

# Consumption of turtle grass seeds and seedlings by crabs in the western Gulf of Mexico

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**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 laboratory and field feeding experiments, we investigated consumption of seeds and seedlings of the sub-tropical seagrass species turtle grass *Thalassia testudinum* along the Texas coast. More turtle grass seedlings were removed from uncaged tethers than caged tethers and time-lapse photography captured a spider crab and pinfish near the tethered seedlings. In laboratory experiments, blue crabs and spider crabs readily consumed  $35.0 \pm 9.3\%$  and  $36.9 \pm 6.02\%$  (mean  $\pm$  SE) of offered turtle grass seedling tissue, respectively, but hermit crabs did not consume seed or seedling tissue. 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. Seedling growth experiments 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.

**KEY WORDS:** Turtle grass · Blue crab · Seed · Seedling · Fruit · Consumption

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## 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). Long-term interactions between consumers and plants can drive evolutionary changes in reproductive tissue characteristics (Janzen 1969). However, many animals also act as seed dispersers, making it difficult to discern between consumption that will result in seed death and consumption that will enhance seed dispersal (Lovett-Doust & Lovett-Doust

1988). Confusion between the two has been clarified for many species by following the fate and growth of eaten seeds (Janzen 1971, Chapman 1989, Andresen 1999). Whereas the prevalence of consumption on 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 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 & Churchill 1988, Fishman & Orth 1996, Nakaoka 2002) and *Posidonia* (Piazzi et al. 2000, Orth et al. 2002, 2007). In these genera, the most common fruit and seed consumers are decapod crustaceans such as crabs (Wigand & Churchill 1988, Fishman & Orth 1996, Holbrook et al. 2000, 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 & Kennedy 1995, Kennish & Williams 1997, Penning 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, 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, 2012, van Tussenbroek & Muhlia-Montero 2013), and the prevalence of fruit and seed consumption in these sub-tropical genera is largely unknown.

Turtle grass *Thalassia testudinum* is a dominant sub-tropical seagrass species in the Gulf of Mexico, Caribbean Sea, and along the southeast coast of the USA (van Tussenbroek et al. 2010). Turtle grass has wide (up to 10 mm), strap-shaped leaves, is dioecious (i.e. plants are separately male and female), and uses hydrophilous (underwater) pollination. After successful pollination, female plants produce fruits containing 1–6 seeds (van Tussenbroek et al. 2010). The relatively large seeds (~5–15 mm) germinate within a buoyant fruit that can detach from the parent plant when mature and 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 & Dunton 1999, Kendrick et al. 2012). 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 pers. obs.).

Whereas several studies have documented fruit and seed production in turtle grass (Moffler et al. 1981, Johnson & Williams 1982, Whitfield et al. 2004, Kahn & Durako 2006), fewer studies have investigated fac-

tors regulating fruit and seed production, growth and seed and seedling survival (Kaldy & Dunton 1999, Kahn & Durako 2006). Kaldy & 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 turtle grass, a common sub-tropical seagrass species. Specifically, we aimed to (1) determine if turtle grass seeds and seedlings are eaten and preferred by local crab species, and (2) estimate seedling growth following partial consumption. We performed laboratory feeding experiments to determine potential consumers and quantify the amount of tissue consumed by each of these consumers, laboratory growth experiments to determine the fate of partially eaten seedlings, and *in situ* tethering experiments in sand and in seagrass beds to compare consumption between substrates. We also quantified carbon (C), nitrogen (N) and phosphorus (P) 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 seed tethering

To assess seedling consumption in the natural environment and compare consumption over different substrates, turtle grass seedlings collected by hand from Traylor Island, Texas (27° 56.883' N, 97° 4.289' W), were tethered in a turtle grass bed and over sand at Steadman Island, Texas (27° 53.123' N, 97° 7.017' 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 × 45 × 25 cm length × width × height, 1 cm mesh size) tethered seeds. For each replicate, 5 seeds were tethered in a turtle grass bed, 5 seeds were tethered over sand, 5 seeds were tethered and caged in a turtle grass bed and 5 seeds were tethered and caged over sand. Each seed was individually tethered to a plastic stake inserted into the sediment using 9-pound test monofilament fishing line. Seeds were tethered for 24 h, after which time 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).

