersed by abiotic and biotic mechanisms. Abiotic dispersal includes transport of floating propagules by wind, water currents, waves and sediment resuspension, and biotic dispersal includes ingestion and excretion of seeds by animals such as waterfowl, sea turtles and manatees. Abiotic transport by water currents is thought to be the dominant mechanism of dispersal (Kendrick et al. 2012), although a recent study highlights the possibility of successful dispersal of *Zostera marina* seeds by waterfowl, sea turtles and fish (Sumoski and Orth 2012). Like the dispersal of terrestrial plants, seagrass seed dispersal is split into two phases: primary dispersal and secondary dispersal. Several studies have investigated primary dispersal of seeds and of dispersal units such as fruits or rhipidia and have reported a wide range of dispersal distances (Orth et al. 1994, Harwell and Orth 2002, van Dijk et al. 2009). While ephemeral genera such as *Halophila* and *Halodule* tend to have small seed shadows, with primary seed dispersal distances on the order of meters, persistent genera such as *Posidonia*, *Enhalus* and *Thalassia* have large seed shadows, with primary dispersal distances of hundreds of kilometers from the parent plant (Kendrick et al. 2012). Only a few studies exist that examine the dynamics of secondary dispersal (Orth et al. 1994, Lacap et al. 2002, Koch et al. 2010), despite the potential for this dispersal phase to shape seagrass population structure and demography.

The primary goal of this study was to quantify the effects of water flow on secondary seed and seedling dispersal for two seagrass species with different reproductive adaptations: turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrighitii*). Specifically, I aimed to (1) quantify secondary seed and seedling dispersal over the range of water velocities obseed in seagrass beds, (2) investigate the influence of substrate type (bare sand or seagrass) on seed and seedling movement, and (3) using *in situ* water velocity measurements during the reproductive seasons for each of these species, estimate potential secondary seed and seedling dispersal distances. I conducted dispersal experiments with turtle grass seedlings and shoal grass seeds in natural seagrass beds and in the laboratory and related dispersal to *in situ* current velocities in south Texas where these two species co-occur. Given the reproductive adaptations of these species, I hypothesized that shoal grass seed secondary dispersal is limited to the parent meadow, whereas turtle grass secondary seed dispersal is possible over greater distances.

Materials and Methods

Experimental Species

Turtle grass (*Thalassia testudinum*) is dominant throughout the Gulf of Mexico and Caribbean Sea. In clear waters, turtle grass can reach depths of 10–15 m (van Tussenbroek et al. 2010). However, along the south Texas coast where this study was conducted, maximum depth is limited to approximately 2 m due to decreased water clarity, and beds are commonly found < 1 m deep. Morphologically, turtle grass is a relatively large species, with strap-like leaves between 0.2–2 cm wide that can grow up to 80 cm long (van Tussenbroek et al. 2010). The turtle grass reproductive season generally spans summertime months, but varies throughout the species range (van Tussenbroek et al. 2006).

In Texas, inflorescences on turtle grass are produced at the base of the shoot in the early summer. Fruit formation, development and maturation occur in June–September, with each fruit containing one to nine pyriform seeds up 15 mm in size, but most commonly having two (Orpurt and Boral 1964; Kaldy and Dunton 2000; van Tussenbroek et al. 2010). Seeds germinate within the fruit. Thus, seedlings are released at the time of dehiscence (Figure 3.1a). Fruits can dehisce (open) while still attached to the parent plant, releasing seedlings near the vicinity of the parent. More commonly, though, fruits detach from the parent plant, are buoyant, and can be transported by currents up to 360 km before dehiscence and settlement of the negatively buoyant seedlings to the substrate (van Dijk et al. 2009). As a result, turtle grass has the potential for long-distance primary dispersal and a large seed shadow. In turtle grass, the dispersal unit during primary dispersal is the fruit and the dispersal unit during secondary dispersal is the seedling.

Shoal grass (*Halodule wrightii*) is a morphologically smaller species (leaves: 2–5 mm wide × 3–30 cm long) than turtle grass and has a wider geographical range, extending throughout the Caribbean and Gulf of Mexico to the east coast of the United States and Bermuda (van Tussenbroek et al. 2010). Whereas shoal grass occurs from the shallow subtidal to 30 m deep in clear water (van Tussenbroek et al. 2010), it is constrained to < 2 m in Texas and can be exposed at low tide. Flower production and fruit and seed development in Texas occur April–July. After successful pollination of a female flower, two fruits are produced at the base of the female shoot. Each of these fruits contains one black, spherical negatively buoyant seed about 2 mm in size (Figure 3.1b), which is released from the fruit at or below the sediment surface adjacent to the parent plant. *Halodule wrightii*, therefore, has localized primary dispersal and, as a result, a relatively small primary seed dispersal shadow. Seeds are surrounded by a hard seed coat and can remain dormant for up to 4 years, forming a seed reserve in the sediment (McMillan 1981). For shoal grass, the propagule capable of primary and secondary dispersal is a seed.

Turtle grass and shoal grass co-occur in mixed beds, but also form separate, large monospecific meadows. *Halodule wrightii* is an early colonizing, pioneer species and is able to tolerate sub-optimal conditions and disturbances that turtle grass cannot (Zieman 1982). Turtle grass, conversely, is a climax species in the Caribbean Sea and Gulf of Mexico (Zieman 1982).

Seed collection and morphology

Turtle grass seedlings were collected during the 2011, 2012 and 2013 reproductive seasons in turtle grass beds in Lower Laguna Madre, Texas, where seedlings are locally abundant (pers. obs.). Seedlings were collected by picking mature fruits from plants and harvesting seedlings after the fruit dehisced, or collecting floating fruits and seedlings with a net from the water's surface. Seedlings were stored in aerated seawater at The University of Texas Marine Science Institute before use in experiments. Turtle grass seedling size is irregular and highly variable, as germinated seedlings have growing leaves upon dehiscence. Therefore, seed measurement techniques used for other species (e.g. Koch et al. 2010) are not appropriate. I used seedling height as a measurement of seedling size, as this factor is likely to influence movement along the substrate and differed substantially among seedlings. Seedling height (mm) was measured as the base of the seed to the tip of the longest leaf, which represented the entire height of the seedling.

Dormant shoal grass seeds were collected during summers in 2012 and 2013 by sieving 9.5 cm wide × 10 cm deep cores taken along the Texas coast as part of the Texas Statewide Seagrass Monitoring Program (texasseagrass.org). Seeds were kept in natural seawater until use in experiments, and for consistency with turtle grass seed measurements, shoal grass seed height was measured as the base to the top of the seed.

In situ *water velocities*

In situ water velocities were measured in natural turtle grass and shoal grass beds along the central Texas coast in summer 2013 during the time of seed and seedling release. SeaHorse tilt current meters (20-cm tall, OkeanoLog, Woods Hole, MA, U.S.A.)

were deployed at each of four sites within Redfish, Aransas and Corpus Christi Bays, TX (Traylor Island, Mud Island, Hog Island and East Flats) at a depth of 80–100 cm in a monospecific turtle grass bed (all four sites), a monospecific shoal grass bed (Traylor Island and Hog Island only) or over sand (Traylor Island and Hog Island only) (Figure 3.2, Table 3.1). SeaHorse tilt current meters utilize three axis accelerometers that take tilt measurements, which are converted to a horizontal velocity vector using MATLAB software (The MathWorks Inc., Natick, MA, U.S.A.), and have been used to accurately measure bottom currents to the nearest tenth of a centimeter (Sheremet et al. 2009). At each of the sites, a SeaHorse current meter logged tilt every 6 min while deployed. Current meters were cleaned periodically to prevent fouling and monitored to confirm that macroalgae or seagrass did not obstruct movement of the tilt meter. Seagrass cores (9.5-cm wide × 10-cm deep, n = 3) were collected after SeaHore tilt meter removal at each site to quantify seagrass shoot density at each tilt meter. Wind speed (m s^{-1}) and direction (0–360°) data near my sites were obtained from a Texas Coastal Ocean Observation Network (TCOON, www.cbi.tamuss.edu/TCOON/) station in Port Aransas, TX from 21 June–30 August 2013 to examine the influence of wind on water velocity at my sites. Wind speed and direction at the Port Aransas TCOON station were measured every 6 minutes.

In situ *secondary dispersal experiments*

Secondary dispersal over the substrate was quantified in natural seagrass beds and over bare sand for turtle grass seedlings and shoal grass seeds at Traylor Island, Redfish Bay, TX during the 2013 reproductive season (Figure 3.2). Turtle grass and shoal grass are both reproductive at Traylor Island (pers. obs.), where they form monopecific meadows, but also co-occur in mixed beds. Experiments were conducted in monospecific seagrass beds with shoal grass seeds on 25 June, 2 July and 4 July 2013 and turtle grass seedlings on 2 August, 5 August and 9 August 2013. At the beginning of each experiment, a 2-m wide PVC frame was hammered into the substrate: the frame consisted of 2 PVC legs, with a third shorter piece of PVC in the center of the frame. A GoPro Hero 2 waterproof video camera (GoPro®, San Mateo, CA, U.S.A.) was mounted to the middle PVC pole ~45 cm above the substrate and positioned facing downward so it overlooked the substrate. Distance on the bottom was calibrated with a ruler, and one seed (or seedling) was placed on the substrate (in seagrass or on bare sand) in the center of the camera's field of view and recorded for ~1 h. Videos were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) to determine distance traveled by the seed. Seed speed (cm s^{-1}) was calculated by dividing the distance the seed traveled by the experiment duration. If the seed moved outside the camera's field of view, the distance traveled and time the seed was in view were used to calculate speed. Four to eight replicates were conducted for each species (turtle grass or shoal grass) and substrate (seagrass or sand) combination. After the conclusion of all experiments, triplicate seagrass cores (9.5-cm wide × 10-cm deep) were collected at the turtle grass and shoal grass dispersal experiment sites to determine shoot densities. Environmental parameters (water temperature, salinity, pH) were measured at the top of the seagrass canopy during the experiments with a YSI 600XL data sonde.

Laboratory secondary seed dispersal experiments

Dispersal of turtle grass seedlings and shoal grass seeds was also quantified under controlled conditions and over a range of current speeds in a recirculating flume (7-m long × 0.5-m wide × 0.9-m deep, Figure 3.3) at the Horn Point Marine Laboratory in Cambridge, MD. Turtle grass seedling movement was quantified during the 2011 and 2012 reproductive seasons and shoal grass seed movement was quantified in 2012 only. The flume was filled to a depth of 50 cm with water from the Choptank River at a natural salinity of approximately 12 ppt and temperature of 26°C. Seed movement was examined over bare sand and in artificial seagrass units (ASUs). Trays (58-cm long × 48-cm wide × 10-cm deep) with quartz sediment of a grain size similar to that in Texas estuaries (120–250 μM) were used for bare sand experiments. Artificial seagrass units (turtle grass: 100-cm long × 50-cm wide; shoal grass: 50-cm long × 50-cm wide) were constructed to mimic natural seagrass beds in Texas (Dunton 1990, Lee and Dunton 1996). Individual shoot construction involved attaching three pieces of polypropylene ribbon (0.4-cm wide × 45-cm long) at one end with electrical tape to mimic the sheath at the base of the shoot. To obtain desired shoot densities, each of these 'shoots' was secured into a hole cut into a piece of flat rigid plastic (3-mm thick) that was covered in thin layer of sand. Turtle grass seedling movement was quantified in three ASU densities: low, intermediate, and high, where the intermediate density ASU (mean ± SD: 962 ± 258 shoots m^{-2}) represented mean shoot density in Texas (Darnell and Dunton 2010), and low (704 shoots m^{-2}) and high density (1,220 shoots m^{-2}) ASUs were 1 standard deviation below and above the intermediate density ASU, respectively. Shoal grass seed movement was quantified in an intermediate shoal grass shoot density (mean ± SD: 3,843 ± 2,977 shoots m^{-2}) only due to time constraints. Shoal grass ASU construction was similar to that of turtle grass, but individual 'shoots' were constructed to mimic shoal grass plants and polypropylene ribbon 'leaves' were 0.1-cm wide × 20-cm long.

Seed (and seedling) movement over sand and in ASUs was tested over a range of current speeds possible in seagrass beds (0–20 cm s^{-1}) and separately over a range of wave heights that simulated calm to slightly windy conditions in a coastal seagrass bed (2.9, 5.2 and 7.6 cm). Wave period was approximately 3 seconds and bottom orbital velocities were 13, 24 and 35 cm s^{-1}, as calculated by Koch et al. (2010) from Infantes et al. (2009). Wave conditions were created by inserting a wave panel and artificial beach into the flume and setting the wave panel to a pre-determined speed (Figure 3.3). Secondary dispersal experiments followed the protocol established by Koch et al. (2010). In experiments examining current speed and seed movement, seeds were placed at the upstream end of the working section, speed was started at 1 cm s^{-1} and slowly increased in 1 cm s^{-1} increments, as would be expected when the current moves after slack tide. When current velocity reached the target velocity, it was maintained at a constant velocity for 2 min. At the end of the 2 min., distance the seed moved was recorded and its speed calculated. If the seed reached the end of the working section before the end of the 2 min, the time to reach the end of the working section was used to calculate speed. Similarly, for wave height experiments, seed movement was quantified over a 2-min. period, unless the seed reached the end of the working section beforehand. Seeds were placed within 10 cm of the upstream end of the tray for experiments examining movement over sand. As a result, maximum possible distances the seed could travel over sand ranged from 40–50 cm. Preliminary experiments indicated that turtle grass seedling orientation affected seed movement, so all turtle grass seedlings used in experiments were initially oriented with the broad side of the leaves perpendicular to water flow.

Statistical analyses

Propagule height between seagrass species was compared using a Welch's Analysis of Variance (ANOVA). Turtle grass and shoal grass speed in field dispersal experiments were analyzed using regression. In the turtle grass field dispersal experiments, mean weighted water velocity and seedling height were the predictor variables. Mean weighted water velocity was the only predictor variable in the shoal grass field dispersal experiments, as seed height was consistent among seeds. *In situ* turtle grass and shoal grass shoot densities were compared among sites that had a SeaHorse tilt current meters using ANOVA. Residuals were normal for all *in situ* analyses, so data were not transformed.

The minimum water speeds necessary to move turtle grass seedlings and shoal grass seeds in laboratory dispersal experiments were analyzed using an analysis of covariance (ANCOVA) with species (turtle grass or shoal grass) as a fixed factor and propagule height as a covariate. Species were analyzed separately for all other laboratory experiments. In laboratory dispersal experiments examining propagule speed over a range of water speeds, turtle grass seedling speed over sand, shoal grass seed speed over sand and shoal grass seed speed in the intermediate density ASU were analyzed separately using ANOVA with the predictor variable of water speed. To achieve normality of the residuals, shoal grass seed speed over sand in the intermediate density ASU was $\log_{10}$ transformed. Turtle grass seed speed over sand was not transformed, as residuals were normal. Turtle grass seedling speeds in the low, intermediate and high density ASUs were analyzed separately using ANCOVA with water speed as a fixed effect and seedling height as the covariate. The residuals were normal in these analyses so data were not transformed.

In laboratory dispersal experiments examining propagule speed over a range of wave heights, shoal grass seed speed over sand and shoal grass seed speed in the intermediate density ASU were examined separately using ANOVA with wave height as the predictor variable. To obtain normally distributed residuals, shoal grass seed speed over sand was square-root transformed and shoal grass seed speed in the intermediate density ASU was $\log_{10}$ transformed before analyses. Turtle grass seedling speed over sand and in the low, intermediate and high density ASUs were analyzed separately using ANCOVA. In these analyses, wave height was a fixed factor and seedling height was a covariate. The residuals were normal, so data were not transformed.

