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Reproductive Ecology and Phenology of *Thalassia testudinum (*Hydrocharitaceae) in Tampa Bay, Florida

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Conservation Biology
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ABSTRACT

Successful sexual reproduction and recolonization are critical in maintaining genetic diversity within seagrass meadows. *Thalassia testudinum* flower, fruit and seedling production were assessed visually at 10 sites within Tampa Bay, Florida approximately every 4 weeks from May to July 2017 to determine if there is spatial and temporal variation in sexual reproductive effort and if location within a meadow effects sexual reproductive effort. Results from this study reveal strong temporal variability throughout the reproductive season. The month of May was observed as peak *Thalassia testudinum* anthesis and June was peak fruiting in Tampa Bay during 2017. Flower and fruit density ranged from 0.19 ±0.75 to 0.54 ± 1.38 per 0.25m^2 and 0 to 0.59±1.550 per 0.25m^2, respectively. Highest and lowest flower densities were observed at CK03 and CB02, respectively and highest and lowest fruit densities were observed at CK01 and both CB02 and WD07, respectively. Mean reproductive effort ranged from 0.51±1.82 0.25m^2 to 1.92±4.86 0.25m^2 with the lowest at JB04 and the highest at EK01. Additionally, results suggest site location, water quality, seagrass density, and localized reproductive variability affect sexual reproductive effort and success. Only 7 seedlings were observed during the survey suggesting that asexual reproduction is the dominant form of meadow growth and expansion in Tampa Bay. These results also suggest that while acreage has reached target goals set forth by the Tampa Bay Estuary Program in the early 1990's, these meadows have not fully recovered and may be susceptible to natural and anthropogenic stressors.

INTRODUCTION

1.1 Background

Seagrasses are marine angiosperms located throughout the coastal and estuarine regions of the world. These meadows are an essential component in the fragile mosaic of the interconnected and interdependent seascape (Moberg and Ronnback, 2003). Seagrass habitats reduce erosion, assist in nutrient cycling, and provide habitat, forage, and nursery grounds for many commercially and economically important fishes during critical periods of their life cycle (Grey and Moffler, 1978; Phillips et al., 1981; Steffe et al., 1989; Jones et al., 1994; Nagelkerken et al., 2002; Moberg and Ronnback, 2003; Duffy, 2006; Orth et al., 2006; Blair et al., 2014; Fulford et al., 2016; Yarbro and Carlson, 2016; Boucek et al., 2017). These submerged grasslands are among the most productive of ecosystems and provide essential cultural, ecological, economical goods and services equated to over $1.9 trillion (Costanza et al., 1997; Orth et al., 2006, Waycott et al., 2009). In Florida alone, the ecosystem services yielded by seagrasses beds are estimated at $20 million dollars annually and it has been suggested that one in five incomes in the Tampa Bay watershed is entirely dependent on healthy bay habitats (Yarbro and Carlson, 2016; Sherwood et al., 2017). However, these heavily relied-upon services are contingent on habitat health and water quality, stimulating the need for global monitoring and conservation efforts (Fulford et al., 2016).

1.2 Marine Habitat Degradation

While seagrass beds cover over 175,000 km^2 globally and occur on every continent except Antarctica, they are often overlooked because they occur underwater (Dawes, 1998; Orth et al., 2006; Waycott et al., 2009; Kendrick et al., 2012; Lamb et al., 2017). In Florida, seagrass ecosystems cover 2.2

million acres extending from the northwestern border of the Panhandle to the Dry Tortugas (Yarbro and Carlson, 2016). However, seagrass ecosystems are generally classified as “poor and declining” and it is estimated that seagrass cover around the world has declined by 29 percent (Waycott et al., 2009; Barbier et al., 2011; Macreadie et al., 2013). Located near the shore, these fragile ecosystems are under significant stress from terrestrial pollution and anthropogenic growth (Waycott et al., 2009; Fulford et al., 2016). Seagrass meadows are also subject to fragmentation from boat propeller scars and biodiversity loss from overfishing, producing changing dynamics of species interactions (Bell et al., 2001; Hughes and Stachowicz, 2004; Orth et al., 2006). Furthermore, nutrient runoff from fertilizers promote light-limiting algal blooms, a scenario which can be devastating to seagrass beds (Moberg and Ronnback, 2003; Papenbrock, 2012; Macreadie et al., 2013).

Further concern for this phytocoenosis is the low taxonomic diversity within the group. There are only four marine families separated into 72 species which constitute only about two-one hundredths of one percent of all angiosperms worldwide (Kendrick et al., 2012). The limited taxonomic diversity of seagrass makes them particularly susceptible to extinction and a priority for ecosystem conservation.

1.3 Sexual Reproduction

Florida seagrass meadows are primarily composed of 4 species: *Thalassia testudinum Banks & Sol. ex K.D. Koenig* (turtle grass), *Halodule wrightii Asch.* (shoal grass), *Syringodium filiforme Kütz.* (manatee grass), and *Halophila engelmannii Asch.* (star grass). Turtle grass, arguably the most dominant seagrass species in the Gulf of Mexico and Caribbean, can reproduce sexually, through underwater flower production, or asexually through the extension of rhizomes and development of clonal ramets, also referred to as short shoots (Tomlinson and Vargo, 1966; Durako and Moffler, 1985; Duarte et al., 1994). Vegetative reproduction by the extension of underground rhizomes or horizontal stems has been assumed to be the dominant method of propagation, however recent use of genetics combined with frequent observations of energetically expensive flowers suggest sexual life stages play important roles in meadow establishment, expansion, and maintenance (Durako and Moffler, 1985; Kendrick et al., 2012).

Thalassia testudinum is dioecious and the gender of each plant can easily be distinguished while reproductively active due to visibly distinct pistillate and staminate flowers (Orpurt and Boral, 1964; Tomlinson, 1969; Grey and Moffler, 1978; Durako and Moffler, 1985b; Durako and Moffler, 1987; Witz and Dawes, 1995). Although studies suggest *Thalassia testudinum* can flower year round in Mexico and the Caribbean Sea, plants living along the Florida Gulf Coast is primarily undergo sexual reproduction from April to July with peak anthesis in early June (Orpurt and Boral, 1964; Tomlinson, 1969; Zieman, 1974; Grey and Moffler, 1978; Moffler et al., 1981; Phillips et al., 1981; Cox and Tomlinson, 1988; Witz and Dawes, 1995).Temperature, salinity, and photoperiod are proposed as significant environmental cues initiating sexual reproduction (Zieman, 1975; Moffler et al., 1981; Phillips et al., 1981; Durako and Moffler, 1985b, 1985c; Durako and Moffler, 1987; Cox and Tomlinson, 1988). Additionally, *Thalassia testudinum* short shoots are thought to reach sexual maturity at about 2 years of age or following production of 15-25 leaves, and therefore, age might also be a biotic factor limiting sexual reproduction (Gallegos et al., 1992; Witz and Dawes, 1995; McDonald et al., 2016).

During summer spring tides, when the staminate flower is fully mature, it undergoes anthesis. Nocturnal, synchronized pollen release has been observed throughout the Gulf of Mexico and Caribbean Sea (Cox and Tomlinson, 1988; van Tussenbroek et al., 2012; van Tussenbroek et al., 2016a). Unlike male flowers, pistillate flowers undergo anthesis during the day. Pollination in *Thalassia testudinum* was originally considered to be entirely hydrophilous, however, *in situ* video recording and mesocosm experiments revealed zoobenthophilous fertilization (Orpurt and Boral, 1964; Durako and Moffler, 1985b; Cox and Tomlinson, 1988; van Tussenbroek et al., 2012; van Tussenbroek et al., 2016a).

If fertilization is successful, seeds develop within a green capsule fruit which can grow to 20-25 mm in diameter and hold 1-6 seeds (Orpurt and Boral, 1964; van Tussenbroek et al., 2016a). Following fruition, the fruit will dehisce and release negatively buoyant seedlings. Because of vivipary, seedlings root in about three days (Orpurt and Boral, 1964; Cox and Tomlinson, 1988; Darnell and Dunton, 2016; van Tussenbroek et al., 2016a). During periods of significant turbulence, buoyant immature fruits may break off from the parent plant and travel large distances by wind and currents (Orpurt and Boral, 1964;

Witz and Dawes, 1995; Darnell and Dunton, 2016; McDonald et al., 2016; van Dijk et al., 2018). This enables substantial spatial distribution, genetically linking populations over long distances. However, erratic and unpredictable long-range dispersal events make the process extremely difficult to study.

Sexual reproduction is essential in maintaining genetic diversity and plasticity, components of species health. High seagrass genetic diversity increases resistance and resilience to natural and anthropogenically-initiated stressors (Hughes and Stachowicz, 2004; Orth et al., 2006; Kendrick et al., 2012). Furthermore, genetic diversity within the phytocoenosis can be paralleled by elevated faunal biodiversity (Hughes and Stachowicz, 2004). Therefore, it is essential to fully understand seagrass reproductive status and develop strategies to preserve the high ecosystem biodiversity.

1.4 Tampa Bay Reproductive Studies

A few studies of *Thalassia testudinum* sexual reproduction were done in the 1970s and 1980s in Tampa Bay (Grey and Moffler, 1978; Phillips et al., 1981; Durako and Moffler, 1985a, 1985b; Witz and Dawes, 1995). Unfortunately, there was a lack of standardized sampling procedures among researchers which resulted in conflicting flower frequency information (Orpurt and Boral, 1964; Tomlinson, 1969; Grey and Moffler, 1978). Much of the data on sexual reproduction of *T. testudinum* in Tampa Bay were collected during a period of poor water quality and significant ecological degradation (Orpurt and Boral, 1964; Tomlinson, 1969; Grey and Moffler, 1978; Phillips et al., 1981; Durako and Moffler, 1985a, 1985b, Durako and Moffler, 1987; Witz and Dawes, 1995; Greening and Janicki, 2006; Greening et al., 2014; Sherwood et al., 2017). By 1982, seagrass beds had lost 7,200 ha. in Tampa Bay, and many beds were in poor health and in severe decline. These conditions might have resulted in reduced sexual reproduction (Greening and Janicki, 2006; Greening et al., 2016). In response to community action and diligent nutrient load reduction efforts from governmental agencies, water quality improved, but seagrasses were slow to responded. In 2006, all segments in the Tampa Bay estuary met the water quality standards of the Tampa Bay Estuary Program. However, it wasn’t until 2014 that seagrass acreage surpassed the restoration targets of 38,000 acres set by the Tampa Bay Estuary Program in 1996 (Greening et al., 2016;

Sherwood et al., 2017). While acreage provides information about where seagrass meadows are located, it fails to reveal the genetic diversity, reproductive capacity, and overall health of the seagrass populations.

Species health is clearly aligned with successful sexual reproduction and genetic diversity which necessitates investigations of sexual reproductive effort. Additionally, the drastic ecological changes that have occurred in the Tampa Bay estuary since the original reproductive surveys require a modern investigation into the reproductive ecology of *Thalassia testudinum* seagrass meadows in Tampa Bay. This study of clonal ramets provides modern information about the reproductive ecology and health of *Thalassia testudinum* populations in the recently recovered seagrass meadows of Tampa Bay and answers critical questions regarding sexual reproduction in a marine habitat.

1.5 Objectives

Specifically, this thesis focused on three specific questions regarding *Thalassia testudinum* flower, fruit and seed production during the 2017 reproductive season at 10 historically studied locations in the Tampa Bay estuary. This study tested the following hypotheses: 1) There is spatial variation in sexual reproductive effort and success in Tampa Bay; 2) Location of short shoots within a seagrass meadow mosaic influences reproductive effort; and 3) Reproductive effort varies temporally throughout the reproductive season.

1.5.1 Thesis Statement

In this research, I examined the sexual reproductivity capacity of *Thalassia testudinum* in Tampa Bay in 2017 and compared it to previous, less extensive surveys in the late 1970’s and 1980’s.

1.5.2 Implications of research

Observations and calculations from this research provide modern information on the reproductive ecology of *Thalassia testudinum* in Tampa Bay, Florida. It establishes standardized reproductive seagrass sampling procedures for Tampa Bay. Completion of this project provided a better understanding of the implications of gender ratios, sexual reproductive effort and success, and spatial variation of reproductive effort and success in Tampa Bay. Because successful reproductive effort provides evidence for seagrass health, this research provides evidence that seagrass ecosystems in Tampa Bay are continuing to recover and is vulnerable to natural or anthropogenic disturbances. Finally, with sea level rise and climate change impending, results from this research provide guidance on which areas of the Tampa Bay estuary should be prioritized for increased management actions.

METHODS

2.1 Site Overview

2.1.1 Site History

Aerial photography collected in the 1950s showed that Tampa Bay had approximately 40,000 acres of seagrass (Johansson et al., 2018). By the mid-1980s, more than half of the seagrass had been lost due to improper nitrogen and sewage management which resulted in large algal blooms, poor water clarity, and light stress (Greening et a1., 2014; Greening et al., 2016). Most significant losses occurred in northern segments of the bay; however, all segments did lose seagrass habitat during the time period (Robison et al., 2020). Through collaborative efforts over the past thirty years by Tampa Bay communities, municipalities and industries, water quality has improved and phytoplankton and macroalgal abundance have declined. Initially, seagrass was slow to respond to enhanced water clarity, but, in 2014 the Southwest Florida Water Management District (SWFWMD) reported seagrass acreage had surpassed what it was in the 1950s. Although the Tampa Bay area still battles with nutrient runoff from storm events, seagrass acreage continues to surpass historic values and most segments reliably meet water quality standards. Tampa Bay is considered a restored estuary and a leader in estuary restoration throughout the country.

2.1.2 Site Description

Tampa Bay is a shallow estuary extending from N28°03’ to N27°30’ and considered the largest open water estuary in Florida with about 400 square miles ($1{,}000^2$ km) of water. The watershed includes over 2,200 square miles ($6{,}700\ km^2$) and receives water from Hillsborough, Pinellas, Polk, Pasco and

Manatee Counties (Johansson et al., 2018). The watershed is mainly urban, but also includes phosphate mining and agricultural activities (Johansson et al., 2018). There are two active ports in the region, the Port of Tampa and Port Manatee, and deep-water channels that enable transit of large ships are occasionally dredged. Freshwater flows into the bay from four major rivers including the Hillsborough River, Little Manatee River, Alafia River, and the Manatee River.

Due to the size, shape, and hydrography of Tampa Bay, it is commonly divided into 4 subregions which include Old Tampa Bay in the northwest region of the bay, Hillsborough Bay in the northeast region, Middle Tampa Bay, and Lower Tampa Bay which extends to Egmont Key (Figure 1). *Thalassia testudinum* is the dominant seagrass in both Lower and Middle Tampa Bay and is common in Old Tampa Bay.