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 cannot detect long-wavelength light (Cronin & 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 seed and seedling consumers and quantify consumption. Specific consumers were chosen based on animal and seed/seedling size and feeding mode. 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 (26° 07' 34.31" N, 97° 11' 17.02" W). One consumer was placed in an aerated 10 gallon (~37.9 l) aquarium with 2 cm of sand that was previously burnt for 5 h in a muffle furnace to remove all organic material and 5 or 10 pre-weighed turtle grass fruits or seedlings. The number of fruits or seedlings offered was determined from preliminary feeding experiments. Fruits were tethered to a buried fishing weight with 2–3 cm of 9-pound test monofilament fishing line to mimic natural fruit height above the substrate, and seedlings were placed directly on the sand substrate. After 24 h, 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. Preliminary experiments indicated that autogenic weight loss of fruit and seed tissue was minimal, so this was not included in final calculations. Potential consumers used in fruit feeding experiments included adult (>100 mm carapace width) and juvenile (<100 mm carapace width) blue crabs *Callinectes sapidus* and potential consumers in experiments with turtle grass seedlings included adult and juvenile blue crabs, spider crabs *Libinia* spp. and hermit crabs *Pagurus* spp.

(Table 1). Adult blue crabs were also used in feeding preference experiments aimed at determining if turtle grass fruits or seedlings are a preferred food item. In these experiments, each crab was offered 5 pieces of pre-weighed pinfish (*Lagodon rhomboides*) tissue and either 5 fruits or 5 seedlings approximating a similar weight as the fish (Table 1). Fish tissue is a known prey for blue crabs, and pinfish are abundant in sea-grass beds throughout the Gulf of Mexico (Darnell 1958).

All crabs used in feeding experiments were collected from local Texas seagrass beds where turtle grass is reproductive. Upon collection, crabs were fed fish ad libitum 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 wk and each crab was used only once.

### Seed growth after consumption

To assess whether consumption ends in seedling death or, alternatively, has the potential to enhance seedling dispersal, the fates of uneaten (control) turtle grass seedlings and partially eaten seedlings from laboratory feeding experiments with blue crabs were monitored in a laboratory growth experiment from 1 August 2011 to 26 September 2011. Six partially eaten seedlings and 6 uneaten seedlings collected from laboratory feeding experiments were kept individually in 100 ml containers with filtered seawater in a Percival I-36VL incubator at 30°C under a 12:12 h light:dark cycle. A digital photograph of each seedling was taken weekly to bi-weekly using a Canon SD 1200 camera and the longest leaf length (mm) on each seed was measured using ImageJ image processing and analysis software (ImageJ, National Institutes of Health).

Table 1. Experimental design of laboratory feeding experiments with turtle grass *Thalassia testudinum* reproductive structures. 'Number offered' refers to the number of reproductive structures offered to 1 potential consumer

Potential consumer	Reproductive structure	No. offered	Replicates
Adult blue crab <i>Callinectes sapidus</i>	Fruit	10	3
	Seedling	10	13
	Preference: Fruit vs. Fish	5 of each type	5
	Preference: Seedling vs. Fish	5 of each type	5
Juvenile blue crab <i>C. sapidus</i>	Fruit	10	3
	Seedling	10	14
Spider crab <i>Libinia</i> spp.	Seedling	10	5
Hermit crab <i>Pagurus</i> spp.	Seedling	5	6

### 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 for analysis of total C, N and P. 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). We 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 our 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. C and N content were measured on a Carlo-Erba EA 1108 Elemental Auto-Analyzer and P content was measured on a Shimadzu UV-2401 PC UV-VIS Recording Spectrophotometer following a modified protocol from Chapman & Pratt (1961). C, N and P 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.