Wind and *in situ* water velocity data were analyzed using Oriana 4 (Kovach Computing Services, Anglesey, Wales, U.K.). Wind data obtained from the Port Aransas TCOON station were binned according to direction (northeast, southeast, southwest and northwest) and speed (0–3, 3.1–6, 6.1–9, and >9 m s^{-1}). Water velocities were examined for each site (Traylor Island, Mud Island, Hog Island and East Flats), substrate (turtle grass, shoal grass and sand) and wind direction and speed combination. The uniformity of the distribution of water flow direction for each site-substrate-wind speed-wind direction combination was tested using Rao's Spacing Test. If data were not uniform ($p<0.05$), the weighted mean vector (i.e. average water direction, degrees) and length of the weighted mean vector (i.e. average water velocity, cm s^{-1}) were calculated. If data were uniform ($p>0.05$), these parameters could not be calculated. Weighted mean water velocity vectors were used for *in situ* secondary dispersal experiments.

Data with residuals that were normally distributed are presented as the mean ± standard error (mean ± S.E.). Data that were transformed to obtain normality of the residuals are reported as the back-transformed mean and back-transformed lower and upper 95% confidance intervals.

Results

Turtle grass seedlings (15.1 ± 0.8 mm) (mean ± S.E.) were significantly taller than shoal grass seeds (2.1 ± 0.1 mm) ($p< 0.0001$). Shoal grass seed height had minimal variation (range: 1.7–2.6 mm), whereas turtle grass seedling height was highly variable (range: 3.2–54.9 mm). *Halodule wrightii* shoot densities at the site of SeaHorse current meter deployment were similar between Traylor Island and Hog Island ($p = 0.28$) and turtle grass shoot densities were similar between current meter deployment sites at Traylor Island, Hog Island, Mud Island and East Flats ($p=0.16$) (Table 3.2).

In situ *water velocities*

Winds were predominantly from the southeast (132 ± 0.4°; Rao's Spacing Test, $U=352.3$, $p<0.01$) and averaged 4.0 ± 0.01 m s^{-1} (Figure 3.4). Average wind speeds from the southwest, northwest and northeast were 2.6 ± 0.02 m s^{-1}, 3.1 ± 0.06 m s^{-1} and 4.3 ± 0.02 m s^{-1}, respectively. Water velocities at each of my sites were generally below 10 cm s^{-1} and average water velocities for each site-substrate-wind direction-wind speed combinations were mostly less than 5 cm s^{-1} (Table 3.3). With southeast winds, water at Traylor Island (in turtle grass, shoal grass and sand), Mud Island (in turtle grass) and East Flats (in turtle grass) moved northwest (Figure 3.5). However, water at Hog Island under southeast winds moved predominantly northeast in shoal grass, northwest over sand and either southwest or northwest in turtle grass (Figure 3.5). Under southeast winds, average weighted water speed at each of the sites remained below 3.5 cm s^{-1}. Mean weighted water speeds were highest under northwest (8.9 cm s^{-1}, shoal grass at Hog Island) and northeast (9.9 cm s^{-1}, turtle grass at Mud Island) winds greater than 9 m s^{-1} (Table 3.3).

In situ ***secondary dispersal experiments***

Turtle grass and shoal grass shoot densities in experimental seagrass beds at Traylor Island were 1,740 ± 170 shoots m^{-2} and 3,291 ± 124 shoots m^{-2}, respectively. Environmental parameters showed minimal variability throughout the experiments; water temperature, salinity and pH were 28.5 ± 0.8 °C, 40.8 ± 1.4 ppt and 8.1 ± 0.1, respectively. Throughout the *in situ* experiments, winds were predominantly from the southeast, water predominantly moved northwest (weighted mean direction: 306°) and overall mean weighted water speed was 2.4 cm s^{-1} (Rao's Spacing Test U=190.1, $p <$ 0.01).

Turtle grass seedling speed over bare sand was not related to mean weighted water velocity (3.7 ± 0.5 cm s^{-1}) or seedling height (water velocity: p=0.59; seedling height: p=0.48). Over the hour-long experiments, seedlings moved an average speed of 0.003 ± 0.001 cm s^{-1} and a distance of 8.8 ± 3.5 cm. Similarly, when seedlings were placed within a turtle grass meadow, weighted mean water velocity and seedling height did not influence turtle grass seed speed (water velocity: p=0.21, seedling height: p=0.13). In seagrass, turtle grass seedlings moved an average of 0.0006 ± 0.0002 cm s^{-1} and 1.8 ± 0.6 cm over the hour-long experiments under an average water velocity of 2.6 ± 0.2 cm s^{-1} (Table 3.4).

The SeaHorse current meter placed in sand at Traylor Island disappeared between 4 July 2013 and 19 July 2013 and water velocity data for the *in situ* shoal grass dispersal experiments over sand were lost. As a result, the effect of *in situ* water velocity on shoal grass seed movement over sand could not be determined. Shoal grass seeds in these hour-long experiments, however, moved an average speed of 0.0009 ± 0.0003 cm s^{-1} and an average distance of 3.0 ± 1.1 cm. When placed within a seagrass meadow, shoal grass seed movement increased with higher mean weighted water velocities ($p=0.01$, $R^2=0.82$). In shoal grass, seeds moved an average of 0.002 ± 0.001 cm s^{-1} and a distance of 4.7 ± 1.8 cm over a range of average weighted water velocities of 3.0–5.2 cm s^{-1} (mean: 3.7 ± 0.3 cm s^{-1}) (Table 3.4). Net movement of turtle grass and shoal grass seeds was generally in the dominant direction of water velocity, where measured.

Laboratory secondary seed dispersal experiments: water speed

In the recirculating flume, turtle grass seedling movement over sand was initiated at a water speed of 6.5 ± 0.4 cm s^{-1}, whereas shoal grass seed movement was initiated at a water speed of 10.7 ± 0.4 cm s^{-1}. Turtle grass seeds initiated movement at a significantly slower water speed than shoal grass seeds, and movement was related to seed and seedling height (species: $p<0.0001$, seed/seedling height: $p=0.04$, species × seed/seedling height: $p=0.72$).

Over sand, increasing flow speed resulted in increased turtle grass seedling movement (turtle grass: $p<0.0001$, Figure 3.6a) and all seedlings reached the end of the 50-cm sand tray by the end of the 2-min experiment at a water speed of 15 cm s^{-1} (Table 3.5). Similarly, turtle grass seedlings in the low density ASU moved faster at higher water speeds, and seedling height influenced seed speed (water speed: $p=0.003$; seedling height: $p=0.02$; water speed × seedling height: $p=0.55$, Figure 3.6b). Seedlings exposed to

10, 15 and 20 cm s^{-1} water speeds moved 0.06 ± 0.02, 0.15 ± 0.03, and 0.21 ± 0.03 cm s^{-1} and distances of 7.8 ± 2.0, 17.9 ± 3.5 and 25.4 ± 3.7 cm, respectively.

In the intermediate and high density ASUs, turtle grass seedling movement was only tested at water speeds of 10 and 20 cm s^{-1}. In the intermediate density ASU, seedling speed was similar among water speeds and seedling height did not influence seedling speed (water speed: p=0.60; seedling height: p=0.84; water speed × seedling height: p=0.90, Figure 3.6b). Seedlings moved 10.1 ± 3.2 cm (0.08 ± 0.03 cm s^{-1}) and 15.1 ± 3.9 cm (0.13 ± 0.03 cm s^{-1}) at 10 and 20 cm s^{-1}, respectively. In the high density ASU, however, both water speed and seedling height positively influenced seedling speed. Seedlings moved faster at 20 cm s^{-1} than at 10 cm s^{-1} and taller seedlings moved faster than shorter seedlings (water speed: p=0.02; seedling height: p=0.03; water speed × seedling height: p=0.63, Figure 3.6b). Seedlings moved distances of 2.9 ± 1.1 and 10.6 ± 3.1 cm at 10 and 20 cm s^{-1}, respectively. I noted that even at the highest water speed tested (20 cm s^{-1}), turtle grass seedlings in the low, intermediate and high density ASUs did not reach the end of the ASUs (Table 3.5).

Over sand, increased water speeds resulted in increased shoal grass seed movement (p<0.0001, Figure 3.6c) and all seeds reached the end of the 50-cm sand tray by the end of the 2-min experiment at a water speed of 15 cm s^{-1}. Shoal grass seeds also moved faster at higher water speeds in the ASU (p=0.003, Figure 3.6d). In the ASU, at water speeds of 10, 15 and 20 cm s^{-1}, seeds moved 0.02 (0.009–0.05) cm s^{-1} (back-trasformed mean (back-transformed lower 95% confidance interval– back-transformed upper 95% confidance interval)], 0.15 (0.07–0.34) cm s^{-1} and 0.29 (0.13–0.63) cm s^{-1}, respectively. The distance moved by shoal grass seeds through the ASU also showed a positive trend with water speed, where seeds exposed to 10, 15 and 20 cm s^{-1} moved 3.0 ±

1.0 cm, 20.3 ± 5.5 cm and 34.3 ± 1.9 cm, respectively. Even at the highest water speed tested (20 cm s^{-1}), shoal grass seeds did not reach the end of the ASU (Table 3.5).

Laboratory secondary seed dispersal experiments: wave height

Neither turtle grass seedlings nor shoal grass seeds moved over sand or in ASUs when exposed to 2.9 cm waves. Seed/seedling movement was initiated at a wave height of 5.2 cm for both species. Over sand, turtle grass seedlings moved faster at a wave height of 7.6 cm than at 5.2 cm, but movement was not affected by seedling height (wave height: p=0.01; seedling height: p=0.11; wave height × seedling height: p=0.61). When exposed to 5.2-cm and 7.6-cm waves, seedlings moved 0.3 ± 0.05 cm s^{-1} and 0.6 ± 0.1 cm s^{-1}, respectively. Turtle grass seedlings moved an average of 32.9 ± 4.8 cm over the 2-min experiment when exposed to 5.2 cm waves. When exposed to a 7.6 cm waves, all seedlings reached the end of the 50-cm sand tray before the end of the 2-min experiment; average time for seedlings to reach the end of the tray was 80 ± 9.0 seconds (Table 3.6).

In the low density ASU, turtle grass seedling speed was not affected by wave height or seed height (wave height: p=0.14; seedling height: p=0.44; wave height × seedling height: p=0.24). Seedlings moved 0.11 ± 0.01 cm s^{-1} and a distance of 13.6 ± 1.6 cm with 5.2 cm waves, and 0.2 ± 0.05 cm s^{-1} and a distance of 24 ± 5.7 cm with 7.6 cm waves. Similarly, at the intermediate ASU density, wave height and seedling height did not influence seedling speed (wave height: p=0.10; seedling height: p=0.79; wave height × seedling height: p=0.49). At wave heights of 5.2 cm and 7.6 cm, seedlings moved 0.08 ± 0.02 cm s^{-1} and 9.5 ± 2.9 cm, and 0.18 ± 0.03 cm s^{-1} and 21.3 ± 3.9 cm, respectively. When exposed to 5.2 cm waves, all turtle grass seedlings moved against the main direction of flow, whereas seedlings exposed to 7.6 cm waves moved with the main direction of flow. Similar to the low and intermediate ASU densities, seedling speed was unaffected by wave height and seed height in the high ASU density (wave height: p=0.11; seed height: p=0.99; wave height × seed height: p=0.41). Seedlings exposed to 5.2 cm waves moved 0.04 ± 0.01 cm s^{-1} and 4.7 ± 1.3 cm, and seedlings exposed 7.6 cm moved 0.11 ± 0.02 cm s^{-1} and a distance of 13.7 ± 2.6 cm (Table 3.6).

Shoal grass seeds moved faster over sand with 7.6 cm waves than 5.2 cm waves ($p<0.0001$). When exposed to 5.2 cm waves, seeds moved 0.03 (0.002–0.08) cm s^{-1} and 15.4 ± 2.8 cm over 2 min. At a wave height of 7.6 cm, all shoal grass seeds reached the end of the 50-cm sand tray by the end of the 2-min experiment and moved 42.3 ± 0.4 cm over 45.5 ± 6.1 sec for an average seed speed of 0.27 (0.17–0.41) cm s^{-1}. Similarly, in the ASU, shoal grass seeds moved faster when exposed to 7.6 cm waves than with 5.2 cm waves (p=0.01). At a wave height of 5.2 cm, seeds moved 0.02 (0.01–0.05) cm s^{-1} and a distance of 3.3 ± 0.9 cm. At 7.6 cm wave height, seeds moved 0.13 (0.05–0.28) cm s^{-1} and 16.7 ± 4.6 cm through the ASU (Table 3.6). Similar to turtle grass, when exposed to 5.2 cm waves, all shoal grass seeds moved against the main direction of flow, whereas seeds exposed to 7.6 cm moved with the main direction of flow.

Discussion

I quantified the effects of water flow on secondary seed and seedling dispersal along the substrate for two seagrass species with different reproductive adaptations: turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*). Results from my *in situ* experiments indicate that under normal hydrodynamic conditions during the time of seed release in Texas (mean water velocity: < 5 cm s^{-1}), shoal grass secondary seed dispersal is likely limited to the parent meadow and turtle grass secondary seedling dispersal distance along the substrate is only on the order of meters, compared to primary dispersal, which has the potential to reach long distances (on the order of kilometers). Turtle grass and shoal grass secondary seed dispersal dynamics are species-specific and likely related to propagule morphology (Figure 3.7).

Wind and water velocity

Wind stress is the primary determinant of water levels and water flows in Texas coastal bays (Shideler 1984) and is estimated to be nearly an order of magnitude more important than astronomical tides (Smith 1977). Wind forcing varies seasonally, with weak prevailing winds from the southeast during summer months, interspersed with infrequent, yet strong northerly components (Shideler 1984). Data obtained from the Port Aransas TCOON station were consistent with this pattern, with predominant southeast winds and short-term fluctuations to northerly winds from June–August 2013. When exposed to southeast winds, water at most of my sites (Traylor Island, Mud Island and East Flats) largely moved northwest, as was expected. Water over sand at Hog Island also moved northwest under southeast winds, but in seagrass beds, mean flow directions were northeast and southwest. This difference could be explained by the separation of the tilt meters between sand and seagrass habitats at Hog Island. The current meter in sand was located in water within the curve of the island, whereas the two current meters in seagrass beds were exposed off the northern tip of the island. The northern tip of Hog Island is located near two channels and a cut through nearby Harbor Island, which could have produced different hydrodynamic conditions in the seagrass beds than the open sand area, which were separated by approximately 10 m.

Water velocities within seagrass beds at my sites (mean: < 5 cm s^{-1}) were similar to velocities commonly reported in seagrass (< 10 cm s^{-1}, Koch 2001). Seagrass structure reduces water flow, as water is deflected over the canopy resulting in a loss of momentum among the leaves (Fonseca et al. 1982). This can result in water velocities 2-10 times slower within the canopy than outside it (Ackerman 1986). Water velocity was lower in turtle grass than over sand at Traylor Island, but this pattern was not upheld at Hog Island likely due to the different landscape features near the different meters (as noted above).

SeaHorse current meters integrated water velocity from the sediment surface to 20-cm above the sediment. Since water velocity increases with increasing distance from the substrate, it is likely that seeds were experiencing even slower water velocities at the benthic boundary layer, which produces a nearly stagnant thin layer of water just above the sediment surface (Koch 2001). However, wave action within seagrass beds increases turbulence and can reduce the boundary layer (Koch et al. 2006). Despite the flow-reducing potential of seagrasses, water velocities have been reported as high as 100 cm s^{-1} within the canopy, although average velocity usually remains low (Koch 2001). The fastest mean weighted water velocities we measured (9.9 cm s^{-1}) occurred with strong (> 9 m s^{-1}) northerly winds and were sustained up to 1.5 h. Higher resolution flow measurements are necessary to better relate seed movement to local hydrodynamic conditions and calculate overall transport.