This study assessed sexual reproductive effort and success by measuring flower, fruit, and seedling production at 10 sentinel sites characterized by well-established and persistent seagrass beds dominated by *Thalassia testudinum*. These sites are located in three segments of Tampa Bay: Lower Gandy Flat (GF01) and Weedon Island (WD07) in Old Tampa Bay, Coquina Key (CK01; CK03) and Cockroach Bay (CB02; CB06) in Middle Tampa Bay, and Joe Bay (JB03; JB04) and Egmont Key (EK01; EK03) in Lower Tampa Bay (Figure 1, Table 1). Most sites had a mean depth of <1.5 meters. At Coquina Key, also referred to as Coquina Key Flat, CK01 was shallow (z <1.5m) and CK03 was deep (z>1.5m). These sites were chosen because they represent different regions of Tampa Bay. Many had been surveyed for sexual reproductive effort by previous studies (Grey and Moffler, 1978; Moffler et al., 1981; Phillips et al., 1981; Durako and Moffler, 1985a, 1985b; Durako and Moffler, 1987; Witz and Dawes, 1995).

2.2 Reproductive Survey

Reproductive ecology of *Thalassia testudinum* was assessed visually at 10 sites approximately every 4 weeks beginning in May 2017 and continuing through July 2017 to capture peak anthesis (Orpurt and Boral, 1964; Grey and Moffler, 1978; Durako and Moffler, 1985). All sites were surveyed over four-day time intervals to maintain quality assurance. At each of the 10 sites, a minimum of 40 quadrats

($25x25\ cm^2$) were randomly dropped within the seagrass beds where *Thalassia testudinum* was present. Random quadrats were chosen in lieu of transects to provide more spatial coverage within a seagrass bed. For each quadrat, observers recorded where in the seagrass bed the quadrat was located (interior seagrass bed or edge of bed), percent basal cover of each seagrass species present (1-100%), and reproductive effort or the count of *Thalassia testudinum* reproductive structures observed. The reproductive stage of shoots could vary in a quadrat; therefore, observations were recorded based on a categorical system implemented by Grey and Moffler (1978). This system records reproductive effort though categorization of reproductive units into a six-phase developmental sequence: bud, anthesis (denoting flower gender), post-anthesis, fruit, fruit dehiscence, and seedling (Grey and Moffler, 1978). Abundance of *Thalassia testudinum* reproductive structures in each phase and gender of every observed open flower was recorded for each quadrat. Reproductive effort was determined following the survey and was defined as the sum of all reproductive structures within a quadrat. Included in the calculation are buds, flowers in anthesis and post-anthesis, fruits, dehisced fruits, and seeds. Although no longer reproductively active, dehisced fruits were included in the calculation because they degrade rapidly and signal very recent seed release. Flower density included buds and open flowers. Because male flowers degrade rapidly after pollen release, post-anthesis structures were not included in the analysis. Gender determination was based on floral morphological features as outlined by Orpurt and Boral (1964) and Tomlinson (1969) (Figure 2). To maintain quality assurance, all observers initially surveyed 5 quadrats together for visual calibration of percent basal coverage and reproductive effort prior to data collection. Furthermore, GoPro photos were taken at each site to create a physical documentation of the flowering regime and to document fine scale textural differences among locations (Figure 3).

In addition to visual flowering surveys, three measurements of salinity, temperature, dissolved oxygen concentration, and pH of the overlying bottom water were collected using a Eureka Manta-2 model sub2 water quality sonde. Water samples were also collected for laboratory analysis using DEP Standard Operating Procedures (https://floridadep.gov/dear/quality-assurance/content/dep-sops). Optical water quality analyses included turbidity (ntu), color (tcu), and chlorophyll-a (mg/L), however they were not included in the analysis of this study.

2.3 Statistical Analysis

Analyses were performed to examine differences in reproductive effort, including flowering, fruiting, dehiscence, and seed set, among site locations, seagrass bed location, and survey month. All data were initially assessed for homogeneity of variance with the Barttlet Test which revealed that the variance of the count data was not approximately equal to the mean. To account for heteroscedasticity with overdispersion, negative binomial regression models were developed to assess variation in the counts of reproductive stages observed among sites, short shoot location, seagrass densities, and month to determine the strongest predictor of reproductive effort and success (Table 2). Negative binomial regressions included a loglink function, which is common among count analyses. Models were then ranked using the statistical package MuMin (https://cran.r-project.org/web/packages/MuMIn/index.html), which develops and ranks models by weights through the calculation of AICc (Akaike's Information Criterion). The lower the AICc value, the greater the model weight and support for that model based on the data (Akaine, 1973; Hurvich and Tsai, 1989). Inferences were made on negative binomial regression models with cumulative AICc weights of 0.95 (Burnham and Anderson, 2002). Models within the 95% confidence model set were then assessed for goodness of fit (Burnham and Anderson, 2002). Goodness of fit for the greatest weighted model(s) were determined using the R package DHARMA (https://cran.r-project.org/web/packages/DHARMa/index.html), which stimulates residuals from generalized linear models and conducts residual diagnostic tests that can be interpreted similar to residuals from a linear regression. Models were considered a good fit if $p>0.05$, dispersion ~1, indicating predicted and observed residuals approximately aligned. Post-hoc tests were then performed on models to determine differences between predictor variables. Estimates, standard errors and 95% confidence intervals were generated by the emmeans package (https://cran.r-project.org/web/packages/emmeans/index.html). Inferences were based on effect sizes of predictor variables from the confidence model sets generated during model ranking and strong evidence for that effect was considered if the 95% confidence interval did not overlap zero. All statistical analyses were run using packages downloaded through R Studio version 3.6.1.

2.4 Tables and Figures

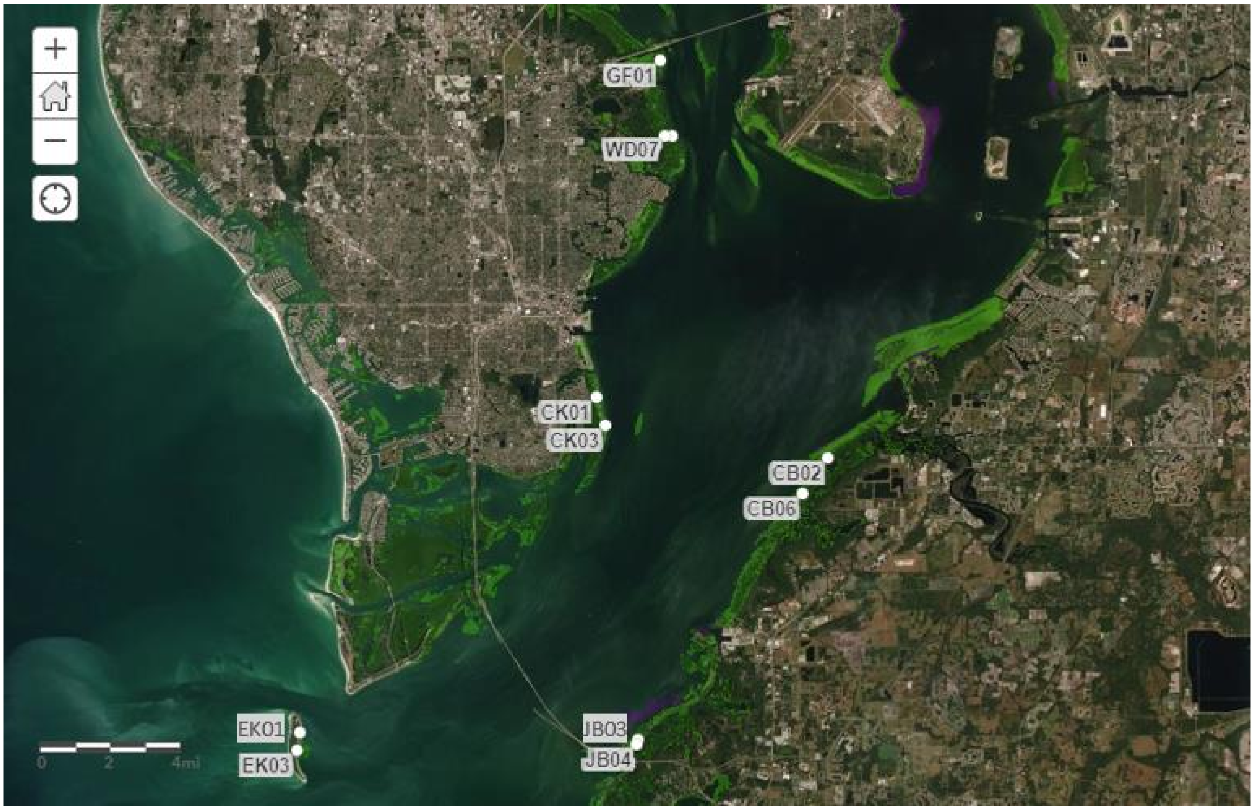


Figure 1. *Map of sites surveyed for Thalassia testudinum short-shoot sexual reproduction in Tampa Bay, Florida. Sites were surveyed once monthly from May 2017- July 2017 and were chosen based on historical site locations.*

Table 1*. List of site locations surveyed during the summer of 2017.*

Bay Segment	**Region**	**Site Name**	**Latitude**	**Longitude**
Old Tampa Bay	Lower Gandy Flat	GF01	27.87054	-82.591
Old Tampa Bay	Weedon Island	WD07	27.83975	-82.5888
Middle Tampa Bay	Coquina Key	CK01	27.73262	-82.6208
Middle Tampa Bay	Coquina Key	CK03	27.72112	-82.6268
Middle Tampa Bay	Cockroach Bay	CB02	27.70768	-82.5137
Middle Tampa Bay	Cockroach Bay	CB06	27.69289	-82.5254
Lower Tampa Bay	Joe Bay	JB03	27.59221	-82.6018
Lower Tampa Bay	Joe Bay	JB04	27.59026	-82.6021
Lower Tampa Bay	Egmont Key	EK01	27.59507	-82.7581
Lower Tampa Bay	Egmont Key	EK03	27.58788	-82.7596

Figure 2*. Visual representation of female (left) and male (right) Thalassia testudinum flowers observed in Tampa Bay, Florida.*

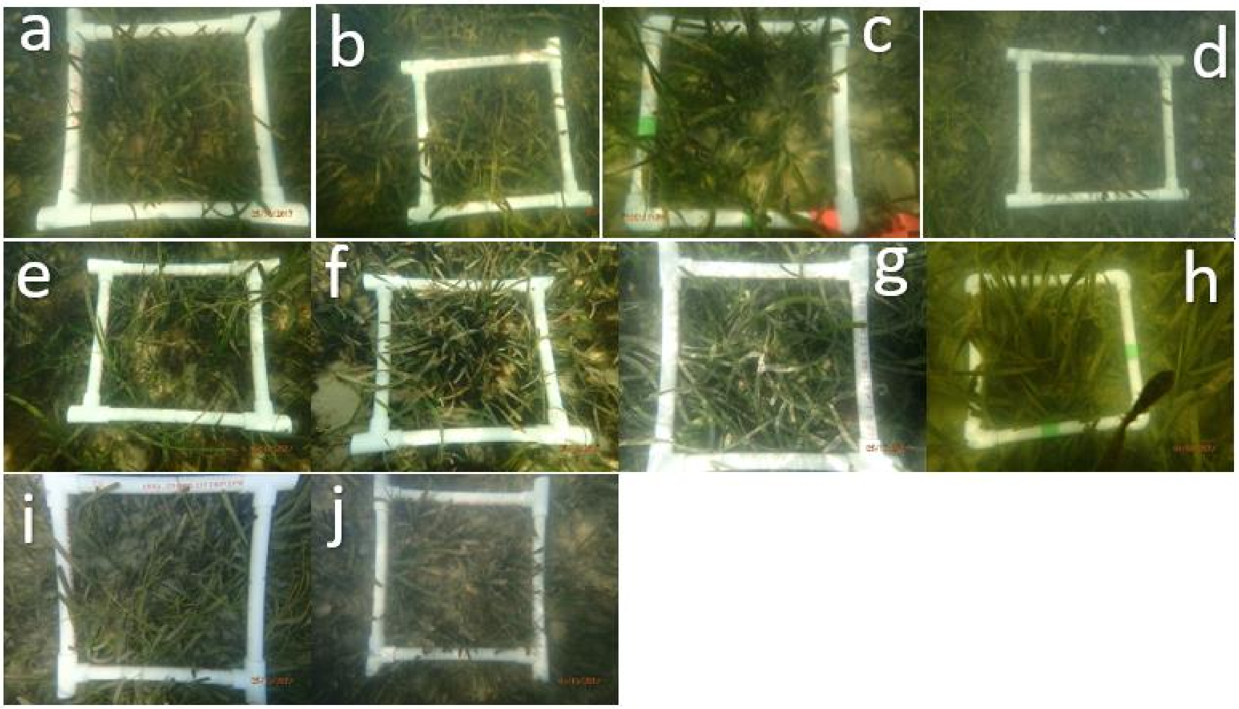


Figure 3. *Collage of images representative of the diversity and density and fine textural difference at each location surveyed in Tampa Bay, Florida. Images were taken at the following locations: a- GF01; b- WD07; c- CK01; d- CK03; e- CB02; f- CB06; g-JB03; h- JB04 i- EK01. All photos were taken during the 2017 survey period.*

Table 2*. Summary of negative binomial models developed to assess variation in Thalassia testudinum sexual reproductive effort and success in Tampa Bay. Site denotes survey location, %Cover and %Cover^2 denote seagrass density, quadrat location denotes location of quadrat within a seagrass bed, and month denotes survey month.*

Reproductive Variable	Model Number	Predictors	WT[a]	FM[b]
Reproductive Effort	m1	Quadrat location	T12, TA1	
	m2	Cover%	T12, TA1	
	m3	Cover%+ Cover%^{2}	T12, TA1	
	m4	Site	TA1	
	m5	Site& Quadrat Location	TA1	T5, F5
	m6	Site+ %Cover	TA1	
	m7	Site+ Cover%+ Cover%^{2}	TA1	
	rm	Month	TA1	
	rm	Month + Site	TA1	F11
Flower Density	fm12	Site& Quadrat Location	T6, TA1	
	fm3	Site+ Cover%+ Cover%^{2}	T6, TA1	F7
	fm1	Quadrat location	T6, T12, TA1	
	fm2	Site+ %Cover	T6, TA1	
	fm13	Site	T6, TA1	
	fm6	Cover%	T6,T12,TA1	
	locfm3	Cover%+ Cover%^{2}	T6, T12, TA1	
	fmm1	Month	TA1	
	fmm2	Month + Site	TA1	F12
Fruit Density	fruit1	Site	T7	
	fruit2	Site+ %Cover	T7	
	fruit3	Site+ Cover%+ Cover%^{2}	T7	
	fruit4	Cover%+ Cover%^{2}	T7	
	fruit5	Cover%	T7	
	fruit6	Quadrat location	T7	
	fruit7	Site& Quadrat Location	T7	T8, F8
	fruitm1	Month	TA1	F14
	fruitm2	Month + Site	TA1	

FM[b]=Full model summaries and/or goodness of model fit graphs are reported in the tables (T) or Figures (F) listed
WT[a]=Table (T) that reports model ranking

RESULTS

3.1 Environmental Data

Mean salinity at locations surveyed in Tampa Bay ranged from 28-34psu, but the mean salinity declined at all locations throughout the sample period, May through July (Table 3). The greatest ranges in salinity were recorded at Cockroach Bay sites CB02 and CB06; these ranges were 4.0 and 3.8, respectively. The lowest ranges in salinities were recorded at Coquina Key locations CK01 and CK03, with ranges of 0.9 and 1.3, respectively (Table 3). Mean temperatures ranged from 29-33 °C during the summer of 2017, but highest mean temperature was recorded at CK01 (Table 3).