### Statistics

On each of 3 different days, tethered seeds were placed in the field. Four treatment combinations were used: herbivore access (inside or outside a cage), factorially with 2 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. We pooled the results from the 3 dates and used a Fisher's exact test to compare herbivore access (inside versus outside a cage), pooling substrates for this test. We then 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 we 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. We

were then able to use ANOVA to analyze this new variable,  $\text{logit}(\text{final weight}/\text{initial weight})$ . Neither a log transformation nor a square-root transformation normalized the residuals; see Warton & 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 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 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 are presented as mean  $\pm$  SE.

## 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), 2 seedlings and 1 seedling were removed from uncaged turtle grass and sand tethers, respectively, during the second experiment (3 Sep-

tember 2013), and 2 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 ( $p = 0.15$ ). 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 02:11 and 09:55 h. Water temperature, salinity and pH were all within normal ranges for our study site and were consistent among experimental dates (mean  $\pm$  SE water temperature:  $32.66 \pm 0.10^\circ\text{C}$ , salinity:  $42.1 \pm 0.31$ , pH:  $8.63 \pm 0.12$ ).

### Laboratory feeding trials

Adult blue crabs ( $n = 3$ , carapace width: 105–185 mm) crushed  $70.0 \pm 11.5\%$  of offered fruits and consumed  $14.2 \pm 4.7\%$  of tissue in turtle grass fruit feeding trials. We observed that adult blue crabs *Callinectes sapidus* 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 ( $p = 0.24$ , Fig. 1) and consumed a similar percent of seedling tissue ( $p = 0.76$ , Fig. 1). Adult blue crabs ( $n = 13$ , carapace widths: 110–190 mm) crushed  $56.2 \pm 10.6\%$  of seedlings and consumed a total of  $35.0 \pm 9.3\%$  of seedling tissue. Juvenile blue crabs ( $n = 14$ , carapace widths: 40–90 mm) crushed  $77.9 \pm 5.9\%$  of offered seedlings and consumed a total of  $42.3 \pm 5.7\%$  of seedling tissue, and spider crabs ( $n = 5$ , carapace widths: 28–52 mm) crushed  $80.0 \pm 8.3\%$  of offered seedlings and consumed a total of  $36.9 \pm 6.02\%$  of turtle grass seedling tissue. Hermit crabs *Pagurus* spp. ( $n = 6$ ) 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 ( $n = 5$ ) consumed  $100 \pm 0\%$  of fish and  $45.3 \pm 12.5\%$  of fruit/seed tissue and crushed  $38.0 \pm 9.7\%$  of fruits. When offered fish and seedlings, crabs ( $n = 5$ ) consumed  $100 \pm 0\%$  of fish and  $21.1 \pm 13.9\%$  of seedling tissue and crushed  $18.0 \pm 11.1\%$  of seedlings.

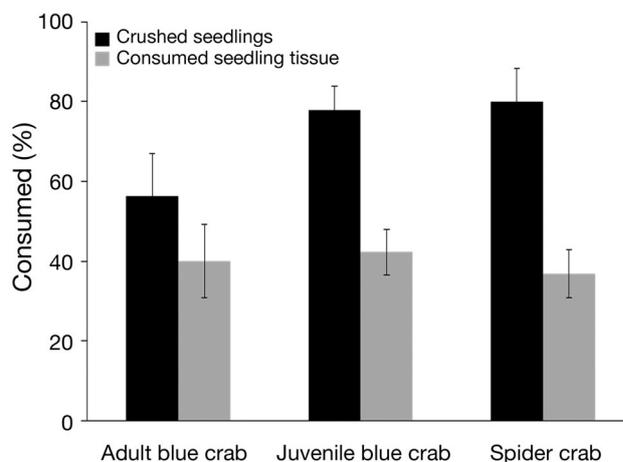


Fig. 1. Percent of crushed turtle grass *Thalassia testudinum* seedlings and seedling tissue consumed in laboratory experiments by adult blue crabs *Callinectes sapidus*, juvenile blue crabs and spider crabs *Libinia* spp. See Table 1 for number of seedlings offered and number of replicates. Values are means  $\pm$  SE