Potential seed dispersal

Using mean dispersal distances from the laboratory flume experiments, my results suggest that daily turtle grass seedling dispersal potential over sand with water speeds between 4–20 cm s^{-1} could range from 54 to 3,283 m d^{-1}. However, in the presences of ASUs, estimated daily dispersal are considerably lower at similar flow regimes and range from 56 to 183 m d^{-1} in low density turtle grass and 21–76 m d^{-1} in high density turtle grass. Daily dispersal estimates from my *in situ* dispersal experiments, however, are markedly lower. Using mean transport distances observed in the field experiments, estimated potential seedling dispersal over sand is only 2.1 m d^{-1} when exposed to a mean water velocity of 3.7 ± 0.5 cm s^{-1} and, in seagrass, only 0.4 m d^{-1}, when exposed to a mean water velocity of 2.6 ± 0.2 cm s^{-1}. The disparity between seed transport distances in the flume and seagrass can be explained by several factors. In the flume, seedlings were exposed to water of a constant speed that was unidirectional and relatively laminar, and the substrate was relatively flat. As a result, secondary seedling dispersal distances in my flume experiments were likely dramatically overestimated compared to my *in situ* measurements. Fluid dynamics in natural seagrass systems are more complicated than within the flume: hydrodynamic flow is rarely, if ever, consistently unidirectional, water velocity fluctuates, and wave action and topographical features produce turbulence. Whereas seedlings in the flume consistently moved with the direction of the water flow, I observed seeds in the *in situ* experiment moving forward, then backward in small increments, with a resulting net forward movement.

Variable patterns in seed transport were also noted in the flume when seedlings were exposed to waves. Wave height dispersal experiments also overestimated turtle grass seedling dispersal over the substrate compared to *in situ* experiments, but to a lesser degree: estimated seedling dispersal over sand after exposure to 5.2 and 7 cm waves was 242–432 m d^{-1}, and in seagrass this was reduced to 98–173 m d^{-1} (low density), 68–153 m d^{-1} (intermediate density) and 34–98 m d^{-1} (high density). Unfortunately, I do not have *in* *situ* wave measurements during my dispersal experiments for direct comparison, but bottom orbital velocities in the flume for 5.2 and 7 cm waves were 24 and 35 cm s^{-1}. The comparison of flume experiments to my *in situ* dispersal experiments demonstrates that experiments conducted an artificial settings are helpful for understanding processes, but may not accurately represent the magnitude of patterns occurring in nature.

Shoal grass seed dispersal was also overestimated in the flume compared to my *in situ* dispersal experiments. Using mean transport data from the flume, estimated shoal grass seed dispersal potential over sand with water speeds between 10–20 cm s^{-1} was 27–2,577 m d^{-1}. In the intermediate ASU, this was reduced to 22–247 m d^{-1}. Estimated dispersal over sand from the *in situ* experiments, however, is only 0.7 m, and in seagrass, was 1.1 m at a water speed of 3.1 ± 0.3 cm s^{-1}. Similar to turtle grass seedlings, seeds of shoal grass had unidirectional movement in the flume, but both forward and backward motion was observed in the natural environment, with net forward movement with the dominant direction of water flow.

Seed morphology and reproductive adaptations

Reports of terrestrial seed secondary dispersal range from a few centimeters (Watkinson 1978) to several meters from the point of origin (Feldman and Lewis 1990) and transport distances are often greater over bare soil than within vegetation (Redbo-Tortensson and Telenius 1995). My *in situ* results indicate that secondary dispersal of turtle grass seedlings and shoal grass seeds during natural wind and water velocity conditions is likewise limited, and dispersal is similarly greater over bare sand than within seagrass structure. Average velocities at each of my sites (< 5 cm s^{-1}) are likely not sufficiently strong enough to move seeds long distances, but may move seeds on the order of centimeters to meters.

Only a few studies exist that have examined seagrass secondary dispersal, but all have reported localized movement on the order of meters. Orth et al. (1994) reported that the small (2-4 mm), barrel-shaped seeds of *Zostera marina* in Chesapeake Bay were mostly retained within 5-m plots over a 2-month period, despite periodic high estimated current velocities (20 cm s^{-1}). Orth et al. (1994) also suggest that micro-topographic features of the sediment such as ripples, pits and mounds trap seeds and prevent dispersal of these seeds that lack morphological features to enhance dispersal (e.g. wings or plumes). I also observed shoal grass seed entrapment in the troughs of ripples in the sediment and adjacent to shoal grass shoots. Koch et al. (2010) reported that secondary seed dispersal of the mesohaline species *Ruppia maritima*, *Potamogeton perfoliatus* and *Stukenia pectinata*, is minimal and that the small size and spherical shape of these seeds promotes seed settlement and retention within the parent meadow. The limited dispersal and similar size and shape of shoal grass seeds to *R. maritima*, *R. perfoliatus* and *S. pectinata* further indicate that this morphology limits seagrass seed secondary dispersal over the substrate (Bakker et al. 1996).

Turtle grass seedlings in the laboratory had lower velocity thresholds for movement than shoal grass seeds, and results from both the flume and *in situ* dispersal experiments suggest that turtle grass seedlings have the potential to disperse farther over the substrate than shoal grass seeds. Lacap et al. (2002) investigated secondary seedling dispersal of *Thalassia hemprichii*, a closely related ‘twin species’ to turtle grass that is distributed throughout the western Pacific and West Indian Ocean. *Thalassia hemprichii* has similarly sized pyriform seedlings as turtle grass, and Lacap et al. (2002) reported that bottom dispersal for *T. hemprichii* approached 1 m d^{-1}. These results are similar to my field experiments, which estimated turtle grass seedling dispersal distanced between 0.43–2.1 m d^{-1}.

Turtle grass seedlings are much larger and morphologically more complex than shoal grass seeds. On average, turtle grass seedlings from my experiments were 7.5 times taller than shoal grass seeds. Whereas the small, round shape of shoal grass seeds likely restricts these seeds to very low velocity hydrodynamic conditions adjacent to the substrate, the larger size and complex shape of turtle grass seedlings likely exposes them to higher water velocities in the water column (Koch et al. 2006). The broad leaves characteristic of turtle grass also likely provide a wide surface over which the force of water can act. These leaves are often curled (pers. obs.), which also likely generates lift (Dijkstra 2012). Although turtle grass seedlings may move farther over the substrate than shoal grass seeds, certain seedling characteristics seem to inhibit long distance secondary dispersal.

Many species of freshwater monocots (Kaul 1978), dicots and terrestrial plants develop fine hairs on the base of the seedling that trap sand or sediment and promote anchoring. In both laboratory and *in situ* experiments, I observed sand grain attachment to these hairs on the base of the turtle grass seedlings. Lacap et al. (2002) reported that *Enhalus acoroides* seedlings also form a ‘hairy mass at the base’ of the seedling that assists with anchoring to the substrate after two to five days of dispersal and a maximum distance traveled of 2.04 m. Further, Koch et al. (2010) observed that seedlings of all 3 species tested produced a root that trapped sand grains, which were up to 4 times as heavy as the seed itself. In both laboratory and *in situ* experiments I also commonly observed turtle grass seedling leaves becoming stuck within the leaves of adult shoots or on the downstream side of a shoot. As a result of the vertical velocity gradients in seagrass canopies, vertical pressure gradients can occur downstream of individual seagrass shoots. These vertical pressure gradients generate a vertical ascending flow (Koch et al. 2006). Specifically in the flume, I observed seedlings becoming ‘stuck’ in the low-pressure area immediately downstream of a shoot, either until the end of the experiment or until some point when the seed was ejected vertically into the water column where it continued moving downstream. Additionally, turbulence is greater over bare sand than in seagrass structure (Koch et al. 2006). This increased turbulence over bare sand likely also promoted seedling movement and may help explain the greater seedling movement over bare sand than in seagrass structure.

As in many terrestrial plants, seagrass seed dispersal distance is an important reproductive adaptation. Whereas buoyant turtle grass fruits are adapted for long distance seed dispersal, shoal grass seeds are adapted for building a persistent seed bank. Negatively buoyant shoal grass seeds are released adjacent to the parent plant (primary dispersal) and sometimes below the substrate (Inglis 2000). For those seeds released below the substrate, it is possible that suspension of sediment could transport seeds to the substrate surface where they would then be exposed to water flow. Results from this study indicate that flow-mediated secondary transport of shoal grass seeds is relatively minimal, and seeds are likely quickly buried in the sediment where they can remain dormant for up to 4 years (McMillan 1981). Like terrestrial plants that have this reproductive adaptation, shoal grass is a successional species and occurs in slightly disturbed environments (Zieman 1982) and qualifies as a repeated seedling recruitment (RSR) species (Inglis 2000). In such disturbed environments, having a persistent seed bank is advantageous because it can facilitate persistence following a disturbance to the adult population (Bakker et al. 1996).

In contrast, turtle grass is a climax species that grows in relatively stable environments (Zieman 1982), where a local, persistent seed bank is not necessarily advantageous. Rather, turtle grass disperses seedlings over long distances to enhance the potential of colonizing new areas and reducing sibling competition (Willson and Traveset 2000). As a result, turtle grass is considered an initial seedling recruitment (ISR) species, where sexual recruitment is likely responsible only for initial population establishment (Inglis 2000). Results from this study indicate that long distance dispersal in turtle grass is achieved primarily by current-mediated transport of fruits, rather than secondary seedling dispersal along the substrate. Once settled, it appears that turtle grass seedlings may disperse relatively short distances (on the order of meters) before anchoring to the substrate. Turtle grass seedlings rely on internal nutrient stores for the first two to six months after dehiscence before becoming phososynthetically self-sufficient (Kaldy and Dunton 1999). It may be advantageous for a seedling to anchor to the substrate quickly, as it would likely orient leaves toward the light and maximize photosynthetic capability.

Although I did not investigate storm-level conditions in this study, it is possible that seeds can be transported with storms over long distances. Koch et al. (2010) suggested that storm activity would widely disperse seeds of *R. maritima*, *R. perfoliatus* and *S. pectinata*, and Kendall et al. (2004) suggested the increased frequency of hurricanes between 1971–1999 has enhanced expansion of *Syrindodium filiforme* in the US Virgin Islands through seed or vegetative fragment transport.

Implications for restoration

The rapid decline in seagrass cover has prompted worldwide management, conservation and restoration actions. Seagrass cover disappeared by 110 km^2 per year between 1980 and 2006 (Waycott et al. 2009), and Short et al. (2011) reported that 14% of all seagrass species are under an elevated risk of extinction. The historical focus on clonal growth and lack of focus on sexual reproduction has left the reproductive life history stages of seagrasses relatively unexplored. A full understanding of all stages of seagrass life history is necessary for successful conservation, management and restoration.

Seagrass restoration and rehabilitation via vegetative transplants has been attempted since the 1940s, but many efforts have been relatively unsuccessful. For example, in a review of over 50 seagrass restoration attempts, Fonseca et al. (1998) reported that many projects required multiple planting efforts to obtain desired coverage. Relatively recent efforts, however, successfully restored *Z. marina* through seed sowing in the Chesapeake Bay (Orth et al. 1994, Harwell and Orth 1999). Successful restoration through seeding requires understanding the reproductive physiology and ecology of the plant. Specifically, it is necessary to understand propagule dispersal in space and time for appropriate restoration and to provide the best chance of re-establishment into an area (Bakker et al. 1996). Results from this study suggest that, under normal hydrodynamic conditions in a central Texas estuary, turtle grass and shoal grass secondary dispersal is on the order of centimeters to meters. Ideal restoration efforts would include propagule placement within suitable habitat or in areas where net flow transports propagules in the direction of suitable habitat. Propagule density should be high if rapid coverage is desired. I suggest that necessary next steps to this research should include understanding factors that break shoal grass seed dormancy, developing the most effective and least disruptive seed collection methods, and determining the most suitable microhabitats for turtle grass and shoal grass seedling growth.

Table 3.1. SeaHorse current meter deployment dates at each site (Traylor Island, Mud Island, Hog Island and East Flats) over each substrate (*Thalassia testudinum*, *Halodule wrightii* or sand).

	Traylor Island			**Mud Island**	**Hog Island**			**East Flats**
	T. testudinum	*H. wrightii*	Sand	*T. testudinum*	*T. testudinum*	*H. wrightii*	Sand	*T. testudinum*
Deployment Dates	2 Aug	21 Jun–4 Jul	25 Jul	11–19 Jul	19 Jul–30 Aug	21 Jun–5 Jul	21 Jun–19 Jul	11 Jul–30 Aug
	5– 9 Aug	25 Jul	2 Aug	25 Jul–1 Aug				
			5–9 Aug	9–23 Aug				
				23–30 Aug				

Table 3.2. Seagrass shoot densities at the location of each of the *in situ* SeaHorse current meters in Redfish, Aransas and Corpus Christi Bays, TX. Turtle grass (*Thalassia testudinum)* and shoal grass (*Halodule wrightii)* shoot densities were similar among sites (all $p > 0.05$).

Species	**Traylor Island**	**Hog Island**	**Mud Island**	**East Flats**	**F**	***P***
Thalassia testudinum Shoot density (shoots m^{-2}, mean ± S.E.)	1,740 ± 188	1,270 ± 141	940 ± 262	1,222 ± 262	1, 2.2835	0.1559
Halodule wrightii Shoot density (shoots m^{-2}, mean ± S.E.)	3,198 ± 410	5,314 ± 1,640	NA	NA	1, 1.5673	0.2788

Table 3.3. Mean weighted water direction (degrees) and speed (cm s^{-1}) at each site (Traylor Island, Mud Island, Hog Island and East Flats), substrate (*Thalassia testudinum*, *Halodule wrightii* and sand) and wind (m s^{-1}) combination. 'NA' indicates that combination was not present in my data, and '–' indicates that data were uniformly distributed (Rao's Uniformity Test > 0.05), so mean weighted water directions and speeds could not be calculated.

	Traylor Island			Mud Island	Hog Island			East Flats
	T. testudinum	*H. wrightii*	Sand	*T. testudinum*	*T. testudinum*	*H. wrightii*	Sand	*T. testudinum*
Southeast wind								
0 – 3 (m s^{-1})	300.5°	306	307.9	267.7	195.8	101.8	324.4	300.6
	1 cm s^{-1}	2.7	1.7	1.2	0.5	0.6	0.6	2.8
3.1 – 6	328.4	305.3	305.4	323.2	246.8	62.9	318.9	297.1
	1	2.8	1.8	1.3	0.8	1.2	0.8	2.9
6.1 – 9	252.3	310.4	308.4	341.5	307.6	62.5	321.4	294.9
	0.9	2.4	3	1.5	1.5	2.8	1	2.9
> 9	NA	NA	NA	NA	NA	NA	NA	NA
Southwest wind								
0 – 3	214.3	306.8	290	294.8	136.1	102.2	280.3	301.8
	1.3	2.8	1.1	0.7	0.6	2.6	0.8	2.7
3.1 – 6	–	304.4	–	51.4	89.7	110.3	266.9	307.1
	–	2.7	–	0.9	0.2	3.7	0.5	2.3
6.1 – 9	NA	325.7	–	49.8	26.8	NA	279	320.4
		2.8	–	4.4	4.2		2.7	3.4
> 9	NA	NA	NA	NA	NA	NA	NA	NA
Northwest wind								
0 – 3	281.2	296.1	331.4	266	194.5	120	275.8	302.6
	2.2	3.2	1.7	1.6	1.1	1.3	0.9	2.9
3.1 – 6	NA	308.2	328	252.1	185.2	–	258.9	308.3
		3	2.2	1.3	1.1	–	0.8	2.4
6.1 – 9	NA	306.6	NA	–	–	–	243.5	–
		2.6		–	–	–	2.6	–
> 9	NA	312.9	NA	NA	NA	293.4	234.6	NA
		2				8.9	3.2	
Northeast wind								
0 – 3	–	295.6	331	253.9	204.7	89.6	304.1	301.3
	–	3.1	2.5	1.6	1.1	1.1	0.8	3
3.1 – 6	–	306.3	316.9	257.8	213.9	3.1	296.7	301.3
	–	3.2	2.5	1.9	1.8	0.6	1.4	2.9
6.1 – 9	NA	278.6	304.5	256.6	212.6	315	297	300.5
		2.8	3.8	3	2.8	1.9	1.6	3.1
> 9	NA	–	–	277.3	238.4	–	–	304.2
		–	–	9.9	2.6	–	–	3.3

Table 3.4. Results of 1-h *in situ* secondary seed/seedling dispersal experiments for turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*) in sand and seagrass at Traylor Island, TX with mean weighted water speed, seed/seedling speed and distance the seeds/seedlings moved over the hour-long experiments (mean ± SE).