3.2 Reproductive Effort Among Site Locations

Locations surveyed had a percent basal seagrass coverage ranging from 6.91±3.78 to 12.83±6.78, with the lowest seagrass coverage at CB06 and greatest coverage at EK01 (Table 4). All locations sampled during the survey in 2017 had reproductively active short shoots (Table 4).

Mean reproductive effort was highly variable among and within locations surveyed in Tampa Bay during the sampling period. Sexual reproduction was often isolated into small, dense patches of reproductively active shoots. This variability is demonstrated by the high frequency of outliers (Figure 4). Mean reproductive effort ranged from 0.51±1.82 to 1.92±4.86 (Table 4). Negative binomial regression models were run to determine if reproductive effort could be predicted by percent cover, location of the short shoot within the seagrass mosaic, and site location. The model with the greatest weight (AICc=3021.6, weight= 0.960) indicated that both site and location influenced reproductive effort. This model revealed there was significantly higher reproductive effort of *Thalassia testudinum* at CK01(est=1.3729; SE=0.321; 95%Ci=0.357, 2.3888), CK03(est=1.2749; SE=0.327; 95%Ci=0.2391, 2.3106), EK01(est=1.5799; SE=0.337; 95%Ci=0.514, 2.6457), EK03(est=1.1427; SE=0.321; 95%Ci=0.1282, 2.1571), JB03(est=1.3619; SE=0.339; 95%Ci=0.2895, 2.4343), and GF01(est=1.1845; SE=0.332; 95%Ci=0.1332, 2.2357), than at JB04 and higher reproductive effort at EK01 than WD07 (est=1.1427; SE=0.306; 95%Ci=0.1033, 2.0375)(Table 5). When the model was assessed for goodness of fit, residual plots indicated that there was significant deviation in observed values compared to expected values. While the dispersion test was not significant (p=0.074), the model is not considered a good fit (Figure 5).

3.2.1 Flower Density

Flowers were observed at all sites surveyed during the summer of 2017 (Table 4). The range of mean flower occurrence was 0.19±0.75 to 0.85±3.56 per 0.25 m^2, with the greatest mean flowering at GF01 and the lowest at CB02 (Table 4). GF01 also had the greatest number of flowering short shoots observed in a single quadrat which displays spatial variability of sexual reproduction in seagrass mosaics (Figure 6).

When site and site characteristics were assessed, model selection results indicated support for two models, site and location of short shoot within the seagrass bed mosaic (AICc=1837.1, weight=0.407) and also percent basal cover and site as strong predictors of flower density (AICc= 1837.2, weight=0.375) (Table 6). Post hoc model results of the model testing predictor variables site and locations determined flower effort at GF01 to be significantly greater than at WD07 (est=1.5703; SE=0.463; 95%Ci=0.105, 3.035). Goodness of fit and dispersion tests for indicate that this model is a good fit for predicting flower density (p=0.136) (Figure 7A). A post hoc analysis was also computed for the other model weighted highly in the confidence model set. Results from this model also indicated that when averaged over percent seagrass coverage, WD07 produced reduced flower quantities than GF01 (est=-1.689; SE=0.462; 95%Ci=0.227, 3.153). Interestingly, this model determined percent cover and percent $cover^2$ to be also significant predictors of flower density (z=2.685, p= 0.00725 and z=-3.240, p=0.0012, respectively). The residual plots of this also resulted in a good of fit (p=0.489) (Figure 7B).

Gender ratios varied greatly among locations surveyed. Male to female ratios ranged from 33 to 0.06, with the greatest disparity of sexes at EK01 (33), GF01 (18), and WD07 (0.06) (Table 4). CK01 had a male to female ratio closest to 1 (Table 4, Figure 16). Although the ratios vary among sites, flowers from both sexes were observed at all locations during the survey (Figure 16). However, at most locations, sexes were isolated and rarely were found coexisting in the same quadrat.

3.2.2 Fruit Density

Thalassia testudinum fruits were observed at all locations surveyed except CB02 and WD07 (Table 5). Densities of fruits were extremely low and ranged from only 0.01±0.09 per 0.25m^2 to 0.59±1.55 per 0.25m^2. Sites located on the Coquina Key seagrass flat, CK01 and CK03, had the greatest fruit density of all locations with fruit density of 0.59±1.55 per 0.25m^2 and 0.39±1.32 per 0.25m^2, respectively (Table 4). The large standard deviations and many outliers display the patchiness of fruit production observed throughout Tampa Bay sites (Table 4, Figure 8).

Models were ranked to determine what combination of site and site characteristics were strongest predictors of fruit production. Like reproductive effort and flower density, the model selection output determined site and location within the seagrass meadow as the strongest predictor of fruit density (Table 7, AICc= 819.1; weight= 0.802). Coquina Key flat short-shoots at CK01 and CK03 produced significantly more fruits than short-shoots at sites EK03 (est=2.035; SE=0.450; 95%Ci=0.6, 3.5 and est=1.53; SE=0.470; 95%Ci=0, 3.0, respectively), JB03 (est=3.013; SE=0.670; 95%Ci=0.9, 5.1 and est=2.508; SE=0.670; 95%Ci=0.3; 4.7, respectively), and JB04 (est=4.12; SE=1.09; 95%Ci=0.7; 7.6 and est=3.636; SE=1.09; 95%Ci=0.2; 7.1, respectively). Because CB02, CB06, and WD07 produced zero or very few fruits throughout the survey, there is a large difference in fruit production at these sites compared to the others. This resulted in large estimates, standard errors, and wide confidence intervals. When analyzed for goodness of fit, some minor deviations were detected. However, despite these minor deviations, residual model passes the dispersion test (p=0.745) indicating a good fit (Figure 9).

3.2.3 Fruit Dehiscence

While fruit and flower development are important, measurements of fruit dehiscence, or the releasing of seedlings from the fruit, is a valuable tool for determining success of sexual reproduction. The density of *Thalassia testudinum* fruit dehiscence observed was extremely low and ranged from 0.01±0.08 to 0.20±0.70 per 0.25 m^2 (Table 4). Although live fruits were not observed at WD07, dehisced fruits were. CK01 had the greatest density of fruits that reached maturity with a mean of 0.20±0.70 dehisced *Thalassia testudinum* fruits per quadrat.

3.2.4 Seedlings

Coquina Key sites, CK01 and CK03, were observed with the greatest number of seedlings with 2 each. One seedling was observed at EK03, GF01, and JB03. Seedlings were not observed at either location in Cockroach Bay, or at WD07, JB04, and EK01. Throughout the survey period, only 7 seedlings were observed (Table 9).

3.3 Variations in Reproductive Effort Throughout the Survey

There was a large disparity in the reproductive effort of Thalassia *testudinum* among survey months during the summer of 2017. Disproportionately greater reproductive effort was observed in May than in June (est=2.39; SE= 0.125; 95%Ci= -2.68, -2.093) or July (est=3.46; SE=0.142; 95%Ci=-3.79, -3.127), and effort was significantly greater in June than in July (est=1.07; SE= 0.156; 96%Ci=-1.44, -0.709) (Figure 10). EK01 had the greatest reproductive effort of all sites during May, but by June, reproductive effort had drastically declined (Table 10). While Coquina Key Flat sites, CK01 and CK03, did not have the greatest reproductive effort in May, reproductive effort was the most stable at these locations throughout the survey in 2017 (Table 10). However, likely due to the limited observations of fruit observations at many sites, predicted model residuals failed to align with observed values (p=0.0024) (Figure 11).

3.3.1 Flower Density

Peak anthesis in *Thalassia testudinum* occurred in May at all sites (Table 10). Not only was flowering in May significantly greater than in the following months, but it was the only month in which flowers were observed, apart from two flowers seen at EK01 in July. Model selection determined that both month and site were strongest predictors of *Thalassia testudinum* flowering (Table A1). However, the rarity of non-zero data points in June and July resulted in large estimates and standard errors and wide confidence intervals during post-hoc analyses. Additionally, goodness of fit tests displayed positive alignment of predicted and observed residual values (p=0.7156) indicating the model was a good fit (Figure 12).

3.3.2 Fruit Production

Thalassia testudinum fruits were observed every month during the survey. In the month of May, fruits were only observed at CB02 and EK01 (Table 10). Peak fruit counts for all locations was observed during the month of June (Table 10). Locations on Coquina Key, CK01 and CK03, had the greatest mean *Thalassia testudinum* fruit numbers with 1.40± 2.18 and 1.02±2.07 fruit numbers per 0.25 m^2 (Table 10, Figure 13). Negative binomial regression models determined June fruit numbers to be greater than May (est=4.35; SE=0.752; 95%Ci= 2.585; 6.11) and July (est=1.76; SE=0.282; 95%Ci=-2.453, -1.13). Furthermore, July fruit production was also statistically greater than in May (est=2.56; SE=0.759; 95%Ci=0.77, 4.33). Observed residuals from this model were plotted against and aligned with simulated expected values resulting in a good fit of the model (p=0.7364; Figure 14).

3.4 Variations in Reproductive Effort Within a Seagrass Bed

Site was removed as a predictor to determine which site characteristic variable was responsible for the variability in sexual reproduction throughout sites in Tampa Bay. These models were used to determine if position in a seagrass bed or percent basal coverage of seagrass were strong predictors of *Thalassia testudinum* reproductive effort and flowering density. Due to low fruit observations throughout the survey, fruit density was omitted from this analysis. Model selection results indicated that mean percent seagrass coverage was not a strong predictor, but location within a seagrass bed was (Table 12A, B). Shoots in the interior of the seagrass bed had an average of 0.55± 1.89 flowers and 1.65 ± 3.34 mean reproductive effort per quadrat (Table 11). This was significantly greater than flowers (est=0.603; SE=0.214; 95%Ci= -1.02, -0.184) and reproductive effort (est=0.611; SE= 0.141; 95%Ci= -0.887, -0.335) observed on the edge of the seagrass bed which had an average of 0.3±1.14 flower and 0.89±2.38 reproductive effort per 0.25m^2 quadrat (Table 11). Residual plots from both models were assessed to determine goodness of fit. Predicted residuals aligned with observed residuals in both models (Figure 15). Additionally, both models passed the dispersion test deeming them a good fit (Figure 15).

3.5 Tables and Figures

Table 3*. Mean ± SD (n=3) for May, June and July salinity and mean temperature data recorded at sites surveyed.*

Mean Salinity and Temperature at Locations in Tampa Bay, Florida

	Salinity (psu)					Temperature (°C)
Site	**May**	**June**	**July**	**Range**	**Mean**	**Mean**
CB02	31.93	30.13	27.65	4.0	30.34 ± 3.81	29.04 ± 3.12
CB06	33.34	32.32	29.63	3.8	31.31 ± 1.92	30.07 ± 2.07
CK01	31.28	30.59	29.55	0.9	30.33 ± 0.80	33.24 ± 12.86
CK03	31.66	30.76	29.65	1.3	30.46 ± 1.04	28.44 ± 2.18
EK01	35.05	34.79	33.53	1.4	34.08 ± 0.82	29.70 ± 1.72
EK03	34.86	34.72	33.31	1.6	33.90 ± 0.84	29.45 ±2.14
GF01	29.32	28.20	27.43	2.2	28.19 ± 0.97	30.45 ±2.36
JB03	34.82	33.45	31.08	3.4	33.16 ± 3.28	24.70 ± 3.00
JB04	35.01	33.67	30.53	3.7	32.05 ± 2.37	29.83 ± 2.08
WD07	30.15	28.80	27.49	2.8	30.13 ± 4.25	30.52 ± 2.63

Table 4. *Table of site and reproductive data. Numbers represent mean ± standard deviation of percent basal coverage of seagrass, flower density, fruit density, density of dehiscence, total seeds observed, and reproductive shoot counts during the survey period per 0.25 m^2 quadrat, and mean male to female ratio of flowers observed per quadrat at each site.*

Site	Basal Coverage (%)	Flower Density (count)		Fruit Density (count)	Density Dehiscence (Count)	Seeds (count)	Reproductive Effort (count)
	Mean	Mean	Male/Female Ratio	Mean	Mean	Sum	Mean
CB02	7.96±3.37	0.19±0.75	3.5	0±0	0±0	0	0.85±1.98
CB06	6.91±3.78	0.47±1.32	7.83	0.01±0.09	0±0	0	0.92±2.33
CK01	12.07±5.76	0.41±1.28	0.66	0.59±1.55	0.2±0.7	2	1.84±2.79
CK03	11.72±6.11	0.54±1.38	0.31	0.39±1.32	0.01±0.08	2	1.79±3.15
EK01	12.83±6.76	0.47±1.72	33	0.21±0.75	0.06±0.27	0	1.92±4.86
EK03	11.35±5.71	0.53±1.32	0.59	0.08±0.34	0.08±0.32	1	1.39±2.38
GF01	11.26±5.32	0.85±3.56	18	0.17±1.03	0.02±0.15	1	1.63±4.18
JB03	9.1±4.56	0.55±1.67	3.3	0.03±0.21	0.04±0.24	1	1.73±3.56
JB04	11.47±4.66	0.32±1.19	0.25	0.01±0.1	0±0	0	0.51±1.82
WD07	11.82±5.93	0.2±0.63	0.06	0±0	0.01±0.09	0	0.94±1.8