### Seedling growth after consumption

Control seedlings grown in the laboratory had significantly longer leaves than partially eaten seedlings (time:  $p < 0.001$ , type:  $p < 0.001$ , time  $\times$  type:  $p < 0.001$ ; Fig. 2). At the beginning of the growth experiment, longest leaf lengths for control and partially eaten seedlings were  $10.4 \pm 1.6$  and  $6.3 \pm 1.3$  mm, respectively. Leaves of control seedlings grew steadily and reached  $35.6 \pm 6.3$  mm by the end of the 2-month experiment. All but one of the partially eaten seedlings lost their leaves (Fig. 2). 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:  $p < 0.001$ ; %N:  $p < 0.001$ , %P:  $p < 0.001$ , Fig. 3). %N and %P in seeds were 2.5 and nearly 4 times the respective values for fruits. Seeds contained the highest C ( $40.5 \pm 0.3\%$ ), N ( $2.2 \pm 0.2\%$ ) and P ( $0.32 \pm 0.04\%$ ) content; leaves had intermediate C ( $32.2 \pm 0.3\%$ ), N ( $1.6 \pm 0.04\%$ ) and P ( $0.12 \pm 0.01\%$ ); and fruits had the lowest C ( $17.8 \pm$

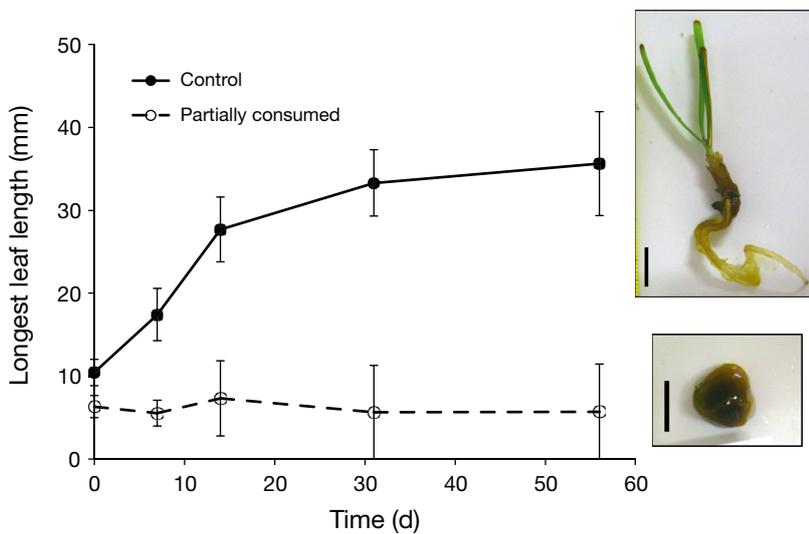


Fig. 2. Longest leaf lengths of control (uneaten) and partially consumed turtle grass *Thalassia testudinum* seedlings from laboratory feeding experiments with adult blue crabs *Callinectes sapidus*. Photographs are examples of control (top) and partially eaten (bottom) seedlings on the last day of the experiment. Values are means  $\pm$  SE;  $n = 6$ . Scale bars are 10 mm