In Situ Secondary Dispersal Experiments					
Species	**Substrate**	**Water Speed (cm s^{-1})**	**Seed Speed (cm s^{-1})**	**Distance Moved (cm)**	**n**
Thalassia testudinum	Sand	3.7 ± 0.5	0.003 ± 0.001	8.8 ± 3.5	8
	Seagrass	2.6 ± 0.2	0.0006 ± 0.0002	1.8 ± 0.6	4
Halodule wrightii	Sand	NA	0.0009 ± 0.0003	3.0 ± 1.1	5
	Seagrass	3.7 ± 0.3	0.002 ± 0.001	4.7 ± 1.8	7

Table 3.5. Results of laboratory secondary seed/seedling dispersal experiments with water speed in a recirculating flume for turtle grass (*Thalassia testudinum*) seedlings and shoal grass (*Halodule wrightii*) seeds. Dispersal measurements include average water speed, seed/seedling speed and distance the seeds/seedlings moved. Experimental duration was 120 sec (see Methods for details). All turtle grass seedlings and shoal grass seeds in the Artificial Seagrass Unit (ASU) treatments remained within the experimental working section over the 120 sec experimental duration. Data are presented as mean ± SE unless data were transformed to obtain normality of the residuals. If data were transformed to obtain normality of the residuals, the back-transformed mean and back-transformed lower 95% confidance interval and upper 95% confidance interval are presented.

Laboratory Secondary Dispersal Experiments						
Species	Substrate	Water Speed (cm s^{-1})	Seed Speed (cm s^{-1})	Distance Moved (cm)	Duration (sec)	n
Thalassia testudinum	Sand	4	0.09 ± 0.08	7.1 ± 5.1	114 ± 6	9
		5	0.11 ± 0.07	8.3 ± 3.9	115 ± 5	15
		6	0.14 ± 0.09	6.9 ± 4.3	110 ± 7	15
		7	0.21 ± 0.11	11.7 ± 4.8	108 ± 7	17
		8	0.45 ± 0.19	17.8 ± 6.5	93 ± 12	12
		9	0.96 ± 0.35	28.1 ± 9.6	69 ± 18	7
		10	0.71 ± 0.20	25.2 ± 5.1	86 ± 10	19
		11	1.6 ± 0.42	40.2 ± 9.4	44 ± 19	5
		12	1.8 ± 0.48	40.7 ± 8.7	42 ± 20	5
		13	2.0 ± 0.46	46 ± 3.5	41 ± 20	5
		15	2.3 ± 0.26	48.8 ± 0.9	33 ± 7	19
		20	4.2 ± 0.37	49.4 ± 0.7	13 ± 1	19
	ASU- Low	10	0.06 ± 0.02	7.8 ± 2.0	120	9
		15	0.15 ± 0.03	17.9 ± 3.5	120	7
		20	0.21 ± 0.03	25.4 ± 3.7	120	8
	ASU- Intemediate	10	0.08 ± 0.03	10.1 ± 3.2	120	8
		20	0.13 ± 0.03	15.1 ± 3.9	120	8
	ASU- High	10	0.02 ± 0.01	2.9 ± 1.1	120	8
		20	0.09 ± 0.03	10.6 ± 3.1	120	8
Halodule wrightii	Sand	10	0.03 (0.01–0.06)	3.8 ± 0.7	120	6
		12	0.08 (0.04–0.16)	14.2 ± 4.1	120	10
		15	0.73 (0.45–0.86)	47.0 ± 2.7	70 ± 10	10
		17	1.74 (1.36–2.22)	50.7 ± 0.2	31 ± 4	10
		20	3.00 (2.74–3.27)	50.7 ± 0.2	17 ± 1	10
	ASU- Intermediate	10	0.02 (0.009–0.05)	3.0 ± 1.0	120	3
		15	0.15 (0.07–0.34)	20.3 ± 5.5	120	3
		20	0.29 (0.13–0.63)	34.3 ± 1.9	120	3

Table 3.6. Results of laboratory secondary seed/seedling dispersal experiments with wave height in a recirculating flume for turtle grass (*Thalassia testudinum*) seedlings and shoal grass (*Halodule wrightii*) seeds. Dispersal measurements include wave height, seed/seedling speed and distance the seeds/seedlings moved. Experimental duration was 120 sec (see Methods for details). All turtle grass seedlings and shoal grass seeds in the Artificial Seagrass Unit (ASU) treatments remained within the experimental working section over the 120 sec experimental duration. Data are presented as mean ± SE unless data were transformed to obtain normality of the residuals. If data were transformed to obtain normality of the residuals, the back-transformed mean and back-transformed lower 95% confidance interval and upper 95% confidance interval are presented.

Laboratory Secondary Dispersl Experiments: Wave Height

Species	Substrate	Wave Height (cm)	Seed Speed (cm s^{-1})	Distance Moved (cm)	Duration (sec)	n
Thalassia testudinum	Sand	5.2	0.3 ± 0.05	32.9 ± 4.8 cm	117 ± 5	10
		7.6	0.6 ± 0.1	40 ± 1.4	80 ± 9.0	10
	ASU- Low	5.2	0.11 ± 0.01	13.6 ± 1.6	120	4
		7.6	0.2 ± 0.05	24 ± 5.7	120	4
	ASU- Intermediate	5.2	0.08 ± 0.02	9.5 ± 2.9	120	4
		7.6	0.18 ± 0.03	21.3 ± 3.9	120	4
	ASU- High	5.2	0.04 ± 0.01	4.7 ± 1.3	120	3
		7.6	0.11 ± 0.02	13.7 ± 2.6	120	3
Halodule wrightii	Sand	5.2	0.03 (0.002–0.08)	15.4 ± 2.8	120	10
		7.6	0.27 (0.17–0.41)	42.3 ± 0.4	45.5 ± 6.1	10
	ASU-Intermediate	5.2	0.02 (0.01–0.05)	3.3 ± 0.9	120	4
		7.6	0.13 (0.05–0.28)	16.7 ± 4.6	120	4

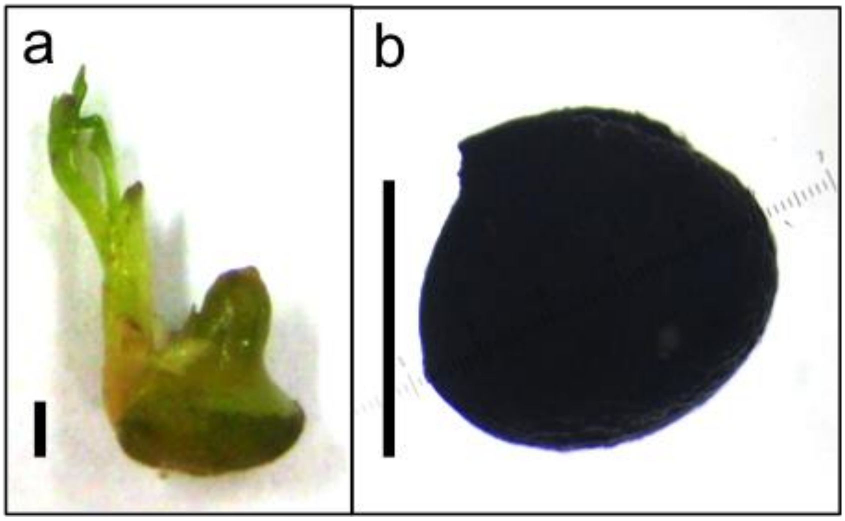

Figure 3.1. Examples of a turtle grass (*Thalassia testudinum*) seedling (a) and a shoal grass (*Halodule wrightii*) seed (b). Scale bar = 2 mm.

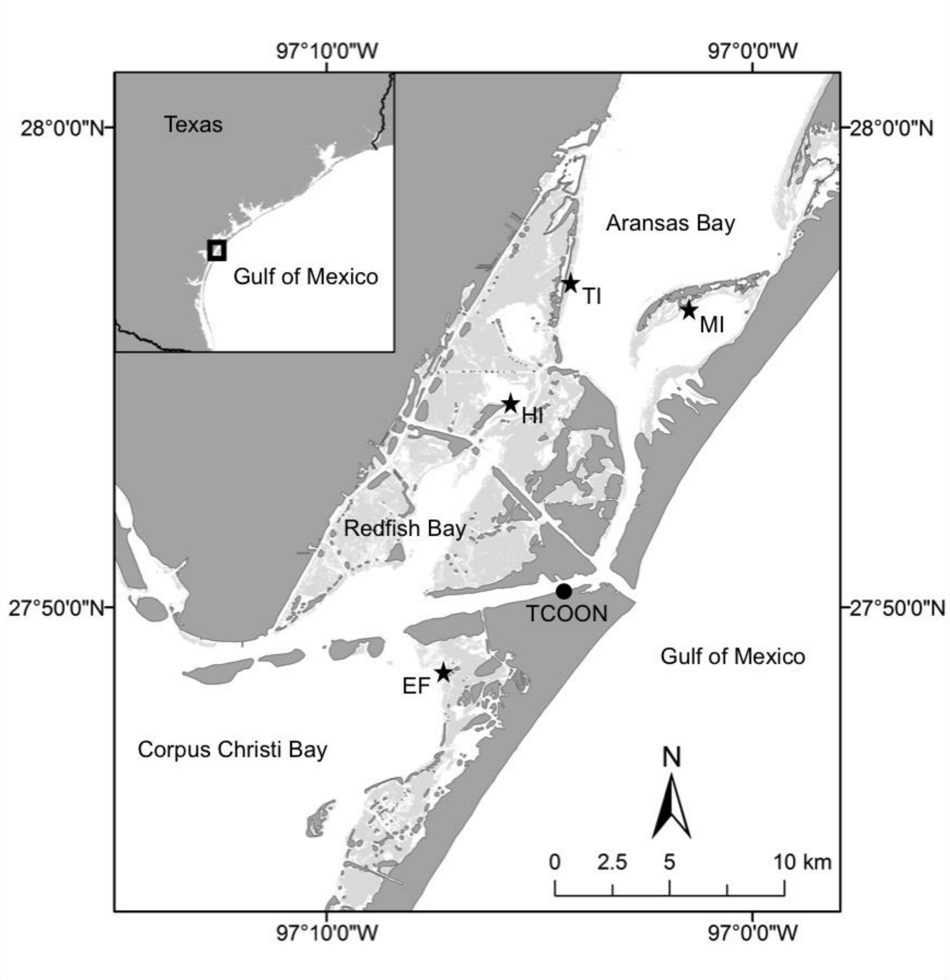

Figure 3.2. SeaHorse current meter deployment locations in Redfish, Aransas and Corpus Christi Bays, Texas. Dark gray represents land, light gray represents continuous seagrass cover obtained from the NOAA Coastal Services Center Benthic Habitat Mapping 2004/2007 Benthic Data Set, and stars represent SeaHorse current meter locations. Current meters were deployed in turtle grass (*Thalassia testudinum*) at Traylor Island, Mud Island, Hog Island and East Flats, and in shoal grass (*Halodule wrightii*) at Traylor Island and Hog Island only (TI = Traylor Island, MI = Mud Island, HI = Hog Island, and EF = East Flats). Wind data were obtained from the Texas Coastal Ocean Observation Network (TCOON, www.cbi.tamuss.edu/TCOON/) station in Port Aransas, TX, denoted by the black circle.

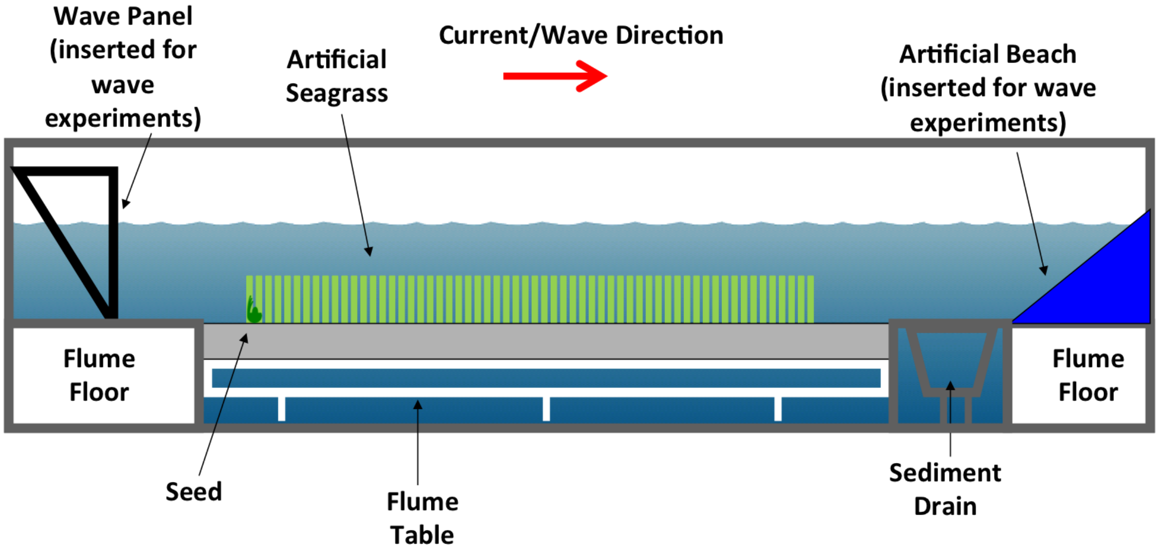

Figure 3.3. Diagram of the recirculating flume at the Horn Point Laboratory in Cambridge, MD with artificial seagrass and a turtle grass (*Thalassia testudinum*) seed depicted at the upstream end of the working section. Water is recirculated through a pipe below the working section (not depicted). Diagram provided by D.M. Booth.

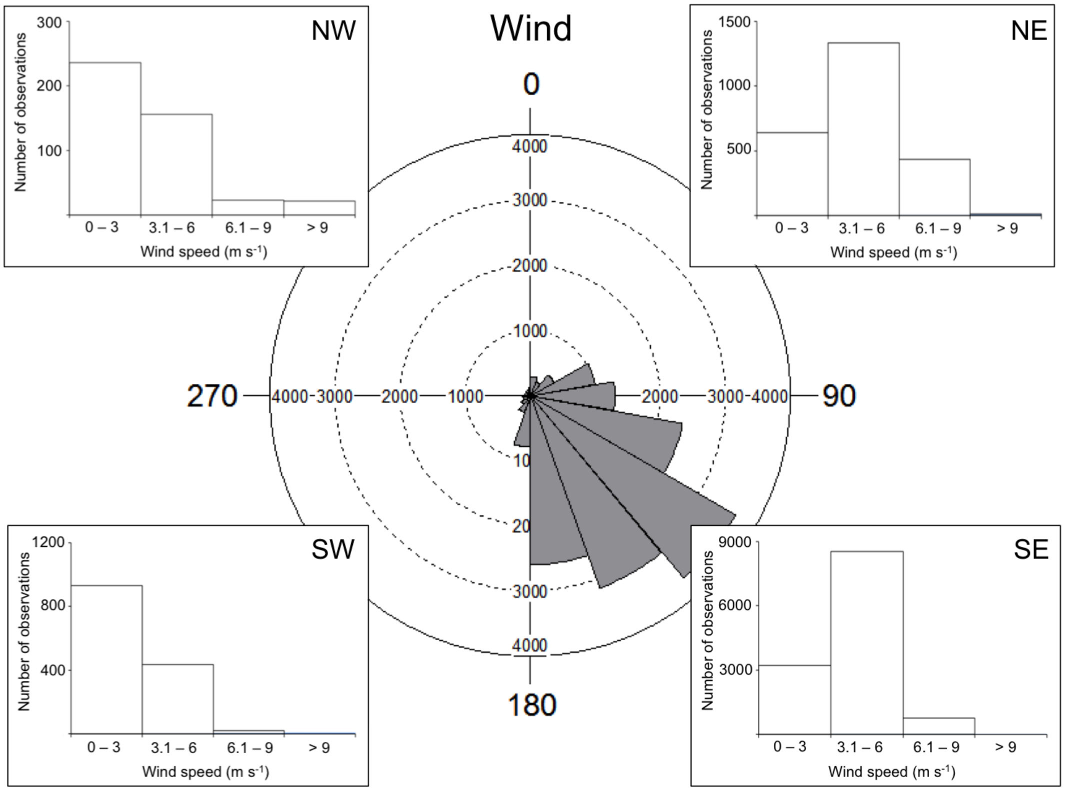

Figure 3.4. Histograms of wind direction and speed obtained from the Texas Coastal Ocean Observation Network (TCOON, www.cbi.tamuss.edu/TCOON/) station in Port Aransas, TX at the time of SeaHorse current meter deployment (21 June – 30 August 2013). The circular histogram displays wind direction, which was predominantly from the southeast during deployment of the current meters. Numbers around the outer circle represent angle (degrees) and numbers within the outer circle represent the number of observations. Linear histograms display wind speeds from the northeast (NE), southeast (SE), southwest (SW) and northwest (NW).