Table 5*. Negative binomial model output comparing reproductive effort between sites visited in summer 2017. Among all other models for reproductive effort, this model was weighted highest with a weight of 0.960 (Table A1). Table displays estimate, standard error, and upper and lower confidence levels.*

contrast	estimate	SE	LCL	UCL
CB02 - CB06	-0.0927	0.3	-1.0405	0.8552
CB02 - CK01	-0.7239	0.281	-1.6138	0.166
CB02 - CK03	-0.6258	0.289	-1.5391	0.2874
CB02 - EK01	-0.9309	0.299	-1.8784	0.0166
CB02 - EK03	-0.4937	0.28	-1.3807	0.3934
CB02 - GF01	-0.5355	0.294	-1.4657	0.3948
CB02 - JB03	-0.7129	0.302	-1.6669	0.2411
CB02 - JB04	0.649	0.334	-0.4081	1.7061
CB02 - WD07	0.1395	0.3	-0.8097	1.0888
CB06 - CK01	-0.6312	0.285	-1.5326	0.2701
CB06 - CK03	-0.5332	0.292	-1.4564	0.3901
CB06 - EK01	-0.8382	0.302	-1.7949	0.1185
CB06 - EK03	-0.401	0.285	-1.3014	0.4994
CB06 - GF01	-0.4428	0.297	-1.3837	0.4981
CB06 - JB03	-0.6203	0.305	-1.5847	0.3442
CB06 - JB04	0.7417	0.337	-0.3251	1.8084
CB06 - WD07	0.2322	0.305	-0.7323	1.1967
CK01 - CK03	0.098	0.273	-0.7665	0.9625
CK01 - EK01	-0.207	0.285	-1.1073	0.6933
CK01 - EK03	0.2302	0.265	-0.6086	1.069
CK01 - GF01	0.1884	0.279	-0.6946	1.0714
CK01 - JB03	0.0109	0.287	-0.8971	0.919
CK01 - JB04	1.3729	0.321	0.357	2.3888
CK01 - WD07	0.8634	0.286	-0.0428	1.7696
CK03 - EK01	-0.305	0.291	-1.2262	0.6162
CK03 - EK03	0.1322	0.273	-0.7323	0.9967
CK03 - GF01	0.0904	0.286	-0.8151	0.9959
CK03 - JB03	-0.0871	0.294	-1.0171	0.8429
CK03 - JB04	1.2749	0.327	0.2391	2.3106
CK03 - WD07	0.7654	0.295	-0.167	1.6977
EK01 - EK03	0.4372	0.285	-0.4637	1.3381
EK01 - GF01	0.3954	0.297	-0.5442	1.335
EK01 - JB03	0.2179	0.304	-0.7453	1.1812
EK01 - JB04	1.5799	0.337	0.514	2.6457
EK01 - WD07	1.0704	0.306	0.1033	2.0375
EK03 - GF01	-0.0418	0.279	-0.9235	0.8399
EK03 - JB03	-0.2193	0.287	-1.126	0.6875
EK03 - JB04	1.1427	0.321	0.1282	2.1571
EK03 - WD07	0.6332	0.284	-0.2649	1.5313
GF01 - JB03	-0.1775	0.299	-1.1248	0.7699
GF01 - JB04	1.1845	0.332	0.1332	2.2357
GF01 - WD07	0.675	0.299	-0.2716	1.6216
JB03 - JB04	1.3619	0.339	0.2895	2.4343
JB03 - WD07	0.8525	0.307	-0.1174	1.8223
JB04 - WD07	-0.5095	0.338	-1.5802	0.5613

Confidence level used: 0.95

Confidence level adustment: Tukey method for comparing family of 10 estimates

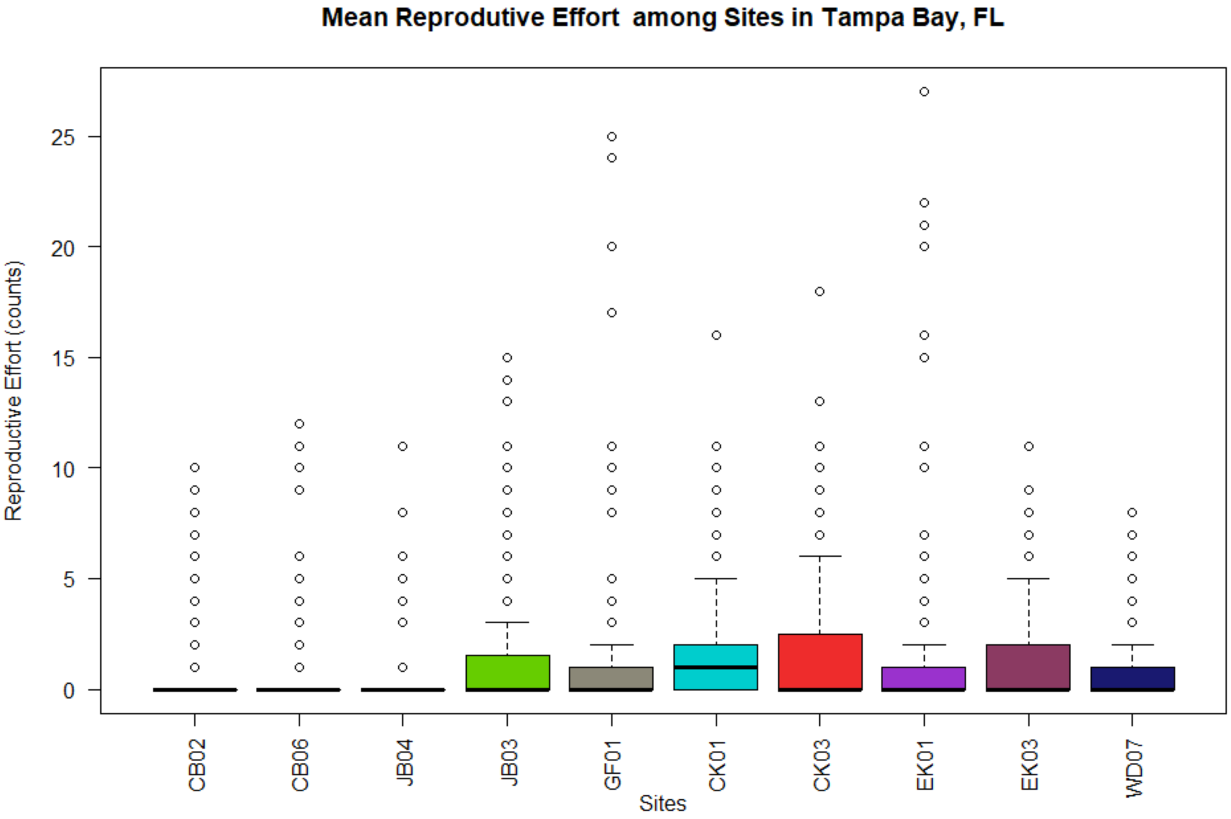
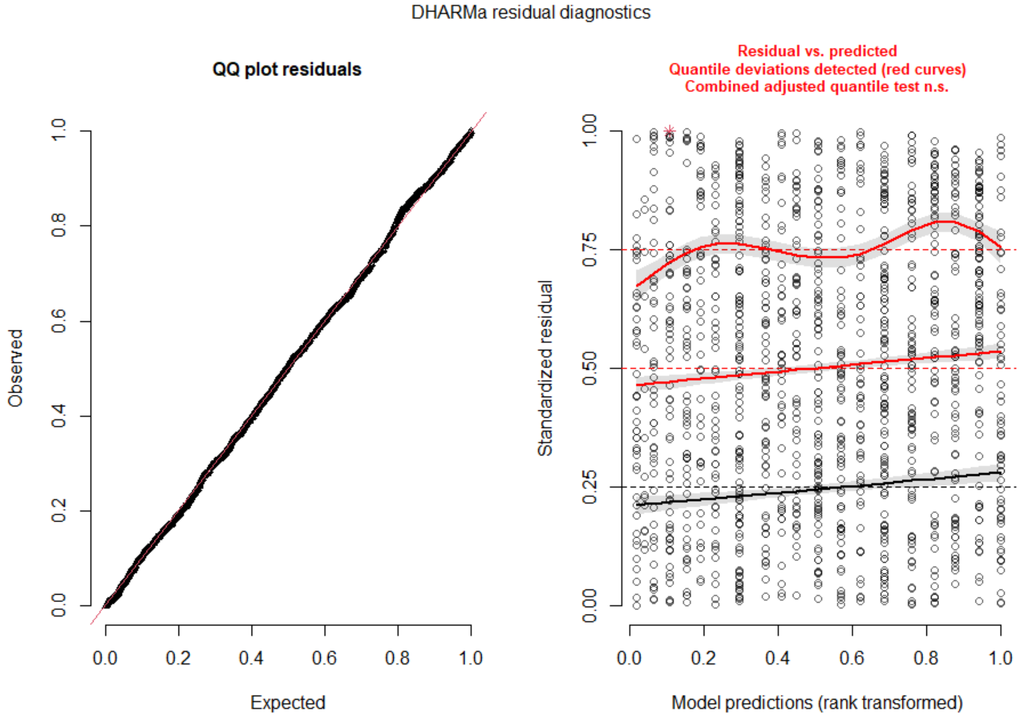


Figure 4. *Box and whisker plot of reproductive effort of* Thalassia testudinum *shoots in quadrats surveys.*



DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 0.8434, p-value = 0.074

Figure 5*. Residual plots (n=1000) of the reproductive effort model. Although deviations were detected, residuals pass dispersion test and p=0.108 indicating it is a good fit. Red lines represent predicted values and black lines represent observed residuals.*

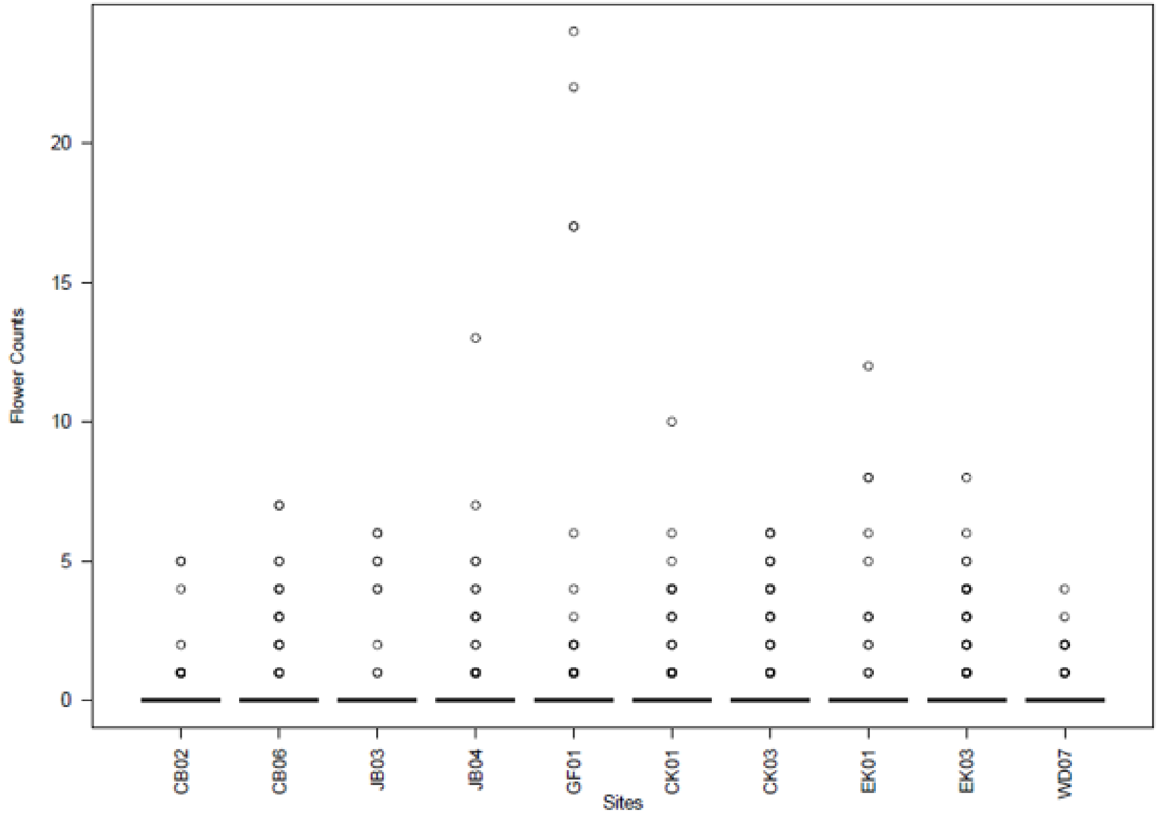
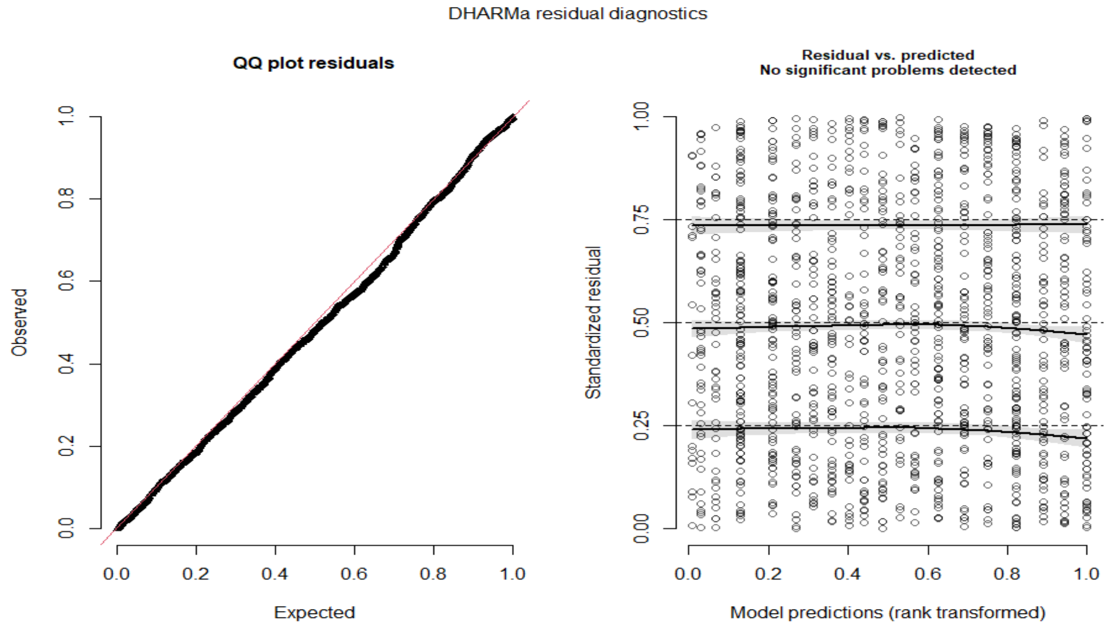


Figure 6. *Box and whiskers plot of flower density per 0.25m² for all sites surveyed.*

Table 6*. Model selection results for negaitve binomial regressions models run to determine the strongest predictor of flower density.*