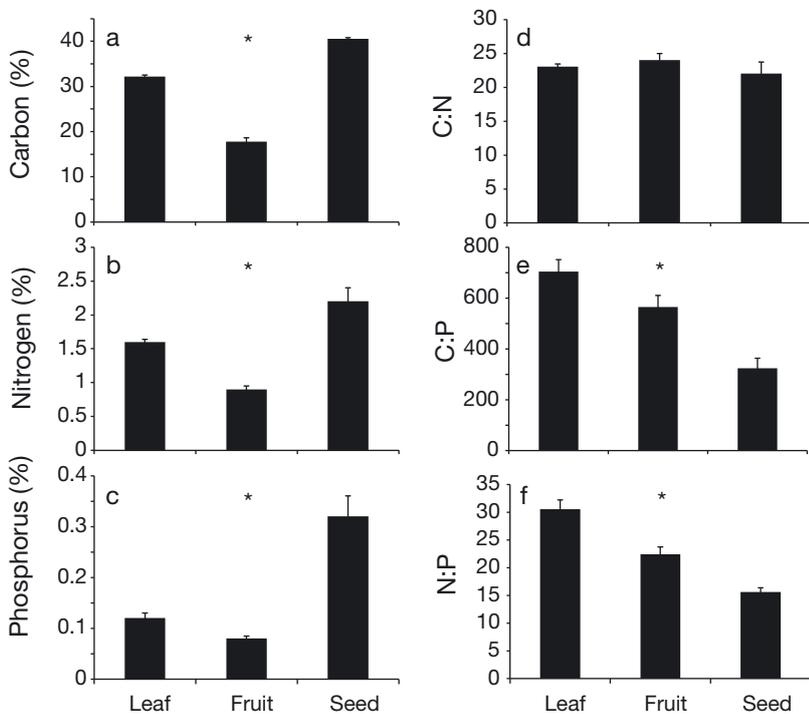


Fig. 3. Elemental composition of turtle grass *Thalassia testudinum* leaf, fruit and seed tissue. \*Indicates significant differences among the tissues. Values are means  $\pm$  SE;  $n = 5$

0.8%), N ( $0.9 \pm 0.05\%$ ) and P ( $0.08 \pm 0.005\%$ ) (Fig. 3a,b,c). Molar C:N ratios (ca. 22:1) were similar among tissue types ( $p < 0.29$ , Fig. 3d), but both C:P ( $p < 0.001$ , Fig. 3e) and N:P ( $p < 0.001$ , Fig. 3f) were

significantly different. Seeds had the lowest C:P ( $323.4 \pm 39.3$ ) and N:P ( $15.6 \pm 0.8$ ) ratios, which reflected their high phosphorus content. Fruit tissue had intermediate nutrient ratios (C:P:  $565.0 \pm 44.9$ ; N:P:  $22.4 \pm 1.3$ ) and leaves had very high ratios (C:P:  $704.2 \pm 46.3$ ; N:P:  $30.5 \pm 1.7$ ), 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 ( $p = 0.77$ ) (Fig. 4).

#### 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 2). Despite manual removal, seeds encased within fruits were still more nutritious than the fruits surrounding them. The %C ( $p < 0.001$ ), %N ( $p < 0.001$ ) and %P ( $p < 0.001$ ) were all greater for seed tissue than fruit tissue, and average %C, %N and %P were 2 to 3 times as high for seeds ( $41.1 \pm 0.2$ ,  $2.1 \pm 0.1$ ,  $0.29 \pm 0.01\%$ , respectively) compared to fruits ( $20.1 \pm 0.9$ ,  $0.95 \pm 0.06$ ,  $0.10 \pm 0.005\%$ , respectively). 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 ( $p < 0.001$ ) and N:P ( $p < 0.003$ ) ratios. Fruit C:P ( $565.5 \pm 28.5$ ) and N:P ( $22.1 \pm 1.5$ ) were higher than seed C:P ( $376.5 \pm 17.6$ ) and N:P ( $16.2 \pm 0.7$ ), reflecting the fruit's lower phosphorus content.

Table 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). Values are means  $\pm$  SE

	C (%)	N (%)	P (%)	C:N	C:P	N:P
Attached fruits	17.8 $\pm$ 0.78	0.87 $\pm$ 0.05	0.08 $\pm$ 0.005	24.03 $\pm$ 0.96	565 $\pm$ 44.9	23.5 $\pm$ 1.3
Manually removed fruits	20.9 $\pm$ 0.93	0.95 $\pm$ 0.06	0.1 $\pm$ 0.005	25.8 $\pm$ 0.64	565.5 $\pm$ 28.5	22.1 $\pm$ 1.5