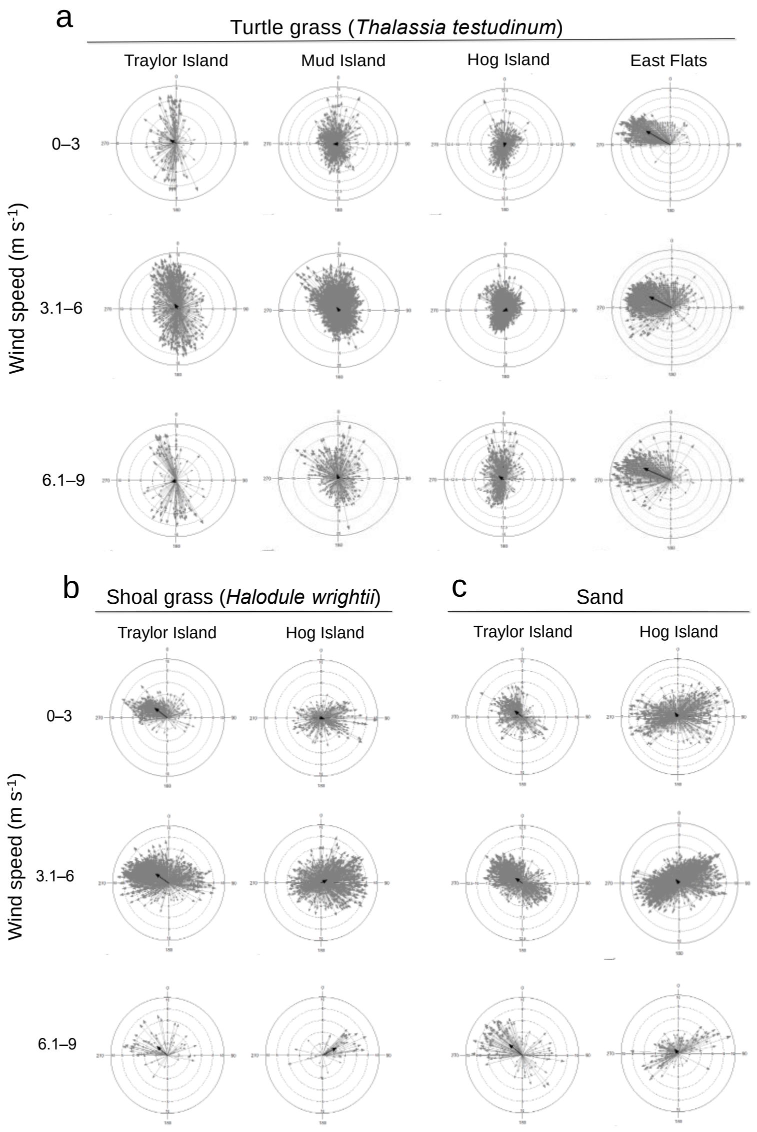

Figure 3.5. Water velocity (cm s^{-1}) in turtle grass (*Thalassia testudinum*) beds (a), shoal grass (*Halodule wrightii*) beds (b) and sand (c) at sites at Traylor Island, Mud Island, Hog Island and East Flats during the months of seed release in 2013 under binned wind speed (m s^{-1}) from the southeast. Each gray arrow represents one water velocity (direction and speed) measurement. Black arrows represent mean weighted water velocity. Figures are not shown for wind speeds > 9 m s^{-1}, because conditions did not exist or mean weighted water velocity could not be calaculated (Rao's Uniformity Test, p>0.05).

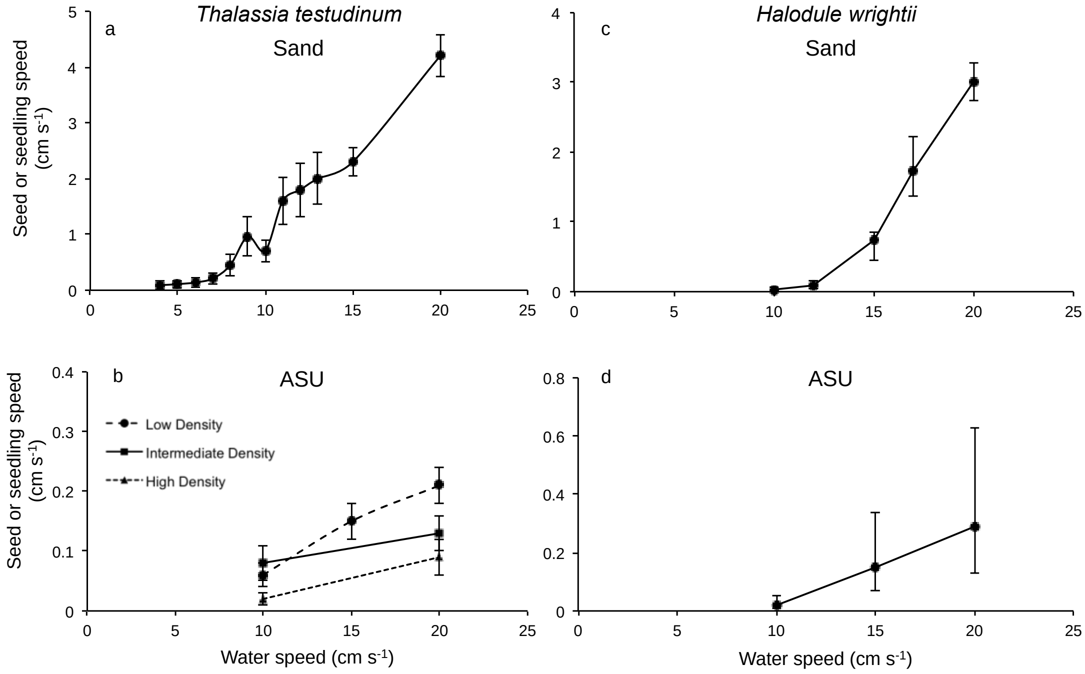

Figure 3.6. Results of laboratory secondary dispersal experiments with turtle grass (*Thalassia testudinum*) seedlings and shoal grass (*Halodule wrightii*) seeds. Turtle grass mean seed speeds (mean ± SE, cm s^{-1}) in sand (a) and artificial seagrass units (ASU) (b) are shown over a range of water speeds (cm s^{-1}). Shoal grass data were transformed to obtain normality of the residuals for statistical analyses. These data are presented as the back-transformed means and back-transformed lower and upper confidance intervals. Experiments were conducted in a recirculating flume at the Horn Point Laboratory in Cambridge, MD. Turtle grass seedling speed increased with water speed in sand ($p<0.05$), and in the low ($p<0.05$) and high density ($p<0.05$) ASU treatments, but not the intermediate density treatment ($p>0.05$). Shoal grass seed speed increased with water speed in sand ($p<0.05$) and the intermediate density ASU ($p<0.05$).

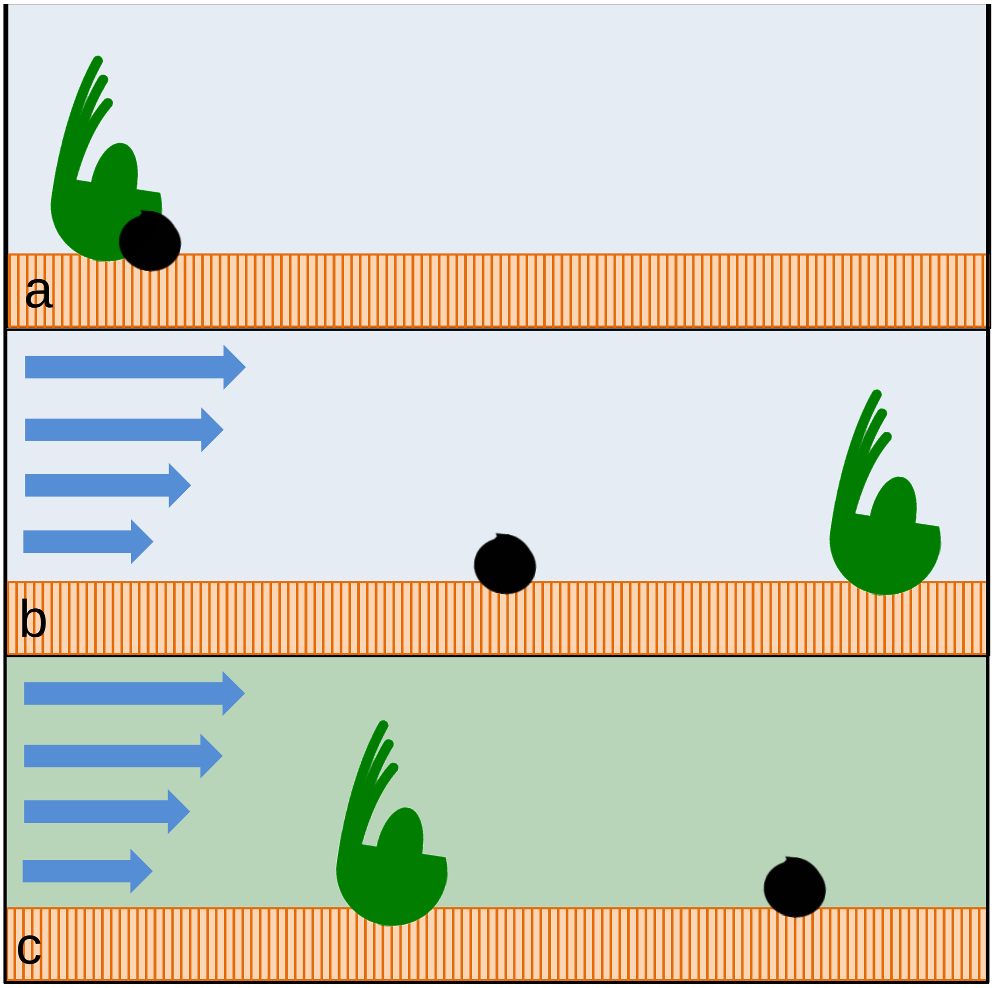

Figure 3.7. A simple conceptual diagram depicting turtle grass (*Thalassia testudinum*) seedling and shoal grass (*Halodule wrightii*) seed movement along the substrate under no flow conditions (a), and over bare sand (b) and in seagrass (c) under normal water flow conditions in Texas.

Chapter 4: Consumption of Turtle Grass and Shoal Grass Seeds and Seedlings by Crabs in the Western Gulf of Mexico

Abstract

Seed consumption by animals can limit reproductive success and recruitment of seagrasses. Consumption of seeds by crustaceans has been reported for several temperate seagrass species, but its prevalence for sub-tropical seagrass species remains unknown. Using local crab species, I investigated consumption of sub-tropical turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*) seeds and seedlings in laboratory and field feeding experiments along the Texas coast. More turtle grass seedlings were removed from uncaged tethers than caged tethers. Time-lapse photography captured a spider crab and pinfish near the tethered seedlings. In laboratory experiments, blue crabs and spider crabs readily consumed > 35% of offered turtle grass seedling tissue, respectively and mud crabs consumed 29.1 ± 8.3 % of shoal grass seed tissue. Hermit crabs did not consume turtle grass or shoal grass seeds. Observations indicate that blue crabs broke open turtle grass fruits and ate the seeds within. Seeds contained 250% and 400% more nitrogen and phosphorus, respectively, than the fruits encasing them. The enhanced nutritional value of turtle grass seeds and seedlings relative to fruit and leaf tissue may be the major driver for the observed patterns in consumption. Laboratory experiments of turtle grass seedling growth indicate that consumption by blue crabs severely reduces seedling growth and survival. As in terrestrial ecosystems, propagule consumption by benthic animals could potentially limit seedling survival and recruitment of sub-tropical seagrass species, but the significance of this process is not well understood at this time.

Introduction

Consumption of flowers, fruits, and seeds of terrestrial plants can dramatically reduce plant reproductive output and recruitment success, while at the same time providing an important food source that sustains a variety of animals (Janzen 1971, Harper 1977, Brown et al. 1979). Long-term interactions between consumers and plants can drive evolutionary changes in reproductive tissue characteristics (Janzen 1969, Smith 1970). However, many animals also act as seed dispersers, making it difficult to differentiate between consumption that will result in seed death and consumption that will enhance seed dispersal (Lovett-Doust and Lovett-Doust 1988). Confusion between the two has been clarified for many species by following the fate of eaten seeds (Janzen 1971, Chapman 1989, Forget 1996, Andresen 1999). Whereas the prevalence of consumption of terrestrial plant reproductive tissues is well documented, far fewer studies have examined the effects of consumption on seagrass reproductive tissues.

Seagrasses are a geographically widespread group of over 70 species of submerged marine vascular angiosperms that can propagate clonally and reproduce sexually. Sexual reproduction in seagrasses was traditionally considered rare (den Hartog 1970, Les 1988), but it is now considered important for both establishing and maintaining seagrass beds (Kendrick et al. 2012), highlighting the necessity to understand factors that affect seagrass reproductive and recruitment success. Consumption of seagrass flowers, fruits, and seeds has been described for several seagrass species. The majority of previous studies, however, have focused on temperate genera such as *Zostera* (Wigand and Churchill 1988, Fishman and Orth 1996, Nakaoka 2002) and *Posidonia* (Piazzi et al. 2000, Orth et al. 2002, Orth et al. 2007). In these genera, the most common fruit and seed consumers are decapod crustaceans such as crabs (Wigand and Churchill 1988, Holbrook et al. 2000, Fishman and Orth 1996, Orth et al. 2006) and shrimp (Wassenberg 1990, Nakaoka 2002). As is common among vertebrates, crustacean feeding decisions are also driven by factors such as food availability and quality (Alexander 1986, Ebersole and Kennedy 1985, Kennish and Williams 1997, Pennings et al. 1998). Studies with temperate seagrass species suggest that seed consumption is greater within seagrass structure than over bare sand (Orth et al. 2006, Orth et al. 2007). The few studies focusing on consumption of reproductive tissues in sub-tropical genera (e.g. *Thalassia, Halodule, Syringodium*) concentrate on flowers and pollen (van Tussenbroek et al. 2008, van Tussenbroek and Muhlia-Montero 2012, van Tussenbroek et al. 2012), and the prevalence of fruit and seed consumption remains unknown.

Turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*) are two of the most common sub-tropical seagrass species throughout the Gulf of Mexico, Caribbean Sea, and southeast coast of the United States (van Tussenbroek et al. 2010). The morphology and reproductive biology of these species are markedly different. Turtle grass, the larger of the two species, has wide (up to 10-mm), strap-shaped leaves and is dioecious (i.e. plants are separately male and female) (van Tussenbroek et al. 2010). Turtle grass uses hydrophilous (underwater) pollination, and after successful pollination, female plants produce fruits containing one to six seeds (van Tussenbroek et al. 2010). The relatively large seeds (up to 15 mm) germinate within a buoyant fruit that detaches from the parent plant when mature and can be transported by currents. As a result of this current-mediated transport, turtle grass seeds and seedlings (germinated seeds that have been released from the fruit) have the potential for long-distance, current-mediated dispersal (Kaldy and Dunton 1999, Kendrick et al. 2012). The unit of dispersal is initially the fruit, but seedlings become the unit of dispersal after release from the fruit. Along the central Texas coast where this study was conducted, the reproductive season for turtle grass spans from April, when flowers are produced, to August, when germinated seedlings are released from fruits (K. Darnell, personal observation).

