Multiple stressors result in reduced reproductive effort by *Thalassia testudinum* in Florida Bay, USA

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ABSTRACT: Sexual reproduction remains an understudied aspect of seagrass ecology. We examined spatiotemporal variability in the percentage of short shoots with sexual reproductive structures and the proportion of sites that had flowered as an indicator of *Thalassia testudinum* sexual reproductive effort (RE) across Florida Bay, USA. Short shoots were collected annually during spring within 13 basins across the bay from 2006–2019. The sample period followed 2 very active hurricane seasons and included 2 subsequent major disturbance events, a large-scale die-off of seagrasses in 2015, and the passage of Hurricane Irma in 2017. On average, 4.7% of the collected short shoots had flowered between 2006 and 2019, ranging from 1.3–8.5% at the bay scale and 0–30% at the basin level. Regression analyses indicated that RE varied significantly among basins and years, with high multiyear variability in several basins. RE was negatively correlated with annual heat accumulation, and positively correlated with the number of days below 28°C. Annual heat accumulation rose steadily from 2006–2019; accordingly, bay-wide RE declined. RE was higher in western basins, which were the most affected by recent disturbance events, indicating a potentially important role for sexual reproduction in recovery from disturbance. However, significant reductions in RE following the 2015 die-off and Hurricane Irma show limits to the plasticity and resilience of *T. testudinum*, both in terms of reduced compensatory RE following successive disturbances and reductions in basal RE correlated with rising annual temperatures.

KEY WORDS: Seagrass · Flowering · Disturbance · Die-off · Hurricanes · Resilience · Climate change · Dispersal · Sexual recruitment

1. INTRODUCTION

Seagrasses are flowering plants that can form large clonal communities, known as beds or meadows, which are capable of long-term persistence (Reusch et al. 1999); individuals (genets) may persist for 100s to 10 000s of years or more (Arnaud-Haond et al. 2012). Under stable, low-disturbance conditions, seagrass beds can be maintained primarily through vegetative clonal growth, providing limited opportunity for sexual recruitment by seedlings (Kaldy & Dunton 1999, Vermaat 2009). Because of the difficulty in tracking seedling recruitment within dense seagrass canopies, sexual reproduction had historically been considered unimportant to seagrass meadow maintenance (Duarte et al. 2006), especially for long-lived, large-bodied seagrasses (Campey et al. 2002). However, recent application of molecular tools has shown high levels of genetic diversity within and among seagrass populations, highlighting the role that sexual recruitment can play in bed maintenance and recovery (Kendrick et al. 2012, 2017, Furman et al. 2015). In fact, intermediate levels of disturbance are thought to maximize both reproductive effort and genetic diversity of tropical seagrasses (Cabaço & Santos 2012, McMahon et al. 2017).

Turtle grass *Thalassia testudinum* Banks ex König is a long-lived, large-bodied seagrass that is widely distributed in shallow tropical to subtropical coastal waters of the western Atlantic, extending north to Bermuda, the Caribbean, and the Gulf of Mexico (den Hartog 1970). Across its range, *T. testudinum* is generally the dominant seagrass, forming large beds that can persist for hundreds of years (van Tussenbroek et al. 2006); individual short shoots of this species are thought to live from 8–10 yr (Patriquin 1973, Durako 1994) to over 20 yr (van Tussenbroek 1994). *T. testudinum* exhibits monopodial growth with low branching frequency (Durako 1994, 1995) and one of the lowest rhizome elongation rates of any seagrass (Patriquin 1973, Tomlinson 1974, Williams 1990, Duarte 1991, Kenworthy et al. 2002). Therefore, it is slow to recover from disturbance events via vegetative recruitment (Whitfield et al. 2004). There has been little research examining the contribution of sexual reproduction in *T. testudinum* meadow maintenance and recovery (Gallegos et al. 1992, Kaldy & Dunton 1999, Whitfield et al. 2004, Darnell & Dunton 2016, 2017); however, Whitfield et al. (2004) suggested that *T. testudinum* seedlings were often an important source of new recruits, and genetic diversity may be increased in physically disturbed meadows (van Dijk & van Tussenbroek 2010).