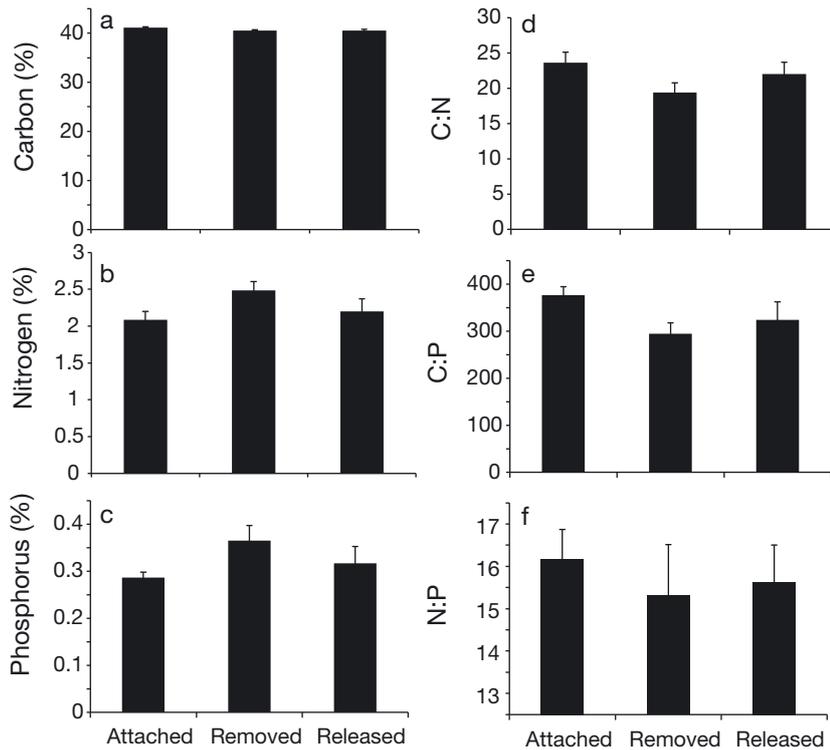


Fig. 4. Elemental composition of turtle grass *Thalassia testudinum* seeds within fruits attached to a shoot (attached, n = 5), seeds within fruits manually removed from a shoot (removed, n = 9) and mature seedlings naturally released from fruits (released, n = 5). Values are means  $\pm$  SE. There were no significant differences ( $p > 0.05$ ) in elemental composition between attached, removed, or released seeds

## DISCUSSION

Our results suggest that turtle grass *Thalassia testudinum* 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. Laboratory experiments demonstrated that common benthic crustaceans consumed turtle grass seeds and seedlings and that partial consumption of turtle grass seeds by blue crabs *Callinectes sapidus* led to seed-

ling death. Although our field results are not conclusive with respect to the significance of seed and seedling consumption 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 our laboratory experiments. Seed and fruit consumption by crabs has been widely documented for terrestrial plants (Wolcott & O'Connor 1992). Consumption by crabs is also known to limit mangrove density and distribution (Lindquist & Carroll 2004). In Australia, for example, grapsid crabs can consume up to 75% of mangrove propagules (Wolcott & 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 & Churchill 1988, Holbrook et al. 2000, Orth et al. 2006). Fishman & Orth (1996) reported that blue crabs,

specifically, are a primary consumer on *Z. marina* seeds in Chesapeake Bay. Blue crabs are common along the eastern and Gulf Coasts of the USA, 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, Louisiana, plant material contributes up to 11% of blue crab gut contents. Additionally, Alexander (1986) reported that 29% of blue crabs collected near Galveston Island, Texas, contained vascular plant tissue in their guts. In a pat-