Similar to turtle grass, shoal grass is dioecious and uses hydrophilous pollination. However, shoal grass is morphologically a much smaller species than turtle grass and has thin, 1–2 mm wide leaves. After successful pollination of the flowers, female shoal grass plants produce 2 fruits, each containing a single small (2-mm), black seed (van Tussenbroek et al. 2010). Seeds are the unit of dispersal for shoal grass, are released at or below the sediment surface, and are surrounded by a hard seed coat, allowing them to remain dormant in the sediment for up to 4 years (McMillan 1981). It has been hypothesized that this reproductive adaptation provides a seed reserve allowing for persistence during adverse conditions (Orth et al. 2006a). Shoal grass flowers are highly reduced and difficult to observe. However, along the central Texas coast, I have observed fruit-bearing shoots in April and May.

Whereas several studies have documented fruit and seed production in turtle grass (Moffler et al. 1981, Johnson and Williams 1982, Moffler and Durako 1987, Whitfield et al. 2004, Kahn and Durako 2006) and shoal grass (McMillan 1981, McMillan 1983, Ferguson et al. 1993, McGovern and Blankenhorn 2007), fewer studies have investigated factors regulating fruit and seed production, growth and seed and seedling survival (Kaldy and Dunton 1999, Kahn and Durako 2006). Kaldy and Dunton (1999), however, hypothesized that turtle grass seedling mortality documented in Lower Laguna Madre, Texas was due to consumption by crabs or fish.

The overall objective of this study was to assess the prevalence of seed and seedling consumption by crabs on sub-tropical seagrass genera. Specifically, I aimed to:

1) determine if turtle grass and shoal grass seeds and seedlings are eaten by local crab species, and 2) estimate seedling growth following partial consumption. I performed laboratory feeding experiments to determine potential consumers and quantify the amount of tissue consumed. With turtle grass only, I tethered seedlings *in situ* in sand and in seagrass beds to compare consumption among habitats and followed the fate of partially eaten seedlings from laboratory feeding experiments. I measured carbon, nitrogen and phosphorus in turtle grass leaf, fruit, seed and seedling tissue to compare elemental composition and assess potential nutritional value between somatic and reproductive tissues.

Materials and Methods

Field seedling tethering

To assess seedling consumption in the natural environment and compare consumption over different substrates, turtle grass seedlings collected by hand from Traylor Island, TX (27° 56’ 52.78” N, 97° 04’ 17.38” W) were tethered in a turtle grass bed and over sand at Steadman Island, TX (27° 53’ 06.95” N, 97° 07’ 01.31” W). Tethering experiments were conducted on 29 August, 3 September and 4 September 2013 (n = 3). Individual replicates consisted of paired uncaged (control) and caged (45 cm long × 45 cm wide × 25 cm tall, 1 cm mesh size) tethered seeds. For each replicate, five seeds were tethered in a turtle grass bed, five seeds were tethered over sand, five seeds were tethered and caged in a turtle grass bed and five seeds were tethered and caged over sand. Each seed was individually tethered to a plastic stake inserted into the sediment using 9-lb test monofilament fishing line. Seeds were tethered for 24 h, and the number of remaining seeds in each uncaged control and caged treatment were counted. Attempts were made to identify potential consumers using time-lapse photography (30-s time interval) with an underwater video camera (Go Pro, Woodman Labs, Inc.). Dive lights covered with red cellophane were used to illuminate the camera's field of view at night to mimic darkness, since many crustacean species are insensitive to red light (Cronin and Forward 1988). A YSI 600XL sonde was used to measure water temperature, salinity and pH during each experiment.

Laboratory feeding trials

Feeding trials were performed in the laboratory to determine potential turtle grass and shoal grass seed and seedling consumers and quantify consumption. Specific consumers were chosen for each seagrass species based on animal and seed size and feeding mode (turtle grass fruits and seedlings are produced and released above the sediment, whereas shoal grass seeds are released at or below the sediment surface). Separate experiments were conducted with turtle grass fruits (containing seeds) that were manually removed from the plant by hand and turtle grass seedlings that were released naturally from the fruit. Fruits and seedlings were collected from Traylor Island and Lower Laguna Madre, TX (26° 07' 34.31" N, 97° 11' 17.02" W). One consumer was placed in an aerated 10 gallon aquarium with 2 cm of sand that was previously burnt for 5h in a muffle furnace at 550 °C to remove all organic material and 5 or 10 pre-weighed (Denver Instrument APX-153) turtle grass fruits or seedlings. The number of fruits or seedlings offered was determined from preliminary feeding experiments. Fruits were tethered to a ~ 2 gram buried fishing weight with 2–3 cm of 9-lb test monofilament fishing line to mimic natural fruit height above the substrate, and seedlings were placed directly on the sand substrate. After 24 h and exposure to a normal light and temperature regime, the consumer was removed and measured, and the fruits or seedlings were re-weighed and the number of crushed fruits or seedlings recorded. The percentage of offered fruits or seedlings that were crushed by the consumer was calculated. Based on wet weights before and after experiments, the percentage of fruit or seedling tissue consumed was also determined. Potential consumers used in turtle grass fruit feeding experiments were adult (>100 mm carapace width) and juvenile (<100 mm carapace width) blue crabs (*Callinectes sapidus*). To assess the effects of consumers on turtle grass seedlings, feeding experiments used adult and juvenile blue crabs, spider crabs (*Libinia* spp.) and hermit crabs (*Pagurus* spp.*)* (Table 4.1). Adult blue crabs were also used in feeding preference experiments, in which each crab was offered five pieces of pre-weighed pinfish tissue (*Lagodon rhomboides*) and either five fruits or five seedlings weighing in total approximately what the total weight of offered pinfish pieces weighed (Table 4.1). Crabs were identified to the lowest taxonomic group possible.

Similar feeding experiments were conducted with shoal grass seeds, in which one consumer was placed in an aquarium with a 2 cm clean sand bottom and five pre-weighed shoal grass seeds. Seeds and seed pieces were re-weighed after 24 h and the number of seeds crushed was recorded. Potential consumers used in shoal grass seed feeding experiments were hermit crabs (*Pagurus* spp.) and mud crabs (Panopeidae) (Table 4.1). Crabs were identified to the lowest taxonomic group possible.

All crabs used in feeding experiments were collected from local Texas seagrass beds with reproducing plants. Upon collection, crabs were fed fish *ad libidum* and then starved for 48 h prior to the experiment to standardize hunger levels. Crabs were held in the laboratory in a tank with running seawater for no longer than 2 weeks and each crab was used only once.

Seed growth after consumption

To assess whether consumption ends in seedling death or has the potential to enhance seedling dispersal, the fate of partially eaten turtle grass seedlings from laboratory feeding experiments with blue crabs was monitored in a laboratory growth experiment from 1 August 2011 to 26 September 2011. Six partially eaten seedlings and six control (uneaten) seedlings were kept individually in 100 mL containers with filtered seawater in a Percival I-36VL incubator at 30°C under a 12:12 light:dark cycle. A digital photograph of each seedling was taken weekly to bi-weekly using a Canon SD 1200 camera (Canon U.S.A., Inc) and the longest leaf length (mm) on each seed was measured using ImageJ image processing and analysis software (ImageJ, National Institutes of Health).

Plant nutritional value

To compare nutritional quality among different plant tissues, turtle grass somatic (leaf) and reproductive (fruit, seed and seedling) tissues were collected during August 2013 from Traylor Island, TX for analysis of total carbon, nitrogen and phosphorus. Specifically, nutritional quality was assessed for: 1) shoots with attached fruits containing developing seeds (e.g. leaf, fruit and seed tissue) and 2) seedlings that were naturally released from the fruit (e.g. seedling tissue). I also analyzed elemental composition of fruits containing developing seeds (e.g. fruit and seed tissue) that were manually removed from the plant by hand, as collected for laboratory feeding experiments, to ensure manual collection did not alter elemental composition and confound my results. Prior to analysis, fruits containing intact seeds were sliced open and seeds were removed for separate analysis. All tissues were dried to a constant weight at 60°C and ground to a fine powder using a mortar and pestle. Carbon and nitrogen were measured with a Carlo-Erba EA 1108 Elemental Auto-Analyzer and phosphorus was measured with a Shimadzu UV-2401 PC UV-VIS Recording Spectrophotometer following a modified protocol from Chapman and Pratt (1961). Carbon, nitrogen and phosphorus data were used to calculate %C, %N and %P and molar C:N, C:P and N:P ratios (e.g. elemental composition) for each tissue.

Statistical analyses

On each of three different days, tethered seeds were placed in the field. Four treatment-combinations were used: herbivore access (inside or outside a cage), factorially with two substrates (bare sand or within a seagrass bed). Five tethered seeds were placed in each treatment-combination. Small sample size limited the statistical analysis of the proportion of tethered seeds that were removed. I pooled the results from the three dates and used a Fisher's exact test to compare herbivore access (insider versus outside a cage), pooling substrates for this test. I used a second Fisher's exact test to compare substrates, pooling herbivore access treatments for this second test.

To obtain normally distributed residuals for the analysis of weight in laboratory feeding experiments, for each trial I divided the final weight (including both eaten and intact seedlings) by the initial weight of those seedlings, and then transformed this proportion with the logit function. I was then able to use analysis of variance (ANOVA) to analyze this new variable, logit(final weight/initial weight). Neither a log transformation nor a square-root transformation normalized the residuals; see Warton and Hui (2011) for an argument in favor of using the logit transformation to normalize the residuals of a proportion that has no underlying binary variable. The fate of seeds (whether it ws eaten or not) in laboratory feeding experiments was analyzed with a generalized linear model with a binomial distribution and a logit link function. In this analysis, crab type was a fixed effect and trial nested within crab type was considered to be a random effect.

Longest leaf length in the laboratory seedling growth experiment was $\log_{10}$ transformed before analysis with ANOVA. In this analysis, seed type (eaten or uneaten) and date were fixed effects and seed number was considered to be a random effect.

Leaf, fruit and seed nutrient variables (proportion C, proportion N, proportion P) were logit transformed to obtain normally distributed residuals before analysis with ANOVA. The residuals of the ratios of the nutrient variables (C:N, C:P, N:P) were normally distributed and not transformed. In these analyses, tissue type was a fixed effect and shoot number was considered to be a random effect. Comparisons of seed nutrient variables between seeds within fruits attached to the plant, seeds within fruits manually removed from the plant and mature seedlings naturally released from fruits were analyzed using ANOVA with seed location as the response variable. Residuals of the seed nutrient data were normally distributed and not transformed. Comparisons of fruit nutrient variables between fruits on the plant and those fruits that were manually removed from the plant were analyzed using ANOVA with fruit location as the response variable. The %C, %N and %P data were logit transformed to obtain normally distributed residuals. Nutrient ratios (C:N, C:P, N:P) were normally distributed and not transformed.

Data with residuals that were normally distributed are presented as the mean ± standard error (mean ± S.E.). Data that were transformed to obtain normality of the residuals are reported as the back-transformed mean and back-transformed lower and upper 95% confidance intervals.

Results

Field seedling tethering

All caged seedlings were recovered intact for all experiments. For uncaged tethers, zero seedlings were removed during the first experiment (29 August 2013), two and one seedlings were removed from uncaged turtle grass and sand tethers, respectively, during the second experiment (3 September 2013), and two seedlings were removed from the uncaged turtle grass tethers during the third experiment (4 September 2013). Seedling loss was greater on uncaged tethers than caged tethers ($p = 0.03$). However, there was no difference in seedling loss between substrates ($p = 0.15$) (Figure 4.1). Time-lapse photography captured a spider crab (*Libinia* spp.) and pinfish (*Lagodon rhomboides*) near the uncaged tethered seedlings, although no animals were photographed actively consuming seedlings. Animals were photographed between 0211h and 0955h. Water temperature, salinity and pH were all within normal ranges for this study site and were consistent among experimental dates (water temperature: 32.66 ± 0.10°C, (mean ± SE) salinity: 42.1 ± 0.31, pH: 8.63 ± 0.12).

Laboratory feeding trials

Turtle grass fruits and seedlings

Adult blue crabs (n = 3, carapace width: 105–185 mm) crushed 70.0 ± 11.5 % of offered fruits and consumed 14.2 ± 4.7% of tissue in turtle grass fruit feeding trials. I observed that adult blue crabs broke open the fruits with their chelae and consumed the internal seeds rather than fruit tissue. Juvenile blue crabs (n = 3, carapace width: 55–75 mm) did not consume any tissue in fruit feeding experiments.

When turtle grass seedlings were offered, adult blue crabs, juvenile blue crabs and spider crabs crushed a similar percent of seedlings ($p = 0.24$, Figure 4.2a) and consumed a similar percent of seedling tissue ($p = 0.76$, Figure 4.2b). Adult blue crabs (n = 13, carapace widths: 110–190 mm) crushed 60.9 (34.8–82.0)% of seedlings and consumed a total of 32.2 (11.1–64.6) % of seedling tissue. Juvenile blue crabs (n=14, carapace widths: 40–90 mm) crushed 83.5 (63.8–93.6) % of offered seedlings and consumed a total of 42.4 (29.2–56.9) % of seedling tissue, and spider crabs (n =5, carapace widths: 28–52 mm) crushed 83.8 (47.3–96.7) % of offered seedlings and consumed a total of 35.9 (20.6–54.7) % of turtle grass seedling tissue. Hermit crabs (n = 6) neither crushed turtle grass seedlings nor consumed turtle grass seedling tissue.

Adult blue crabs exhibited a preference for pinfish tissue over fruits ($p < 0.002$) and seedlings ($p < 0.002$). When offered both fish and fruits, crabs (n = 5) first consumed all fish tissue, then consumed fruit/seed tissue. Crabs ate all of the fish tissue and 33.5 (19.4–48.8) % of fruit/seed tissue. When offered fish and seedlings, crabs (n = 5) consumed all of the fish tissue and 25.2 (10.2–44.0) % of seedling tissue.

Shoal grass seeds

Mud crabs (n = 9, carapace widths: 10–17 mm) crushed 46.7 ± 13.0% of offered seeds and consumed 29.1 ± 8.3 % of seed tissue. Hermit crabs (n =9) did not crush shoal grass seeds or consume shoal grass seed tissue.

Seedling growth after consumption

Control seedlings grown in the laboratory had significantly longer leaves than partially eaten seedlings (time: $p < 0.0005$, type: $p < 0.001$, time x type: $p < 0.001$; Figure 4.3). At the beginning of the growth experiment, longest leaf lengths for control and partially eaten seedlings were 10.2 (6.3–16.3) mm and 6.0 (2.9–12.4) mm, respectively. Leaves of control seedlings grew steadily and reached 32.1 (17.9–57.5) mm by the end of the 2-month experiment. All but one of the partially eaten seedlings lost their leaves (Figure 4.3). The remaining seedling with intact leaves had a longest leaf length of 5.7 mm at the end of the experiment. Nearly all control seedlings produced a prop root, whereas this was not observed for partially eaten seeds.

Plant nutritional value

Seeds and seedlings

Seeds were the most nutritious tissue tested, leaf tissue was the next most nutritious, and fruit tissue was the least nutritious (%C: $p< 0.0001$; %N: $p < 0.0001$, %P: $p < 0.0001$, Figure 4.4). The percentages of nitrogen and phosphorus in seeds were 2.5 and nearly 4 times that of fruits, respectively. Seeds contained the most carbon 40.5 (39.8–41.2) %), nitrogen (2.2 (1.8–2.7) %), and phosphorus (0.31 (0.23–0.42) %), leaves had intermediate carbon (32.2 (31.3–33.0) %), nitrogen (1.6 (1.5–0.7) %), and phosphorus (0.12 (0.10–0.14) %) and fruits had the lowest carbon (17.8 (15.6–20.0) %), nitrogen (0.86 (0.74–1.00) %), and phosphorus (0.08 (0.07–0.12) %) (Figure 4.4a, b, c). Molar C:N ratios (ca. 22:1) were similar among tissue types ($p = 0.29$, Figure 4.4d), but both C:P ($p < 0.0001$, Figure 4.4e) and N:P ($p < 0.0001$, Figure 4f) were significantly different. Seeds had the lowest C:P (323.4 ± 39.3) and N:P (15.6 ± 0.8) ratios, which reflected their high phosphorus content. Fruit tissue had intermediate nutrient ratios (C:P: 565.0 ± 44.9; N:P: 22.4 ± 1.3) and leaves had very high ratios (C:P: 704.2 ± 46.3; N:P: 30.5 ± 1.7), reflecting depleted phosphorus.