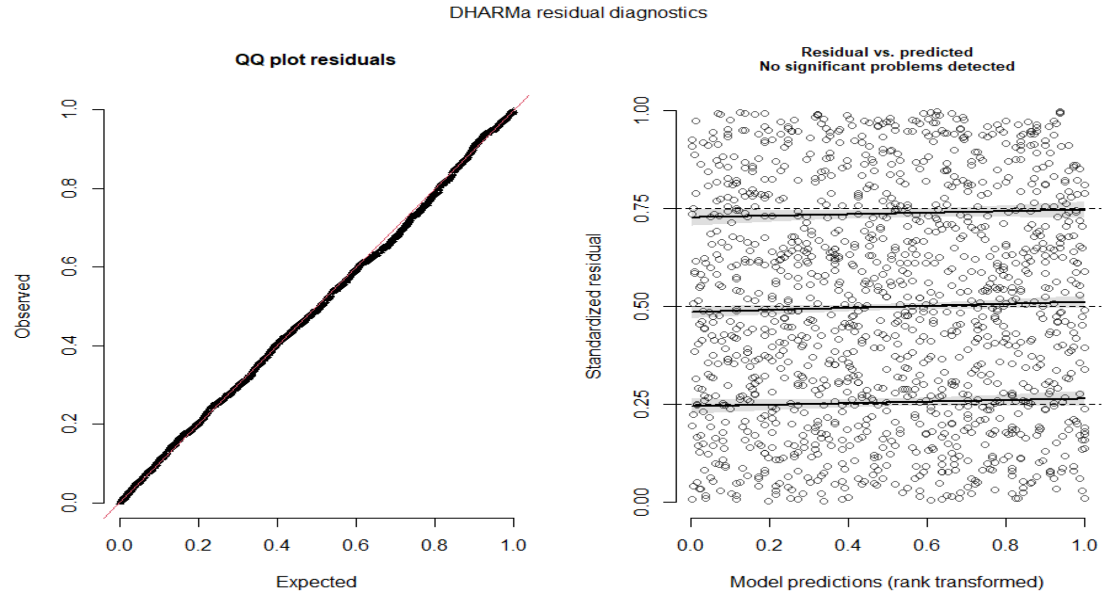
Model Selection Table

Model	df	logLik	AICc	deltaAICc	AICc weight
Site& Quadrat Location	12	-906.42	1837.1	0	0.407
Site+ Cover%+ Cover%^{2}	13	-905.482	1837.2	0.16	0.375
Quadrat location	3	-916.674	1839.4	2.29	0.1130
Cover%+ Cover%^{2}		-916.236	1840.5	3.42	0.074
Site+ %Cover	2	-910.512	1845.3	8.18	0.007
Site	12	-912.001	1846.2	9.12	0.005
Cover%	11	-920.339	1846.7	9.62	0.003



A.

DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 0.92462, p-value = 0.6318



B.

DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 0.88593, p-value = 0.4896

Figure 7. *Residual plots (n=10000) output to determine goodness of fit and test for dispersion for flower density models supported by the 95% confidence model set. A. Residual plots from site and quadrat location model and B. Residual plots from Site, Percent Cover, and Percent Cover2 model. Both plots suggest good model fit for predicting flower density.*

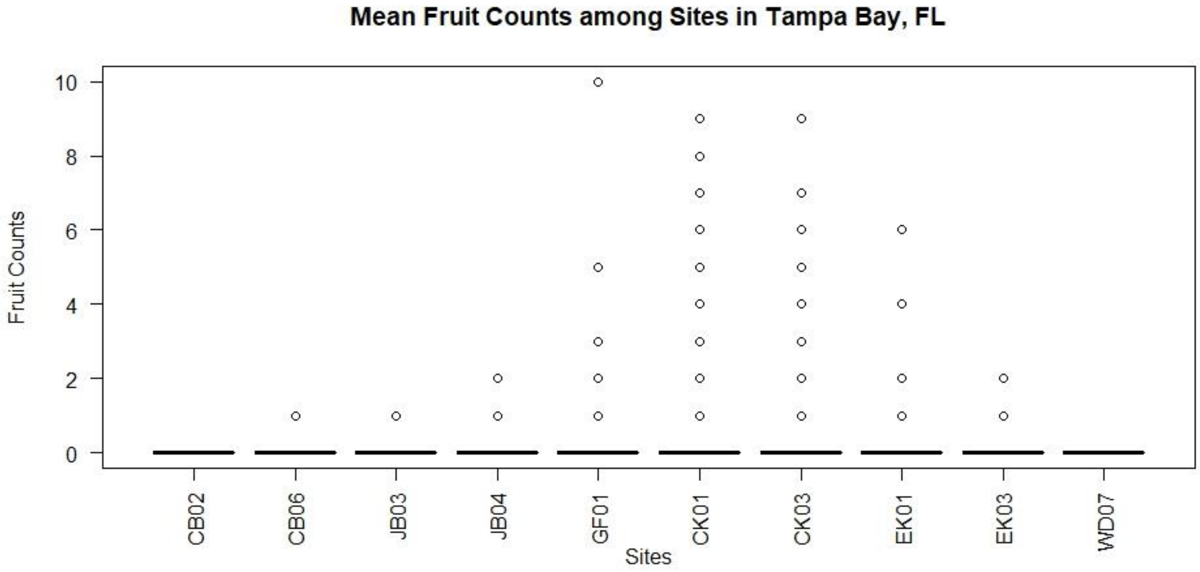


Figure 8. *Box and whiskers plot of mean* Thalassia testudinum *fruit counts observed per 0.25m^2 throughout the survey period.*

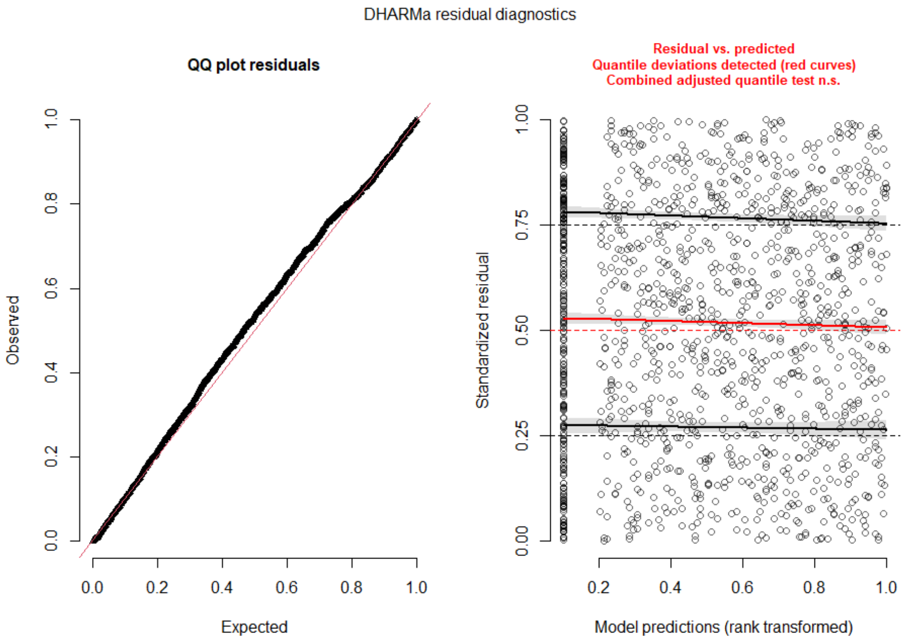
Table 7. *Model selection results for negative binomial regressions models run to determine the strongest predictor of fruit density.*

Model Selection Table

Model	df	logLik	AICc	deltaAICc	AICc weight
Site& Quadrat Location	12	-397.409	819.1	0.00	0.802
Site+ Cover%+ Cover%^{2}	13	-398.688	823.7	4.60	0.080
Site+ %Cover	12	-399.908	824.1	5.00	0.066
Site	11	-401.172	824.5	5.49	0.051
Cover%+ Cover%^{2}	4	-450.319	908.7	89.61	0.000
Cover%	3	-451.669	909.4	90.30	0.000
Quadrat location	3	-454.744	915.5	96.45	0.000

Table 8. *Post-hoc output for model of best fit which assessed the effects of site and locations within a bed on fruit density. Table displays estimate, standard error, and upper and lower confidence levels.*

contrast	estimate	SE	LCL	UCL
CB02 - CB06	-25.412	1.90E+05	-602379.9	602329.1
CB02 - CK01	-29.751	1.90E+05	-602384.2	602324.7
CB02 - CK03	-29.246	1.90E+05	-602383.7	602325.2
CB02 - EK01	-28.816	1.90E+05	-602383.3	602325.7
CB02 - EK03	-27.715	1.90E+05	-602382.2	602326.8
CB02 - GF01	-28.425	1.90E+05	-602382.9	602326
CB02 - JB03	-26.737	1.90E+05	-602381.2	602327.7
CB02 - JB04	-25.609	1.90E+05	-602380.1	602328.9
CB02 - WD07	0.195	2.75E+05	-870814.7	870815
CB06 - CK01	-4.339	1.08E+00	-7.7	-0.9
CB06 - CK03	-3.834	1.08E+00	-7.3	-0.4
CB06 - EK01	-3.404	1.10E+00	-6.9	0.1
CB06 - EK03	-2.303	1.11E+00	-5.8	1.2
CB06 - GF01	-3.013	1.10E+00	-6.5	0.5
CB06 - JB03	-1.325	1.21E+00	-5.2	2.5
CB06 - JB04	-0.197	1.48E+00	-4.9	4.5
CB06 - WD07	25.607	1.99E+05	-628852.4	628903.7
CK01 - CK03	0.505	3.90E-01	-0.7	1.8
CK01 - EK01	0.935	4.30E-01	-0.4	2.3
CK01 - EK03	2.035	4.50E-01	0.6	3.5
CK01 - GF01	1.326	4.40E-01	-0.1	2.7
CK01 - JB03	3.013	6.70E-01	0.9	5.1
CK01 - JB04	4.142	1.09E+00	0.7	7.6
CK01 - WD07	29.946	1.99E+05	-628848.1	628908
CK03 - EK01	0.429	4.50E-01	-1	1.9
CK03 - EK03	1.53	4.70E-01	0	3
CK03 - GF01	0.821	4.60E-01	-0.6	2.3
CK03 - JB03	2.508	6.90E-01	0.3	4.7
CK03 - JB04	3.636	1.09E+00	0.2	7.1
CK03 - WD07	29.441	1.99E+05	-628848.6	628907.5
EK01 - EK03	1.101	5.10E-01	-0.5	2.7
EK01 - GF01	0.391	4.90E-01	-1.2	1.9
EK01 - JB03	2.079	7.10E-01	-0.2	4.3
EK01 - JB04	3.207	1.11E+00	-0.3	6.7
EK01 - WD07	29.012	1.99E+05	-628849	628907.1
EK03 - GF01	-0.71	5.10E-01	-2.3	0.9
EK03 - JB03	0.978	7.20E-01	-1.3	3.2
EK03 - JB04	2.106	1.11E+00	-1.4	5.6
EK03 - WD07	27.911	1.99E+05	-628850.1	628906
GF01 - JB03	1.688	7.10E-01	-0.6	3.9
GF01 - JB04	2.816	1.11E+00	-0.7	6.3
GF01 - WD07	28.62	1.99E+05	-628849.4	628906.7
JB03 - JB04	1.128	1.22E+00	-2.7	5
JB03 - WD07	26.933	1.99E+05	-628851.1	628905
JB04 - WD07	25.804	1.99E+05	-628852.2	628903.8



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DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 0.91069, p-value = 0.7454
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Figure 9. *Residual plot (n=10000) of the fruit density model of greatest weight. Although deviations were detected, simulated residuals align with predicted residuals and pass dispersion test (p=0.745) indicating it is a good fit. Red lines represent predicted values and black lines represent observed.*

Table 9*.Count of Thalassia testudinum seedlings observed during the survey.*

Seed Observations	
Site	Sum
CB02	0
CB06	0
CK01	2
CK03	2
EK01	0
EK03	1
GF01	1
JB03	1
JB04	0
WD07	0

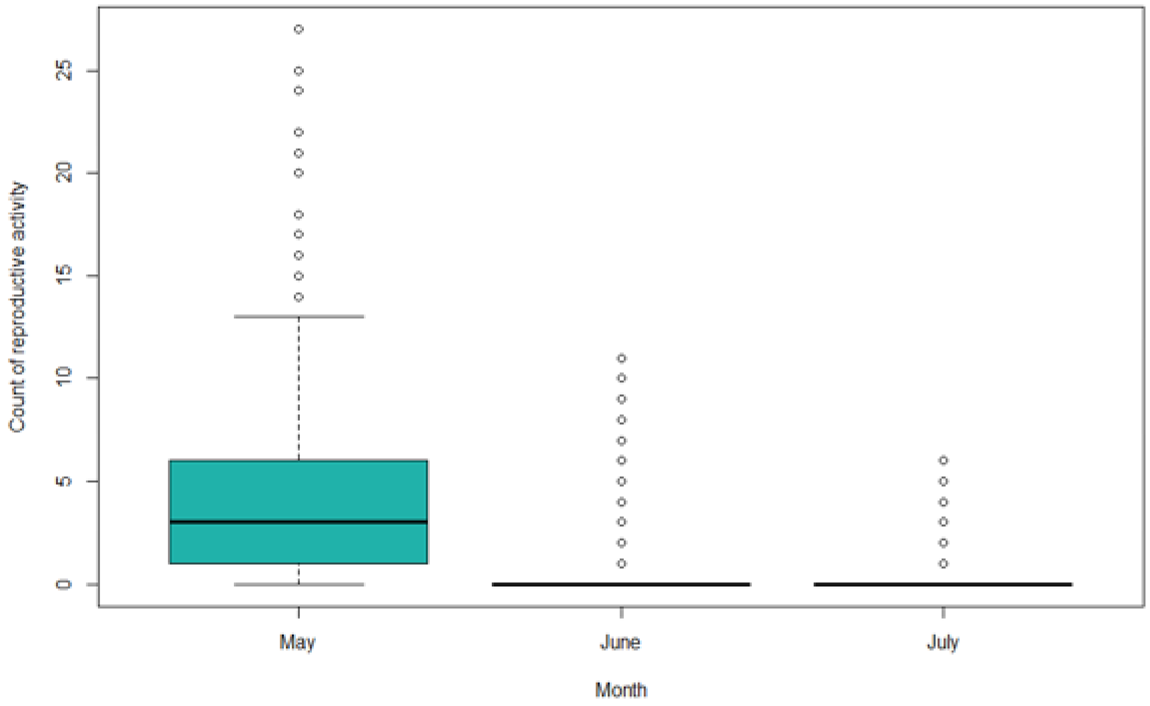
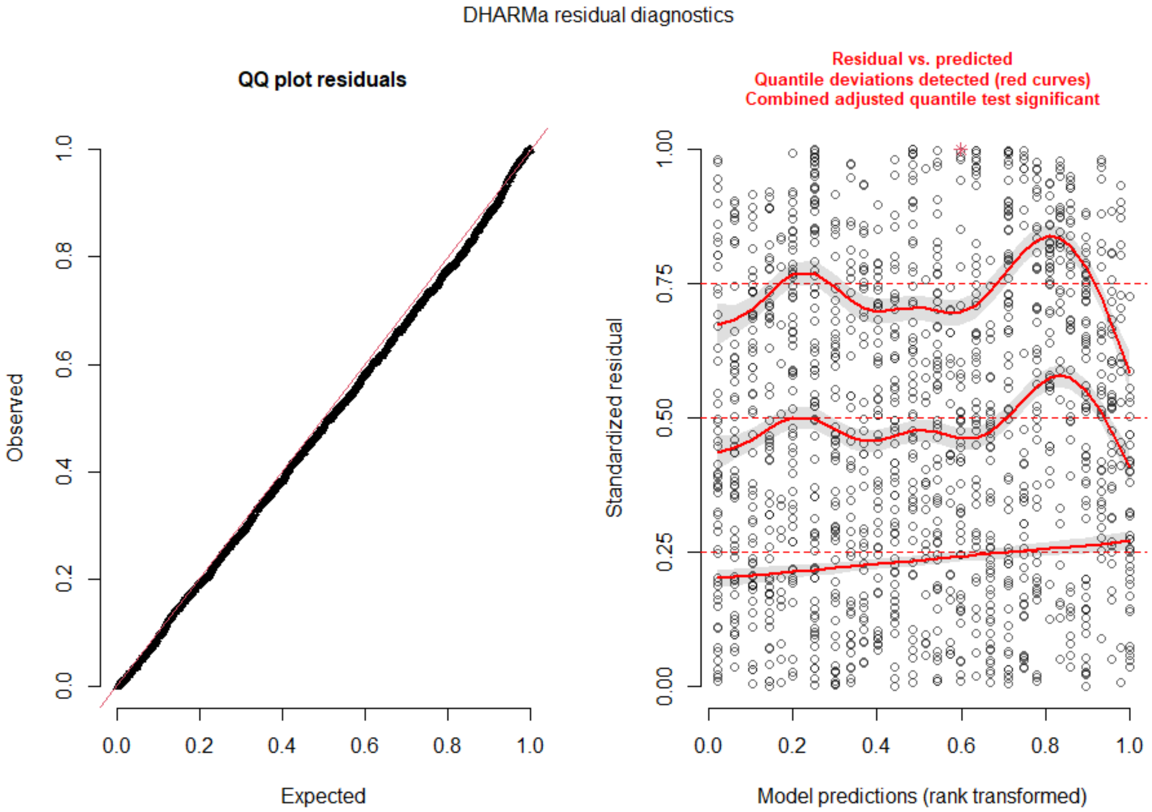