tern consistent with opportunistic feeders, adult blue crabs in our study consumed seed and seedling tissue when offered no other food choice, but when given a choice, preferred pinfish *Lagodon rhomboides* tissue to fruits (and the seeds within) and seedlings. However, in these preference experiments the crabs still crushed and ingested plant tissue once all the fish had been consumed. Juvenile blue crabs (<100 mm carapace width) in our study ate turtle grass seedling tissue, but did not consume turtle grass fruit tissue. Similar to adult blue crabs, plant matter can make up a high percentage of gut contents in juvenile crabs (Seitz et al. 2011). In the present study, we 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 reports of increased *P. australis* seed consumption over seagrass structure compared to bare sand (Orth et al. 2006, 2007), we saw no difference in turtle grass seedling consumption between the substrate types. To avoid a large spatial separation between our substrate treatments and potential confounding factors such as varying depth and water flow, we placed our 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. We have observed broken turtle grass fruits attached to parent plants in turtle grass beds within Corpus Christi Bay, Texas, in July that mimicked fruits broken in our 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. We 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.

We also found that spider crabs *Libinia* 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 *Libinia* spp. is from Nova Scotia to the western Gulf of Mexico and these crabs are primarily found in muddy bottom and seagrass substrates. Our time-lapse photographs of a spider crab near the tethered turtle grass seedlings reinforces that spider crabs are active in our local seagrass beds.

Hermit crabs *Pagurus* spp. did not eat turtle grass fruits or seedlings 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 our 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 5 h in a muffle furnace prior to the experiment to remove all organic material.

Time-lapse photography revealed pinfish near our 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 & Livingston 1984). Kaldy & Dunton (1999) reported that pinfish picked up and spat out turtle grass seedlings in Lower Laguna Madre. 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. Passage through an animal's gut can aid in seed dispersal and/or enhance seed germination success (Lovett-Doust & Lovett-Doust 1988). Following the fate of eaten seeds can eliminate any confusion between seed loss by consumption and ingestion that enhances dispersal (Janzen 1971, Chapman 1989, Andresen 1999). In our seedling growth experiments, all but one of the partially consumed turtle grass seedlings lost their leaves and died, whereas uneaten seedlings grew steadily throughout the experiment. By the end of the experiment, leaves on the remaining partially eaten seedling were only 14% as long as leaves on the uneaten seedlings. 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 seed or seedling consumption by crabs would end in the seed or seedling's 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 (van Dijk 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 & 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.

#### 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 elevated nutrients relative to the parent plants to aid in seedling establishment and development (Tyler & Zohlen 1998). P, 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 & Zohlen 1998). C, N and P content in leaves from fruit-bearing shoots is low compared to normal leaf C ( $35.88 \pm 2.47\%$ ), N ( $2.09 \pm 0.28$ ) and P ( $0.16 \pm 0.03\%$ ) in seagrass from this area (Wilson & Dunton 2012), likely because of the maternal resources invested in the developing seeds (Reekie & Bazzaz 1987).

Turtle grass seedlings become photosynthetically self-sustaining between 2 and 6 mo after dehiscence from the fruit, but prior to that rely on reallocation of carbon resources within the seedling (Kaldy & Dunton 1999). Our higher overall C levels in seeds and recently released seedlings compared to adult leaf tissue supports this notion. Adult seagrass plants can actively absorb N and P through both leaves and roots (Lee & Dunton 1999). However, the relative nutrient uptake ability of different tissues in turtle grass seedlings is unknown. Without a substantial root system to uptake 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. (2014) reported that *P. australis* seedlings rely on internal nutrient stores for up to 4 mo after germination, and Hocking et al. (1980) reported a linear rela-

tionship between loss of N and P and seedling dry matter in the first 9 mo following germination. The similar nutrient content we 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 we analyzed, fruits had the lowest N and P 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 P is likely most important. Although the percentages of both N and P 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). Goecker et al. (2005) found that parrotfish (*Sparisoma radians*) detect elevated N levels in turtle grass leaf content 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 N-rich areas throughout Discovery Bay, Jamaica, preferentially ate turtle grass enriched in P. We 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

Turtle grass seed and seedling loss in our field and 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 suggest that seed and seedling consumption can be an important factor regulating successful recruitment of turtle 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 may impart an under-recognized pressure on sub-tropical seagrass populations.

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