Seed location did not influence nutritional quality. Seeds within fruits attached to the plant, seeds within fruits manually removed from the plant and mature seedlings naturally released from fruits all had similar %C ($p = 0.06$), %N ($p = 0.15$) and %P ($p = 0.08$), C:N ($p = 0.19$), C:P ($p = 0.09$) and N:P ($p = 0.77$) (Figure 4.5).

Fruits

Fruits removed from turtle grass plants for lab feeding experiments did not significantly differ nutritionally from fruits still attached to the plant (%C: $p = 0.05$; %N: $p = 0.45$; %P: $p = 0.11$; C:N: $p = 0.16$; C:P: $p = 0.92$; N:P: $p = 0.60$) (Table 4.2). Despite manual removal by hand, seeds encased within fruits were still more nutritious than the fruits surrounding them. The percentages of carbon ($p < 0.0001$), nitrogen ($p < 0.0001$) and phosphorus ($p < 0.0001$) were all greater for seed tissue than fruit tissue, and average carbon, nitrogen and phosphorus were 2 – 3 times as high for seeds compared to fruits Similar to fruits still on the plant, manually removed fruits and their enclosed seeds had similar C:N ratios ($p = 0.10$), but different C:P ($p < 0.0002$) and N:P ($p < 0.003$) ratios.

Discussion

My results suggest that turtle grass and shoal grass seeds and seedlings may be subject to consumption by crabs in the western Gulf of Mexico. Laboratory seedling growth experiments indicate that turtle grass seedlings do not survive partial consumption, and nutritional analyses suggest that elevated phosphorus content in turtle grass seeds and seedlings may be driving patterns in consumption. Lab experiments demonstrated that common benthic crustaceans consumed turtle grass and shoal grass seeds and seedlings and that partial consumption of turtle grass seeds by blue crabs led to seedling death. Although my field results are not conclusive with respect to the significant consumption of seeds and seedlings by consumers, removal of seagrass propagules has the potential to affect seagrass recruitment and establishment.

Seed consumption by crustaceans

Blue crabs and spider crabs ate turtle grass seeds and seedlings in my laboratory experiments. Seed and fruit consumption by crabs has been widely documented for terrestrial plants (Wolcott and O'Connor 1992). Consumption by crabs is also known to limit mangrove density and distribution (Lindquist and Carroll 2004). In Australia, for example, grapsid crabs can consume up to 75% of mangrove propagules (Wolcott and O'Connor 1992). Several studies on temperate seagrass species such as *Zostera marina*, *Phyllospadix torreyi* and *Posidonia australis* reported that crustaceans are dominant seagrass seed consumers (Wigand and Churchill 1988, Holbrook et al. 2000, Orth et al. 2006). Fishman and Orth (1996) reported that blue crabs, specifically, are a primary consumer on *Z. marina* seeds in the Chesapeake Bay. Blue crabs are common along the eastern and Gulf Coasts of the United States, and similar to other benthic decapod crustacean species, are omnivores and opportunistic feeders whose diet varies spatially and seasonally based on food availability (Laughlin 1982).

The blue crab diet consists of fish, crustaceans, infauna, and plant material. Darnell (1958) reported that in Lake Pontchartrain, LA, plant material contributes up to 11% of blue crab gut contents. Additionally, Alexander (1986) reported that 29% of blue crabs collected near Galveston Island, TX contained vascular plant tissue in their guts. In a pattern consistent with opportunistic feeders, adult blue crabs in this study consumed seed and seedling tissue when offered no other food choice, but when given a choice, preferred pinfish tissue to fruits (and the seeds within) and seedlings. However, in these preference experiments, the crabs still crushed and ingested plant tissue once the fish had been consumed. Juvenile blue crabs (< 100 mm carapace width) in this study also ate turtle grass seedling tissue, but did not eat turtle grass fruit tissue. As is true for adult blue crabs, plant matter can also make up a high percentage of gut contents in juvenile crabs (Seitz et al. 2011). In this study, I observed juvenile crabs attempting, but being unsuccessful at breaking the fruits. The inability of the small, relatively weak chelae of juvenile blue crabs to break open the tough fruit exterior is a likely explanation for the lack of consumption.

Unlike previous reports of increased *P. australis* seed consumption over seagrass structure compared to bare sand (Orth et al. 2006, Orth et al. 2007), I saw no difference in turtle grass seedling consumption between the substrate types. To avoid a large spatial separation between my substrate treatments and potential confounding factors such as varying depth and water flow, I placed the substrate treatments in close proximity (~2 m apart) within a patchy seagrass meadow. This approach may have introduced edge effects (Smith et al. 2008), masking any effects of substrate. I have observed broken turtle grass fruits attached to parent plants in turtle grass beds within Corpus Christi Bay, TX in July that mimicked fruits broken in my laboratory feeding experiments. In both cases, the broken fruits were severed in half with the seeds removed. These severed fruits look distinctly different from mature fruits that have naturally released their internal seeds. I have also observed crushed turtle grass seedlings in Corpus Christi Bay in turtle grass beds and over bare sand during August, the time of peak seed release in Texas.

I also found that spider crabs (*Libnia* spp*.)* consume seagrass seeds. In laboratory experiments, spider crabs consumed as much and crushed as many seeds as blue crabs. Like blue crabs, spider crabs are scavengers and opportunistic feeders and plant tissue can make up as much as 100% of gut contents (Aldrich 1974). The range of *Libnia* spp. is from Nova Scotia to the western Gulf of Mexico. These crabs are primarily found in muddy bottom and seagrass substrates. The time-lapse photographs of a spider crab near the tethered turtle grass seedlings confirms that spider crabs are active in local seagrass beds.

Hermit crabs (*Pagurus spp.)* did not eat turtle grass fruits, turtle grass seedlings, or shoal grass seeds in laboratory feeding experiments. Hermit crabs in the genus *Pagurus* are omnivorous and can utilize different feeding modes to take advantage of available food items. Like many other crustaceans, *Pagurus* spp. can use their chelae to grasp or break apart a food item and move it to their maxillipeds and mandible (Gerlach et al. 1976). However, *Pagurus* spp. are also deposit feeders and suspension feeders, and will sift through the sediment or filter the water column to obtain food (Gerlach et al. 1976). Hazlett (1981) reported that the primary food source for hermit crabs is detritus, suggesting that deposit feeding may be their main feeding mode. In my laboratory experiments, hermit crabs may have utilized another feeding mode, such as suspension feeding. It is unlikely that crabs utilized deposit feeding, as sand in the experimental aquaria was burnt for 5h in a muffle furnace prior to the experiment to remove all organic material.

Time-lapse photography revealed pinfish (*Lagodon rhomboides*) near the uncaged turtle grass tethers. Pinfish are common grazers in coastal seagrass beds and undergo an ontogenetic shift from carnivore to herbivore at around 120 mm total length (Stoner and Livingston 1984). Kaldy and Dunton (1999) reported that pinfish picked up and spat out turtle grass seedlings in Lower Laguna Madre, TX. It is possible that, at larger sizes (> 120 mm), pinfish may consume or damage turtle grass seeds.

Fate of partially eaten turtle grass seedlings

Biotic ingestion of a seed may not ultimately end in its death, as passage through an animal's gut can aid in seed dispersal and/or enhance seed germination success (Lovett-Doust and Lovett-Doust 1988). Following the fate of eaten seeds can eliminate any confusion between seed loss by consumption and dispersal (Janzen 1971, Chapman 1989, Forget 1996, Andresen 1999). In my seedling growth experiments, all but one of the partially consumed turtle grass seeds lost their leaves and died, whereas uneaten seeds grew steadily throughout the experiment. By the end of the experiment, leaves on the remaining partially eaten seed were only 14% as long as leaves on the uneaten seeds. Turtle grass seeds and seedlings are too large to pass through the crab gut tract intact. Crabs feed by crushing, tearing and breaking apart their food into small pieces with their chelae before bringing it to their mandible. Therefore, it is likely that any seed or seedling consumption by crabs would end in death.

Turtle grass seeds germinate within the fruit and do not have a hard seed coat. Passage through and scarification by the gut, therefore, are not necessary for germination. Turtle grass fruits are buoyant and adapted for long distance dispersal. Once released from the plant, they have the potential to be transported hundreds of kilometers by currents (vanDijk et al. 2009). This life history strategy eliminates the need for a biotic dispersing agent. Other seagrass species whose seeds have a hard seed coat, however, may benefit from ingestion by animals. Sumoski and Orth (2012) reported that seeds of *Z. marina*, a species with a hard seed coat, can successfully germinate after passing through the guts of several fish, turtle and waterfowl species and have the potential for biotic dispersal up to 20,000 km. Similar to *Z. marina*, shoal grass seeds are surrounded by a hard seed coat that allows long-term persistence in a seed bank. In laboratory feeding experiments, mud crabs crushed seeds with their chelae and consumed the inner seed tissue. This, combined with the mud crabs' small size and slow speed, suggests that they would not be effective agents for biotic dispersal. As part of a statewide seagrass monitoring program in Texas, I sampled nearly 600 sites for shoal grass seed densities in the sediment. Of the 558 samples I collected, over 400 (73.1%) of the sites contained broken seeds (unpublished data). Although the cause of these broken seeds is unknown, it is possible that a consumer crushed them.

Large consumers that likely consume whole shoal grass seeds may act as biotic dispersing agents (Sumoski and Orth 2012). During the winter months, thousands of waterfowl migrate to the south Texas coast where they feed primarily in seagrass beds. Redhead ducks (*Aythya americana*) in particular feed mostly on underground shoal grass rhizomes (Mitchell et al. 1994), and are therefore likely to consume seeds that are buried in the sediment.

Nutrient content of seeds and seedlings

The higher nutrient content in turtle grass seeds and seedlings than in leaf tissue is not surprising. It is well known for terrestrial plants that seeds have higher nutrient levels than the parent plants to aid in seedling establishment and development (Tyler and Zohlen 1998). Phosphorus, specifically, is necessary for optimal root growth and development and can be exceptionally concentrated in seed tissue versus leaf tissue in habitats where it is less readily available (Tyler and Zohlen 1998). The carbon, nitrogen and phosphorus content in leaves from fruit-bearing shoots are low compared to normal leaf carbon (35.88 ± 2.47%), nitrogen (2.09 ± 0.28) and phosphorus (0.16 ± 0.03%) in seagrass from this area (Wilson and Dunton 2012), likely because of the maternal resources invested in the developing seeds (Reekie and Bazzaz 1987).

Turtle grass seedlings become photosynthetically self-sustaining between 2 and 6 months after dehiscence from the fruit, but prior to that rely on reallocation of carbon resources within the seedling (Kaldy and Dunton 1999). Higher overall carbon levels in seeds and recently released seedlings than in adult leaf tissue supports this notion. Adult seagrass plants can actively absorb nitrogen and phosphorus through both leaves and roots (Lee and Dunton 1999). However, the relative nutrient uptake ability of different tissues in turtle grass seedlings is unknown. Without a substantial root system to take up pore-water nutrients, seedlings may be limited by the amount of nutrients they can acquire, and therefore rely on reallocating their internal nutrient stores for growth. Statton et al. (2012) reported that *P. australis* seedlings rely on internal nutrient stores for up to 4 months after germination and Hocking et al. (1981) reported a linear relationship between loss of nitrogen and phosphorus and seedling dry matter in the first 9 months following germination. The similar nutrient content I measured between turtle grass seeds still encased within the fruit and seedlings released from the fruit is likely because the developing seedlings were collected immediately after release and had not yet utilized their internal nutrient stores for growth.

Among the tissues I analyzed, fruits had the lowest nitrogen and phosphorus content, indicating their primary function is dispersal and seed protection (Janzen 1971). It is possible that the nutritional quality of the seeds relative to the fruits drives the observed blue crab feeding preference for seed over fruit tissue in laboratory experiments. Of the nutrients measured, elevated phosphorus is likely most important. Although the percentages of both nitrogen and phosphorus were elevated in seed tissue, the C:N ratio was similar among tissue types, yet the C:P and N:P were significantly lower in seeds. Consumers can detect subtle differences in nutritional quality of their food (Bjorndal 1980, Preen 1995). Goecker et al. (2005) found that parrotfish detect elevated nitrogen levels in turtle grass leaves and prefer to consume the high nutrient tissue. The importance not only of absolute nutrient content, but also of stoichiometry is becoming increasingly recognized in consumer-plant interactions. For example, Peterson et al. (2012) found that fish in nitrogen-rich areas throughout Discovery Bay, Jamaica, preferentially ate turtle grass enriched in phosphorus. I observed a distinct disparity in the relatively low percent of seedling tissue that was consumed in laboratory feeding experiments versus the relatively high percent of seedlings that were crushed (Fig. 2). It is possible that the crabs may be targeting the nutrient-rich hypocotyl (Kuo et al. 1991) and ignoring the remaining tissue. Seagrasses in the genus *Thalassia* lack an endosperm and, as a result, nutrients are stored in the hypocotyl within the base of the seed or seedling (Kuo et al. 1991).

Conclusion

Seed and seedling loss in my laboratory feeding experiments, combined with growth experiments demonstrates that consumption by crabs ends in seedling death. Observations of broken fruits, seeds and seedlings in local seagrass beds suggests that seed and seedling consumption can be an important factor affecting successful recruitment of turtle grass and shoal grass. Opportunistic species like crabs, whose diets are tightly coupled with food availability, have the potential to dramatically reduce the number of viable recruiting seeds and impart an under-recognized pressure on seagrass populations. I suggest that future research investigate the potential impact of consumption by pinfish on turtle grass seed and seedling survival and possible biotic dispersal of shoal grass seeds by migratory waterfowl, as this could represent an effective method for long distance dispersal of this species (Figuerola et al. 2002).

Table 4.1. Experimental design of laboratory feeding experiments with turtle grass (*Thalassia testudinum*) and shoal grass (*Halodule wrightii*) reproductive structures. 'Number offered' refers to the number of reproductive structures offered to one potential consumer.

Potential consumer	*Reproductive structure*	*Numbered offered*	*Replicates*
Turtle grass (*Thalassia testudinum*)			
Blue crab (*Callinectes sapidus*)	Fruit	10	3
	Seedling	10	13
	Choice: Fruit vs. Fish	5 of each type	5
	Choice: Seedling vs. Fish	5 of each type	5
Juvenile blue crab (*Callinectes sapidus*)	Fruit	10	3
	Seedling	10	14
Spider crab (*Libinia* spp.)	Seedling	10	5
Hermit crab (*Pagurus* spp.)	Seedling	5	6
Shoal grass (*Halodule wrightii*)			
Mud crab (Panopeidae)	Seed	5	9
Hermit crab (*Pagurus* spp.)	Seed	5	9

Table 4.2. Elemental composition of turtle grass (*Thalassia testudinum*) fruits attached to the parent shoot (Attached Fruits, n = 5) and fruits manually removed from the shoot (Manually Removed Fruits, n = 9). Data for carbon, nitrogen and phosphorus were transformed to obtain normally distributed residuals. These data are presented as the back-transformed mean and back-transformed lower and upper 95% confidance intervals. Residuals for the elemental composition ratios were normally distributed and are presented as the mean ± SE.