Figure 10. *Box and whisker plot of mean reproductive effort, or activity, of* Thalassia testudinum *short shoots each month during the reproductive survey.*

Table 10. *Mean densities ± SD of reproductive stages of Thalassia testudinum observed during each month of the survey.*

	Mean Flower Counts			Mean Fruit Counts			Mean Reproductive Effort		
Site	May	June	July	May	June	July	May	June	July
CB02	0.64±1.25	0	0	0	0	0	2.6±2.88	0.17±0.5	0
CB06	2.1±2.11	0	0	0.03±0.19	0	0	4.07±3.44	0.04±0.2	0
CK01	1.68±2.17	0	0	0	1.4±2.18	0.06±0.23	3.7±3.43	2.02±2.78	0.37±0.88
CK03	2.11±2.04	0	0	0	1.02±2.07	0.07±0.33	5.11±4.15	1.24±2.06	0.09±0.3
EK01	2.94±3.52	0	±0.270.04	0.06±0.24	0.07±0.25	0.37±1.05	10.5±7.98	0.11±0.31	0.57±1.27
EK03	1.45±1.87	0	0	0	0.04±0.21	0.16±0.46	3.29±3.01	0.04±0.21	0.36±0.7
GF01	3.24±6.44	0	0	0	0.52±1.77	0	5.47±6.58	0.6±1.81	0.02±0.14
JB03	1.7±2.61	0	0	0	0.13±0.46	0	5.16±4.68	0.13±0.46	0.09±0.35
JB04	0.94±1.91	0	0	0	0	0.02±0.14	1.44±2.91	0	0.04±0.19
WD07	0.72±1.03	0	0	0	0	0	3.28±2.02	0.12±0.33	0
ALL	1.64±2.84	0	±0.080	0.01±0.07	0.43±1.39	0.07±0.40	4.06±4.48	0.6±1.64	0.16±0.60



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DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 0.71706, p-value = 0.0024
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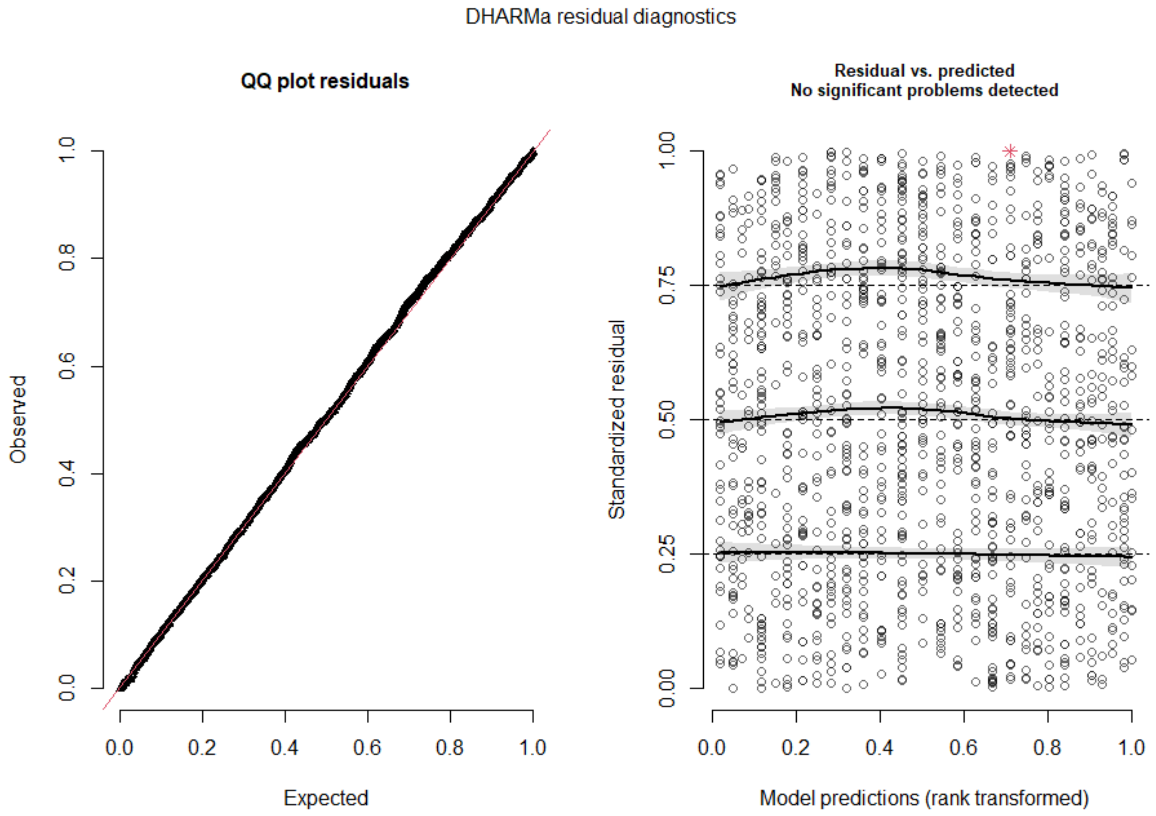
Figure 11. *Plot of residuals (n=10000) from model determining the effects of month and site as the strongest predictors of* Thalassia testudinum *reproductive activity during the summer survey.*



DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 1.0294, p-value = 0.7156

Figure 12. *Residual plot (n=10000) of the model with flower density predictor variables of month and site. No deviations were detected, therefore simulated residuals align with predicted residuals and pass dispersion test (p=0.7156) indicating it is a good fit, dark black lines represent observed residuals.*

Table 11*. Mean percent coverage and reproductive effort of exhibited by Thalassia testudinum short shoots on the edge and interior of seagrass meadows at all sites surveyed.*

Location	Cover (%)	Flower Density (count)	Fruit Density (count)	Total Reproductive Effort
	Mean	Mean	Mean	Mean
Edge	8.76±5.18	0.3±1.14	0.09±0.53	0.89±2.38
Interior	11.78±5.55	0.55±1.89	0.19±0.95	1.65±3.34

Table 12*. Model selection tables to determine the strongest predictor for A. reproductive effort and B. flowering density when site was held constant.*

A.

Model Selection Table

Model	df	logLik	AICc	deltaAICc	AICc weight
Quadrat location	3	-1805.773	3617.6	0.00	0.990
Cover%	4	-1809.859	3627.7	10.18	0.006
Cover%+ Cover%^{2}	3	-1811.415	3628.3	11.28	0.000

B.

Model Selection Table

Model	df	logLik	AICc	deltaAICc	AICc weight
Quadrat location	3	-916.674	1839.4	0	0.604
Cover%+ Cover%^{2}	4	-916.236	1840.5	1.14	0.342
Cover%	3	-920.339	1846.7	5.55	0.038

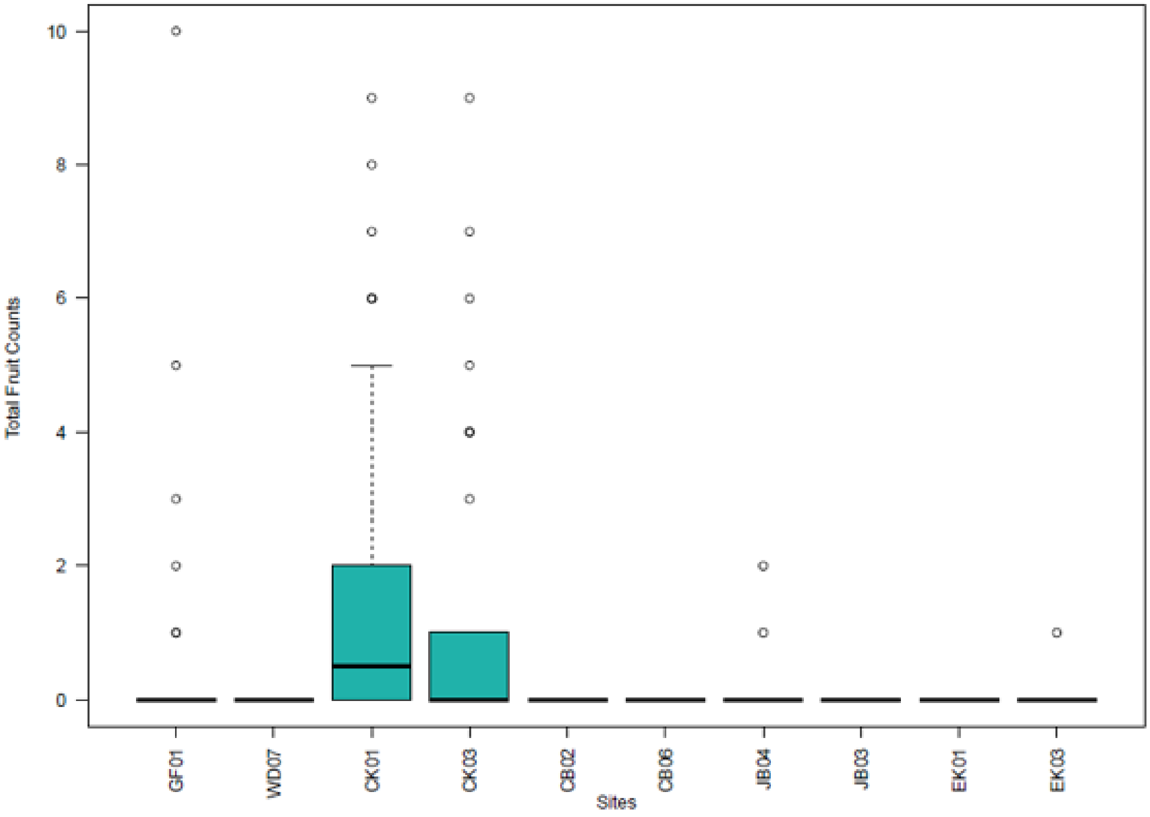
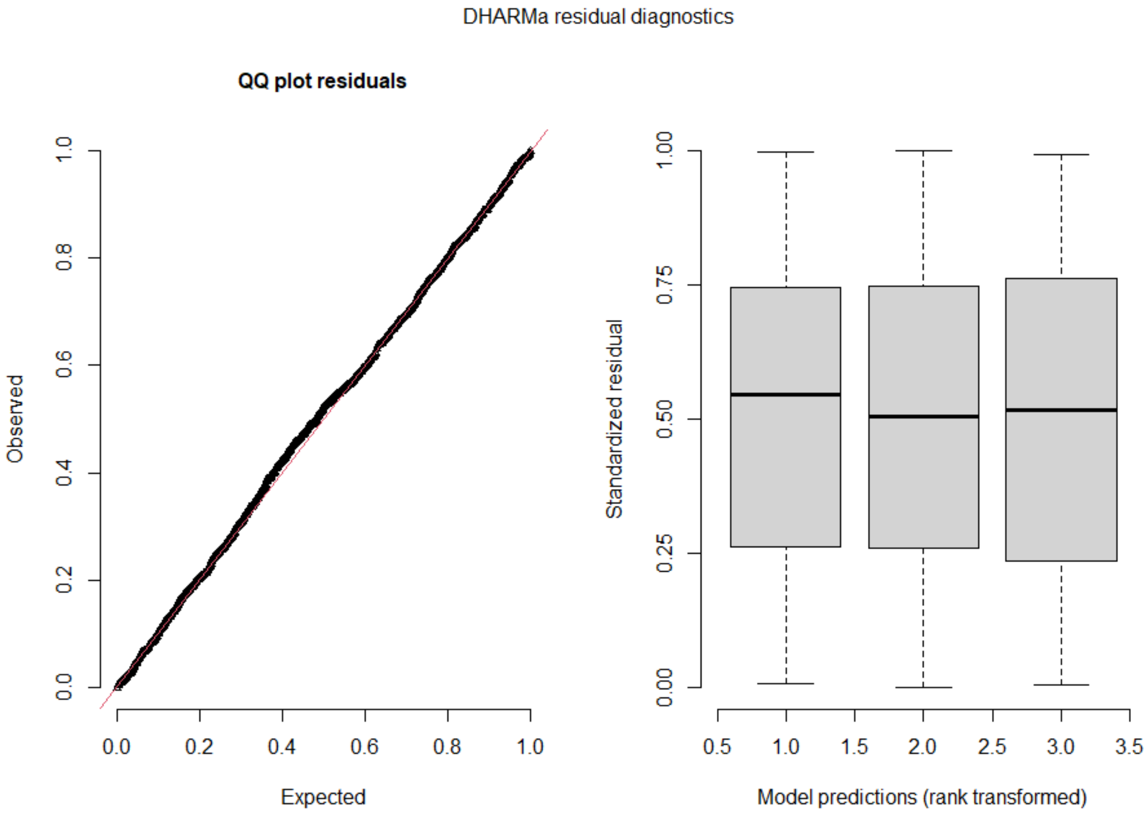
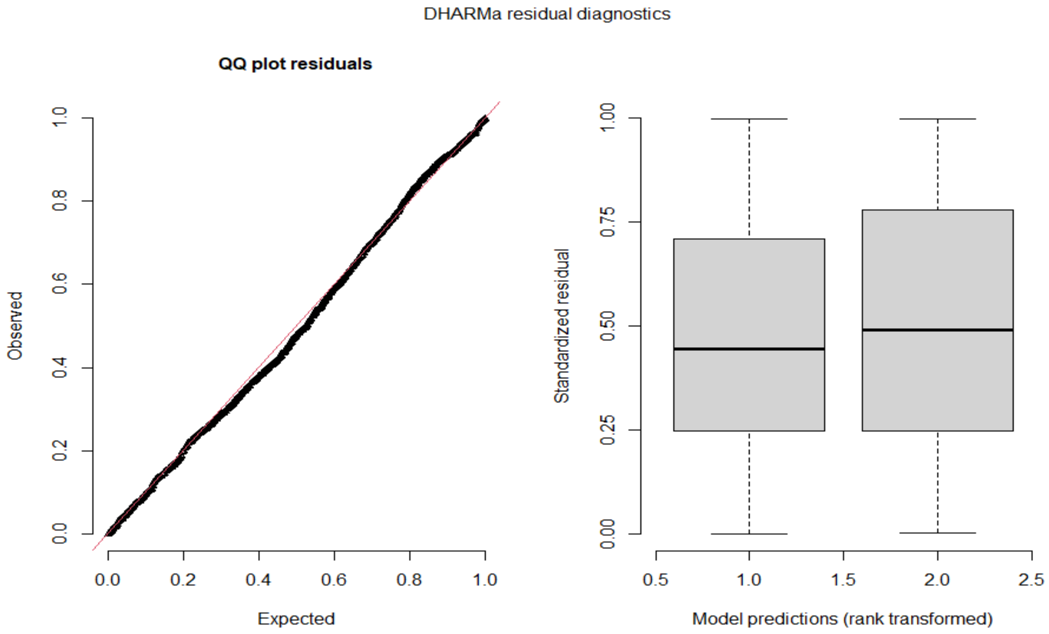


Figure 13. *Box plot of mean June* Thalassia testudinum *fruit counts observed at all sites in Tampa Bay.*



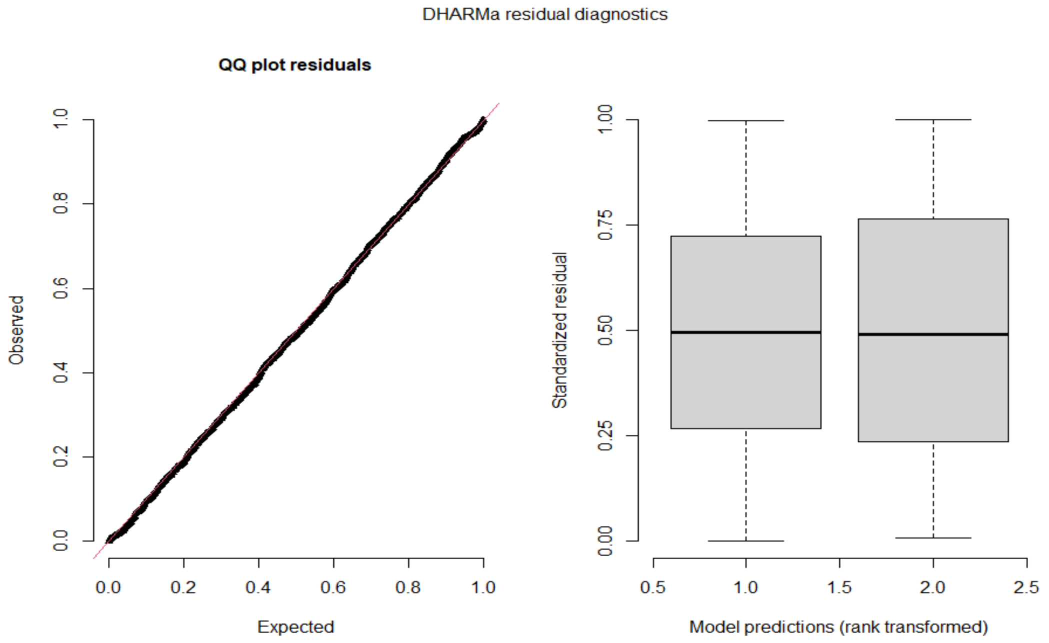
DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 0.91874, p-value = 0.7364

Figure 14. *Residual plot (n=10000) of the model with fruit density predictor variables of month and site. No deviations were detected, therefore simulated residuals align with predicted residuals and pass dispersion test (p=0.7364) indicating it is a good fit, dark black lines represent observed residuals.*



A.

DHARMa nonparametric dispersion test via sd of residuals fitted vs. simulated
ratioObsSim = 0.88653, p-value = 0.172



B.

DHARMa nonparametric dispersion test via sd of residuals fited vs. simulated
ratioObsSim = 0.97484, p-value = 0.8926

Figure 15. *Residual plots (n=10000) of models testing quadrat location as a predictor of A. reproductive effort and B. flower density. No deviations were detected, therefore simulated residuals for both models align with predicted residuals and pass dispersion tests indicating goodness of fit.*

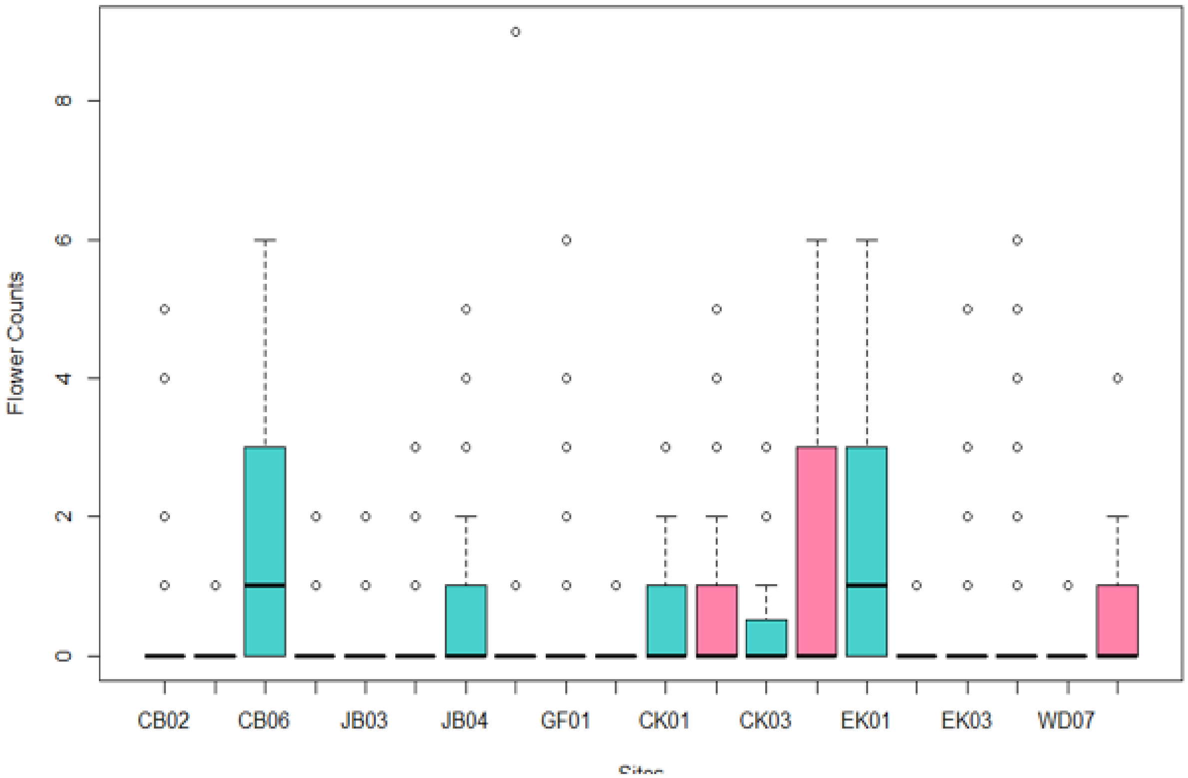


Figure 16. *Mean* Thalassia testudinum *pistillate and staminate flower production during May 2017 for all locations surveyed in Tampa Bay, Florida. Blue bars reflect male flower density while pinks bars reflect female flower density.*

DISCUSSION

4.1 Reproductive Effort Variations Among Site Locations

Overall, sexual reproduction of *Thalassia testudinum* in Tampa Bay, Florida, was lower than previously observed in Tampa Bay (Grey and Moffler, 1978; Durako and Moffler, 1987c). Furthermore, there was limited successful sexual reproduction and seedling recolonization observed during the 2017 reproductive season. As terrestrial plants that migrated back into the water, seagrasses retain adaptations from time on land. Some terrestrial land plants will only undergo sexual reproduction during or following periods of substantial stress. While limited research has been done to understand stress-induced reproductive effort in *Hydrocharitaceae* seagrasses, there is some evidence of increased reproductive capacity following significant disturbances in other seagrass families (Cabaco and Santos, 2012). While seagrass families are genetically distinct and much more research is needed to support this hypothesis, an argument could be made that reduced reproductive effort during 2017 is a result of reduced environmental stress. Whether the temporal inconsistency in reproductive effort is from lack of standardized, *in situ* survey methods among studies or reduced environmental stressors, these finding support the hypothesis that asexual reproduction is the primary form of reproduction and expansion in *Thalassia testudinum* beds. Additionally, these results indicate that Tampa Bay is not be a fully recovered estuary and that meadows might have minimal genetic diversity making them vulnerable to disease and other stressors.

4.1.1. Flower Density

Results from this study determined May to be the time of peak anthesis for *Thalassia testudinum* short shoots in Tampa Bay during the 2017 reproductive season (Table 4, Figure 10). This is similar to findings of Zieman (1975) and Durako and Moffler (1987), but different from the study by Tomlinson (1969) who reported June as the month with the greatest anthesis in Biscayne Bay (Orpurt and Boral, 1964; Tomlinson, 1969; Zieman, 1975; Durako and Moffler, 1975). Temperature is considered one of the strongest drivers of flower initiation (Durako and Moffler, 1987). Therefore, this suggests that climate change and increased sea surface temperatures has not yet begun shifting reproductive cycles in *Thalassia testudinum* populations of Tampa Bay.

Significant spatial variability in *Thalassia testudinum* flower densities was observed at locations surveyed during the 2017 reproductive season (Figure 4; Table 4). Variability is not uncommon in Tampa Bay and has been observed since 1976 (Grey and Moffler, 1978; Durako and Moffler, 1985c; 1987). Salinity, genetic variability, and sexual separation caused by asexual reproduction have been proposed as explanations for this variability (Tomlinson, 1969; Grey and Moffler, 1978; Phillips et al., 1981; Durako and Moffler, 1985c; 1987; Gallegos et al., 1992; Duarte et al., 1994; Darnell and Dunton, 2016). Short shoot age may also be affecting flower production and increasing spatial variability in reproductive effort. Phenological literature suggests *Thalassia testudinum* short shoots reach sexual maturity at two years of age (Gallegos et al., 1992; Witz and Dawes, 1995; McDonald et al., 2016). While the Southwest Florida Water Management District has been mapping seagrass recovery beginning in 2008, the target acreage was not met until 2014. Recently recovered seagrass beds may not have reached sexual maturity, limiting the flowering effort.

Flower densities were notably lower at WD07 and CB02, both of which are directly adjacent to mangrove forests (Figure 17). Mangroves shuttle nutrients, organic matter, and tannic water to the surrounding estuary. These seemingly minor alterations in water quality have been documented to have long-lasting negative effects on seagrass habitats (Livingston, 1984). In another study which sampled biomass from each of the sites surveyed for sexual reproduction, CB02 and WD07 displayed reduced total biomass accumulations compared to other sites in Tampa Bay (Scolaro, unpublished data). Therefore, these isolated decreases in water quality may be inhibiting sufficient carbohydrate accumulation required for energetically expensive sexual reproduction. Generally, it is suggested that healthy turtle grass populations require a minimum 18 percent surface irradiance for growth, yet there is no information on light requirements for sexual reproduction (Lee et al., 2007; McDonald et al., 2016).

Flower production was highest at locations in seagrass meadows located on sandy shoals with a clear separation from land and significant stormwater sources. This separation results in more stable environmental conditions, particularly salinity. Although not significant at both sites, limited flowering at CB02 and CB06 might also be a result of the sites' proximity to the mouth of the Little Manatee River, one of the major rivers delivering freshwater to Tampa Bay. Freshwater pulses following heavy rain events can significantly reduce aboveground biomass and seagrass productivity, which would result in diminished sexual reproduction (Livingston, 1984). Physical water quality measurements taken during the reproductive survey documented the steep decline of salinity at these sites from May to July of 2017 (Table 3). The difference in salinity during the three-month period was >4 psu (Table 6). While these measurements only document moments in time and are within the salinity range for peak growth, it does suggest the possibility of salinity pulses following major rain events common in a sub-tropical summer (Zieman, 1975; McDonald et al., 2016). These pulses may have resulted in stress-induced flower and/ or fruit senescence, abortion, or abscission. Durako and Moffler (1987) also observed decreased salinity followed by declines in reproductive effort in Tampa Bay during the late 1970's suggesting this is not an uncommon phenomenon in Tampa Bay. These results suggest that salinity was the environmental condition limiting sexual reproduction in *Thalassia testudinum* in Tampa Bay.

Percent cover of seagrass was determined to be a significant predictor of flower production (Table 6). Percent cover was positively related to flower density while the square of percent cover was negatively related flower production. This presents a possible "goldilocks density" or ideal seagrass percent coverage, which stimulates the greatest flower productivity. Extremely dense seagrass meadows will develop a shading effect, thus reducing light penetration to neighboring blades. Excess density may also produce stressful environmental micro-climates with reduced flow, elevated temperature, and limited gas exchange. This model also predicts that there is reduced flower production in areas with low percent cover of seagrass. While the model assumes that this is due to percent cover, there may be phenological factors at play. Often in seagrass meadows of Tampa Bay, low percent seagrass abundance is observed on the edge of seagrass beds. This area represents early colonization or bed expansion (Durako and Moffler, 1985b). Therefore, it is unclear which factor, age or density, is more important in determining reproductive effort when simply comparing edge and interior flowering effort.