	Carbon (%)	**Nitrogen (%)**	**Phosphorus (%)**	**C:N**	**C:P**	**N:P**
Attached Fruits	17.7 (15.7–20.0)	0.86 (0.74–1.0)	0.08 (0.07–0.09)	24.03 ± 0.96	565 ± 44.9	23.5 ± 1.3
Manually Removed Fruits	20.3 (18.2–22.6)	0.92 (0.80–1.1)	0.09 (0.08–0.10)	25.8 ± 0.64	565.5 ± 28.5	22.1 ± 1.5

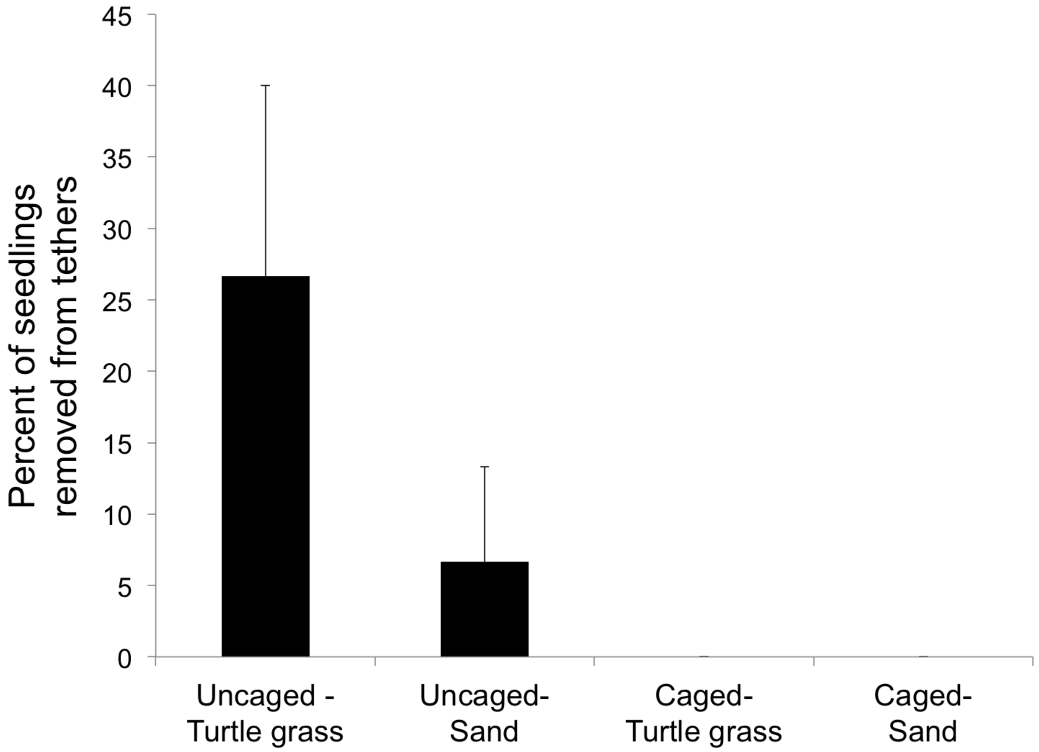

Figure 4.1. Percent of turtle grass (*Thalssia testudinum*) seedlings removed from caged and uncaged tethers in turtle grass and over sand in field tethering experiments at Steadman Island, TX. Values are means ± SE and n = 3.

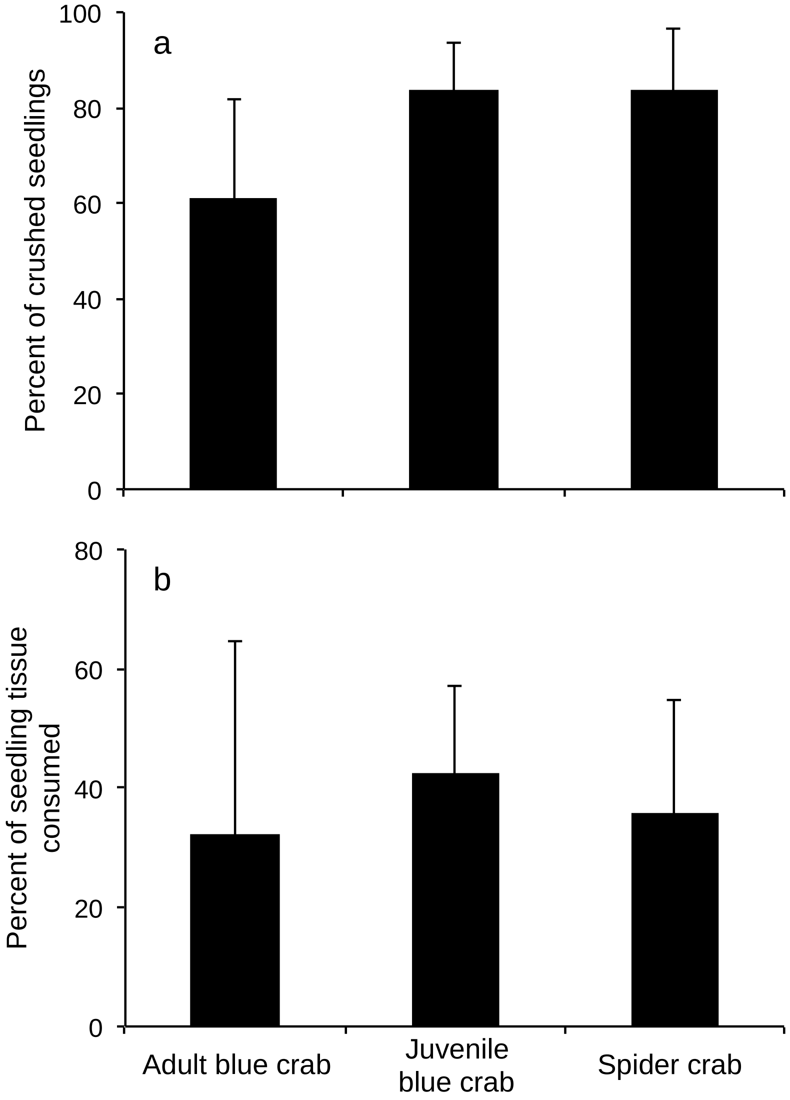

Figure 4.2. Percent of turtle grass (*Thalassia testudinum)* crushed seedlings (a) and seedling tissue consumed (b) in laboratory experiments by adult blue crabs, juvenile blue crabs (*Callinectes sapidus*) and spider crabs (*Libnia* spp.). See Table 1 for number of seedlings offered and number of replicates. Values are the back-transformed mean and back-transformed lower and upper 95% confidance intervals.

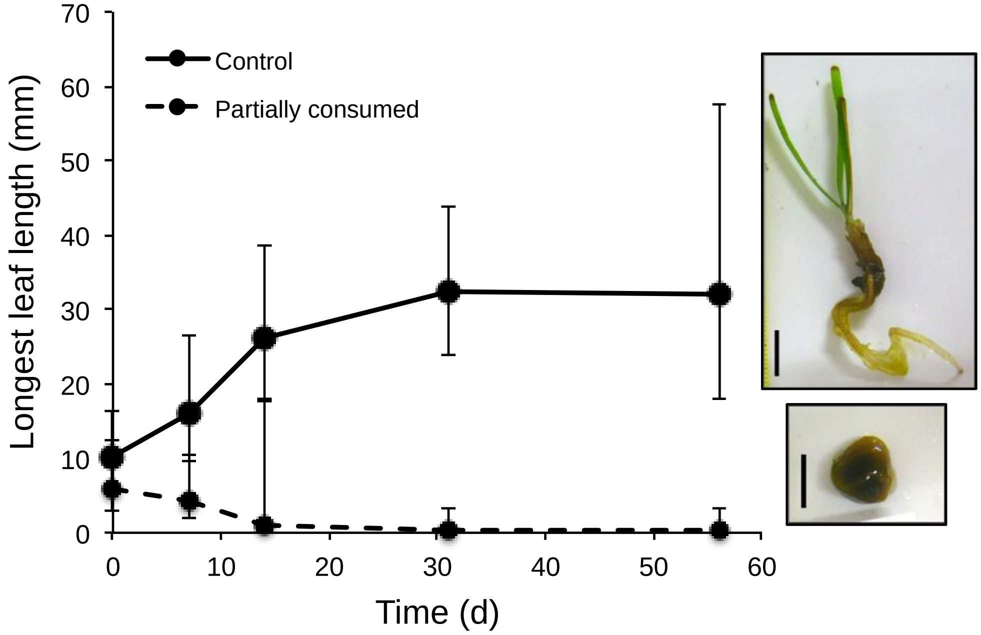

Figure 4.3. Longest leaf lengths (mm) of control (uneaten) and partially consumed turtle grass (*Thalassia testudinum*) seedlings from laboratory feeding experiments with adult blue crabs (*Callinectes sapidus*). Photographs are examples of control (top) and partially eaten (bottom) seedlings on the last day of the experiment. Values are the back-transformed means and back-transformed lower and upper 95% confidance intervals. Scale bars in the photographs are 10 mm.

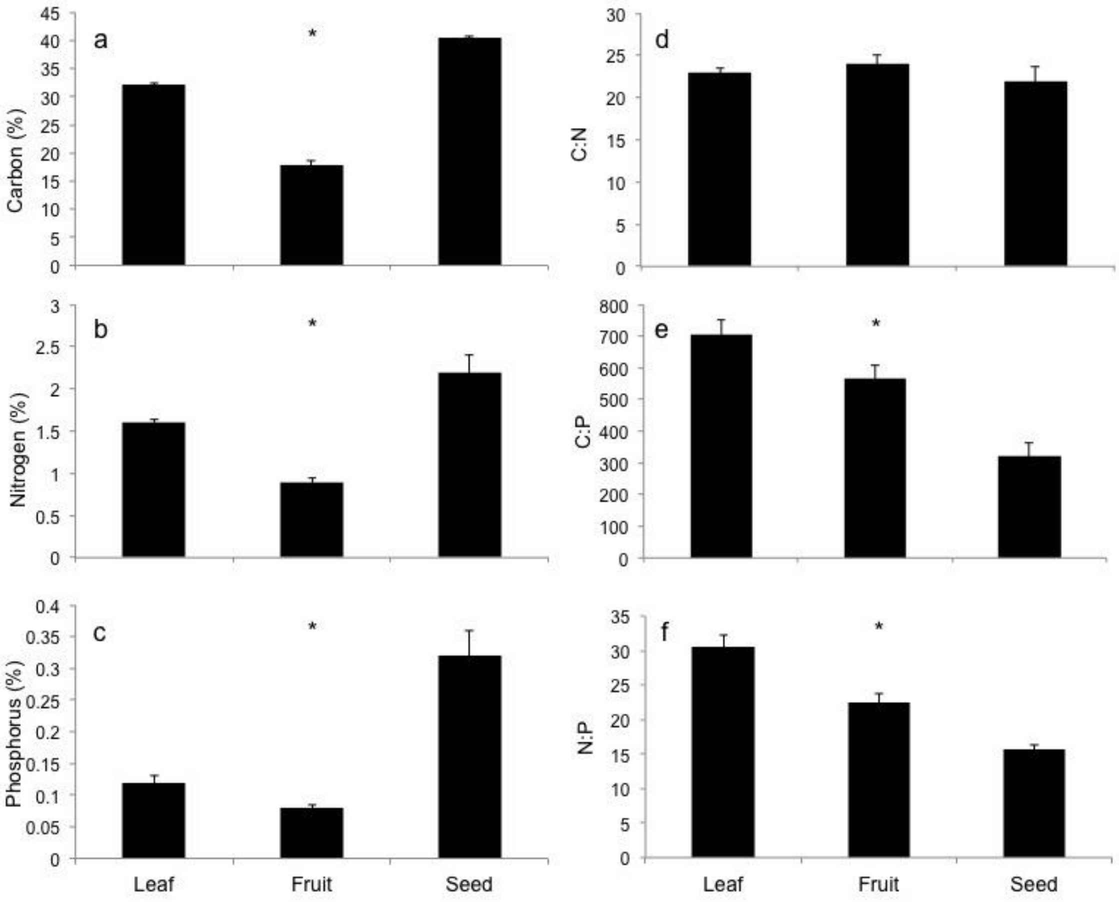

Figure 4.4. The percent carbon (a), percent nitrogen (b), percent phosphorus (c), C:N (d), C:P (e) and N:P (f) of turtle grass (*Thalassia testudinum*) leaf, fruit and seed tissue. '*' indicates significant differences among the tissue types. Data for carbon, nitrogen and phosphorus were transformed to obtain normally distributed residuals. These data are presented as the back-transformed mean and back-transformed lower and upper 95% confidance intervals. Residuals for the elemental composition ratios were normally distributed and are presented as the mean ± SE.

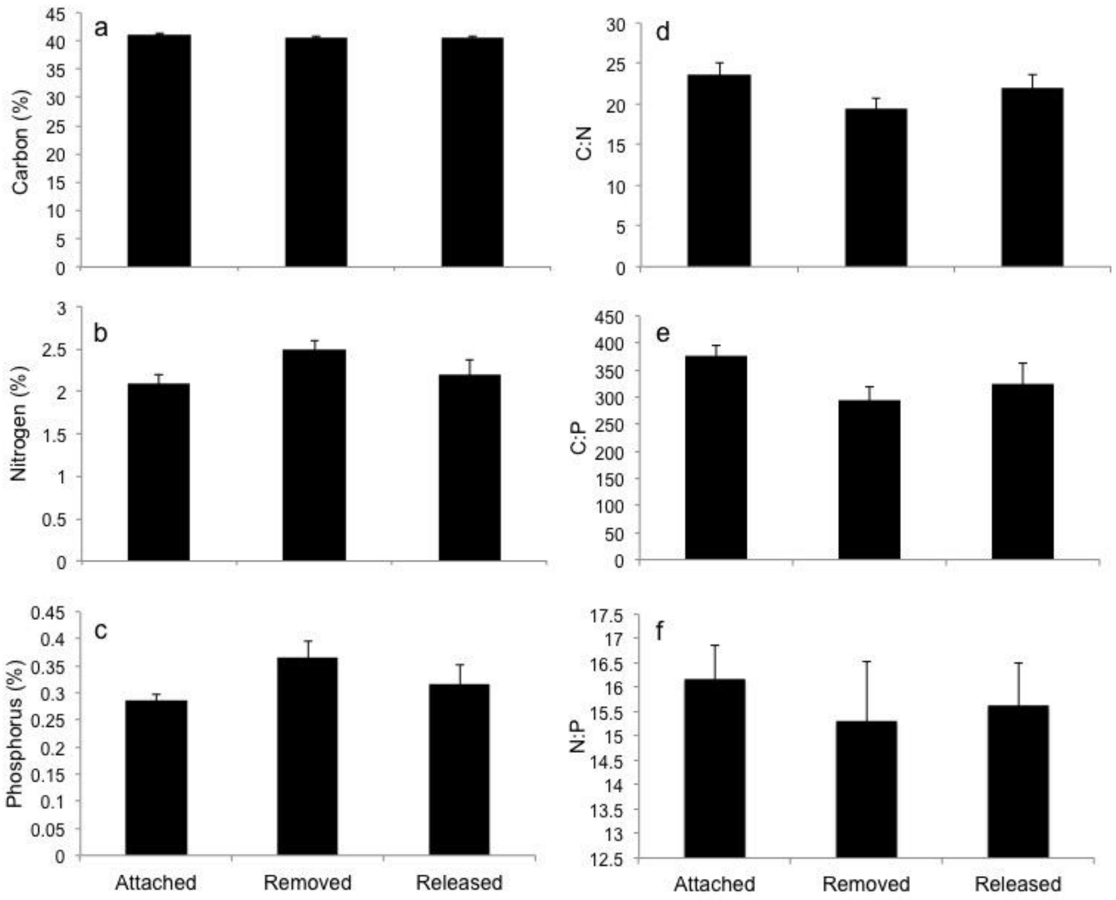

Figure 4.5. The percent carbon (a), percent nitrogen (b), percent phosphorus (c), C:N (d), C:P (e) and N:P (f) of turtle grass (*Thalassia testudinum*) seeds within fruits attached to a shoot (attached, n = 5), seeds within fruits manually removed from a shoot (removed, n = 9) and mature seedlings naturally released from fruits (released, n = 5). Values are means ± SE. There were no significant differences ($p > 0.05$) in elemental composition between attached, removed, or released seeds.

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