4.1.2 Fruit Density

The large standard deviation around mean fruit density depicts the patchiness of the fruiting occurring in a bed mosaic (Table 4). Although not significant, fruiting was notably greater at locations on Coquina Key Flat, at both the deep site and the shallow site, than at any other site. (Table 4, Figure 8, Figure 13). Results from a biomass study conducted at the same locations determined total biomass, both above and below ground, to be significantly greater at the locations on Coquina Key flat than at the other locations except JB03 and JB04, (Scolaro, unpublished data). An increase in biomass may result in increased fruiting due to an abundance of stored carbohydrate resources required for energetically expensive sexual reproduction.

Skewed gender ratios might have caused significant implications of sexual reproductive success. Sites at Coquina Key exhibited the greatest production of fruits during the survey. These were also the only areas which exhibited a similar male to female ratio. Therefore, skewed sex ratios likely result in reduced fruit production and point to a founder effect. If gender ratios are temporally consistent at each site, it might indicate that the initially colonizing material determines the sex ratio and reproductive success of Tampa Bay *Thalassia testudinum*. Large disparities in gender ratios are not uncommon in Tampa Bay. Grey and Moffler also observed large variations of sex ratios among locations surveyed in Tampa Bay in the 1970s and 1980s (Grey and Moffler, 1978; Durako and Moffler, 1985b). However, unlike Grey and Moffler (1978) and similar to Tomlinson (1969) and Cox and Tomlinson (1988), results from this survey revealed gender ratios favoring male flowers (Tomlinson, 1969; Grey and Moffler, 1978; Cox and Tomlinson, 1988). Male plants can produce up to five individual flowers in sequence in one reproductive season, while females only produce one flower due to the high energetic cost of female reproduction (Tomlinson, 1969; Cox and Tomlinson, 1988; Durako and Moffler, 1985b; 1985a; van Tussenbroek et al., 2016b; Darnell and Dunton, 2016). Additionally, staminate anthesis occurs during summer spring tides (Phillips et al., 1981; Cox and Tomlinson, 1988). After releasing pollen sacs, these flowers quickly degrade (Grey and Moffler, 1978; Cox and Tomlinson, 1988). Flower surveys in May 2017 coincided with spring tides. Thus, this survey was conducted at an ideal time to capture the entire population prior to degradation. Regardless of the timing, Coquina Key locations had abundant production of both staminate and pistillate flowers which may have resulted in elevated fruit development (Figure 16).

Spatial separation of male and female flowers may also be a factor in reducing fruit development among sites in Tampa Bay. There were distinct patches of male and female flowers with minimal mixing observed at all locations except sites on Coquina Key. Abiotic pollination results in large quantities of poor quality, pollen production (van Tussenbroek et al., 2010). For seagrass ecosystems, pollen movement is influenced by both hydrodynamic features including currents, turbulence, and pollen buoyancy and meadow structure including canopy height, canopy density, fragmentation, and distance to a flower of the opposite sex (van Tussenbroek et al, 2010; van Tussenbroek et al 2016b; Kendrick et al., 2017). Pollen is estimated to be transported less than 10 meters in a dense seagrass meadow and be active for only a few hours (Kendrick et al., 2017). These conditions present a challenge in sexually isolated meadows and may have reduced pollination in several areas. Segregation of sexes may be influenced by asexual reproductive patterns leading to increased instances of a single ramet. Evidence suggests that all ramets on a genet retain the same gender as the parent short shoot; however, more data is needed to support this hypothesis (Grey and Moffler, 1978).

4.1.3 Dehiscence

Evidence of successful *Thalassia testudinum* sexual reproduction through the observation of dehisced fruits was extremely low among all locations surveyed in Tampa Bay. Following dehiscence, fruit remnants begin to degrade. Additionally, fruit fragments are high in carbohydrate resources and are thus a target for herbivory. Therefore, limited observations may not be a result of failed fruits, but a bias created by the survey method.

On the other hand, many aborted fruits were observed while surveying in June and July. Fruit abortion is not uncommon in reproductive seagrasses and may be a result of decreased pollen quality, self-pollination, or lack of resources, or erratic shifts in environmental conditions (Balestri and and Cinelli, 2003; van Tussenbroek et al., 2016b). Additionally, many peduncles were observed with dark legions close to where the fruit was borne. These observations suggest that a fungus or virus is the impetus for fruit abortion. Fungal growth in and on *Thalassia testudinum* short shoot structures is well documented throughout the literature. Mata and Cebrian (2013) discovered filamentous fungal growth on 20-80 percent of plate samples taken from short shoots in the north-central Gulf of Mexico (Mata and Cebrian, 2013). Endocrine disrupter chemicals (EDCs) are increasingly common in coastal waters because of wastewater effluent. Seagrass habitats have been documented to be adversely affected by these chemicals at relatively low exposures (Sárria et al., 2011; Adamakis et al., 2018). EDCs have been measured at varying concentrations throughout Tampa Bay, where deteriorating sewage infrastructure results in occasional sewage overflows. This increase in EDCs may have prevented normal fruit growth and contributed to immature fruit mortality during the 2017 reproductive survey.

As with inflorescence development, fruit maturation might be influenced by salinity. Several reproductive studies in Tampa Bay have observed mature fruits only at locations with higher salinities (Lewis et al., 1985; Durako and Moffler, 1987c). However, because numbers of dehisced fruits were extremely low at all locations through during this study, more data is needed to draw conclusions on the influence of salinity on fruit maturation.

4.1.4 Seedling

Seedling presence was extremely low at all locations surveyed (Table 9). Turbulence, sedimentation, and herbivory can reduce survivorship of seedlings and alter reproductive success (Balesti and Cinelli, 2003; Tuya et al., 2017). Sites at Weedon Island, WD07, and Cockroach Bay, CB02 and CB06, are heavily utilized by motorized vessels, while locations on Coquina Key are protected by a shallow sandbar. This difference in water movement may explain why seedlings were present in one location and not the other. Additionally, herbivory is reported as another strong environmental regulator of seedling survival (Tuya et al., 2017). Tampa Bay is home to a large population of pinfish, *Lagodon rhomboides.* Diet of the pinfish, considered the most dominant fish species in a *Thalassia testudinum* bed, shifts throughout their life cycle, becoming increasingly dominated by plant material (Stoner, 1980). Fish over 100 mm in length are almost entirely herbivores, feeding directly on seagrass biomass (Stoner, 1980). During this study, observations of large pinfish consuming *Thalassia testudinum* reproductive structure were documented. Structures primarily consumed by these fish are high in sugars and carbohydrates and include fruits and seedlings, which have enough carbohydrate storage to sustain the seedling for up to six months post-dehiscence (Whitfield et al., 2004). This herbivory likely reduced the quantity of seedlings observed throughout the bay and emphasizes the importance of considering the entire trophic community to fully understand seagrass restoration.

Another factor suppressing seedling density might be a result of vivipary. Upon release, seedlings are negatively buoyant, sink and are vulnerable to benthic currents until primary roots form. Due to vivipary, the seedling has already germinated when released, resulting in reduced time required for cotyledon emergence. Shortly after these first leaves emerge, photosynthesis is initiated. If primary roots have not formed, seedlings become buoyant, migrating up the water column, where they become susceptible to wind, waves, and surface currents. In June and July, Tampa Bay often is plagued with afternoon storms which produce strong winds that can sweep seedlings out to sea. This phenomenon is well documented throughout the literature and enables buoyant seedlings of *Thalassia testudinum* to dispersal over long ranges (Whitfield et al., 2004).

4.2 Reproductive Effort Variability Within a Seagrass Bed

Regardless of the site, short shoots located in the interior of a seagrass meadow produced significantly more flowers than those on the edge of the bed (Table 11). As mentioned earlier, the interior seagrass bed is often more established than the short shoots on the edge which are associated with recent colonization (Kenworthy et al.,1982; Durako and Moffler, 1985). Therefore, these results may reflect the age of the short shoots opposed to the location itself.

Additionally, increased flower density in the interior of the mosaic may reflect sexual spatial segregation. It has been suggested that female short shoots are most frequently located on the edge of the seagrass bed, while males occur in the interior (Durako and Moffler, 1985). Furthermore, it has been proposed that male short shoots are typically older than female short shoots due to larger vegetative structure size (Durako and Moffler, 1987). However, there is little documented support for this in the literature and phenological data reveals short shoot sex does not change over time (Scolaro, unpublished data).

4.3 Figures

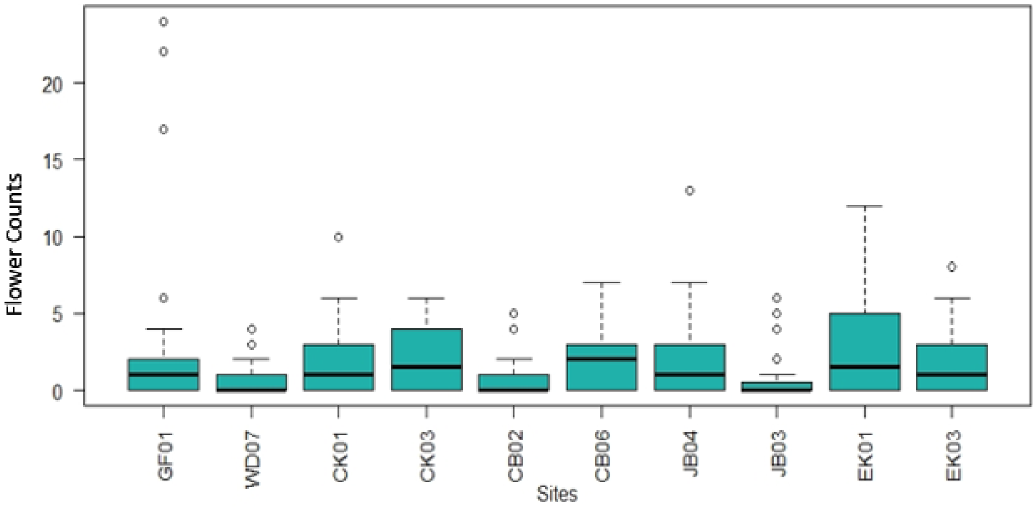


Figure 17. *Mean* Thalassia testudinum *flower densities observed at all sites during May 2017 in Tampa Bay, Florida*

CONCLUSION

Regardless of site location, the month of May was observed as peak anthesis and June was peak fruiting in 2017. This is similar reproductive timing as documented in studies from the 1970s and 1980s. These results suggest that seagrass reproductive cycles have not been affected by climate change. Although timing was similar, results indicated that in a large estuary, reproductive effort and success vary spatially. Seagrass beds adjacent to mangrove forests and freshwater sources resulted in reduced sexual reproduction due to elevated localized water quality shifts, while seagrass beds protected by sandy shoals exhibited the opposite. Future studies should investigate impacts of mangroves and freshwater pulses on reproductive capacity. Researchers should include continuous monitoring of physical water quality parameters, including salinity and light attenuation, and flow monitoring to understand how local environmental conditions and urban flood controls are affecting sexual reproduction of *Thalassia testudinum* short shoots.

Sexual reproduction is dominantly performed by older short shoots in the interior of the seagrass meadow opposed to younger ones on the meadow fringe. Results from this work also point to a "Goldilocks density", or perfect seagrass coverage. This density of seagrass coverage may provide the ideal environment--light, turbulence, temperature-- to enhance sexual reproduction in *Thalassia testudinum.* To further test this hypothesis, research should examine reproductive efforts in the center of seagrass beds, where short-shoot age is greatest, at varying seagrass densities.

Successful sexual reproduction and recolonization are critical in maintaining genetic diversity within seagrass meadows. While a subset of *Thalassia testudinum* short shoots in Tampa Bay, Florida, were observed to be healthy and robust enough to undergo sexual reproduction, the occurrence of successful seedling release, as determined by fruit dehiscence was extremely low. Furthermore, recolonization of seedlings back into the seagrass mosaic was even more rare. This suggests that that asexual reproduction is the dominant form of *Thalassia testudinum* meadow growth and expansion in Tampa Bay, Florida. Therefore, while acreage goals have been met, these meadows are not fully recovered and likely have limited genetic diversity. Reduction in genetic diversity reduces seagrass plasticity and increases vulnerability to stressors including climate change, disease, and other anthropogenic contaminants.

As we begin to experience the full impacts from sea level rise and climate change in Tampa Bay, we will be become more reliant on seagrass meadows as they replace inundated coastal habitats. Therefore, it is imperative that these habitats be a priority for enhanced management which considers reproductive capacity and genetic diversity to ensure seagrass resilience into the future. Managers can use results from this research to prioritize areas which are already exhibiting successful sexual reproduction for increased protection. Additionally, areas with reduced success should be prioritized for restoration with the consideration of introducing increased genetic material in the form of seeds or young seedlings. Increased and more informed management will ensure thriving wildlife and habitats into the future.

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APPENDIX I:

SUPPLEMENTAL TABLE

Table A1. Table of model selection. Models ranked through the AICc calculation.

Model Selection Table

Reproductive Variable	Predictors	df	logLik	AICc
Reproductive Effort	Site + Month	13	-1797.690	3021.6
	Month	4	-1570.885	3149.8
	Site+ Quadrat Location	12	-1786.885	3598
	Site+ Cover%+ Cover%^{2}	13	-1791.999	3610.3
	Site+ %Cover	12	-1793.448	3611.1
	Quadrat Location	3	-1805.773	3617.6
	Site	11	-1798.958	3620.1
	Cover%+ Cover%^{2}	4	-1809.859	3627.7
	Cover%	3	-1811.415	3628.8
Flower Density	Site + Month	13	-615.919	1258.1
	Month	4	-634.977	1278
	Site+ Quadrat Location	12	-906.420	1837.1
	Site+ Cover%+ Cover%^{2}	13	-905.482	1837.2
	Quadrat location	3	-916.674	1839.4
	Cover%+ Cover%^{2}	4	-916.236	1840.5
	Site+ %Cover	12	-910.512	1845.3
	Site	11	-912.001	1846.2
	Cover%	3	-920.339	1846.7
Fruit Density	Site+ Quadrat Location	12	-397.409	819.1
	Site+ Cover%+ Cover%^{2}	13	-398.688	823.7
	Site+ %Cover	12	-399.908	824.1
	Site	11	-401.172	824.5
	Month	4	-415.880	839.8
	Cover%+ Cover%^{2}	4	-450.319	908.7
	Cover%	3	-451.669	909.4
	Quadrat location	3	-454.744	915.5