One of the largest populations of *T. testudinum* occurs in Florida Bay, USA, where it covers more than 2000 km^2 (Fourqurean et al. 2002). Within the bay, *T. testudinum* is the dominant physical feature, and its presence greatly affects physical, chemical, geological, and biological processes in the system (Durako et al. 2002). A genotypic survey of *T. testudinum* in Florida Bay in 2005 showed high levels of genetic diversity with moderate gene flow among subpopulations, suggesting a larger role of sexual reproduction in Florida Bay than previously appreciated (Bricker et al. 2011). However, the relative importance of sexual reproduction in recovery from seagrass die-off or hurricane impacts is unknown.

Sexual reproductive effort in many seagrass species is thought to increase following physical and physiological stress events, whether natural or anthropogenic (Cabaço & Santos 2012); the extent to which *T. testudinum* might follow this pattern is not yet clear. It is known to exhibit significant plasticity in flowering in response to variations in environmental factors such as water temperature, salinity (Durako & Moffler 1987), nitrogen availability (Darnell & Dunton 2017), and hurricane disturbance (Gallegos et al. 1992, but see van Tussenbroek 1994). In addition, *T. testudinum* beds exposed to higher wave energy have higher genetic diversity than more protected sites (van Dijk & van Tussenbroek 2010). Similar to the intermediate disturbance hypothesis, high rates of sexual recruitment by colonizing tropical seagrass species have been observed in meadows with moderate probabilities of tropical storm activity, but storm-related disturbance and competition between genets likely limit reproductive success (through recruitment of new genets) and genetic diversity in high and low probability locations, respectively (McMahon et al. 2017).

Here, we present data on the spatial and temporal variability of sexual reproductive effort (RE) of *T. testudinum* in Florida Bay. We defined site-level RE as the percentage of short shoots at a site with visible reproductive structures; this was aggregated to basin-level via arithmetic mean. Bay-wide RE was calculated as the proportion of sites (pooled across basins) that flowered each year. Short shoots were collected annually during spring (May) at 29–31 sample sites within each of 13 basins across Florida Bay from 2006–2019. The 14 yr sample period includes 3 major disturbance events, which provided an opportunity to assess the effects of repeated disturbance on the plasticity and resilience in RE of *T. testudinum*.

The first set of disturbances were the active hurricane years of 2004 and 2005. The fall of 2005 was the most active North Atlantic hurricane season yet recorded (Virmani & Weisberg 2006), with 3 Category 5 hurricanes (Katrina: 26 August, Rita: 20 September, and Wilma: 24 October) passing within 50 km of Florida Bay. In addition, 4 hurricanes (Ivan, Charlie, Frances, and Jeanne) passed near Florida Bay during the fall of 2004. Temporal analysis of benthic macrophyte cover and water quality (that began in 2005) indicated that hurricanes significantly affected both water quality and seagrass community structure at 15 permanent transect sites across Florida Bay for approximately 2 yr (Cole et al. 2018). The second major disturbance was the widespread 2015 seagrass die-off that affected 88 km^2 of *T. testudinum*-dominated beds in north-central and western Florida Bay (Hall et al. 2016). In the year following this die-off, seagrass communities and water quality were significantly altered (Fredley et al. 2019); sediment re-suspension events and a 3 to 4 mo algal bloom led to altered light regimes in die-off-affected basins. The third major disturbance was Hurricane Irma, which passed over western Florida Bay as a Category 4 hurricane on 10 September 2017. The large pulse of freshwater input from the storm reduced salinities and wind-generated

waves mechanically sheared and uprooted sea-grasses and mangroves, although most direct and early indirect effects of the storm on seagrasses were limited to coastal basins affected by excessive runoff (Wilson et al. 2020). Elevated chlorophyll concentrations (>10 µg l^{-1}), colored dissolved organic matter, and re-suspended fine sediments caused persistent turbid conditions in the central bay for >8 mo post Irma. The close temporal proximity between the 2015−2016 seagrass die-off and Hurricane Irma the following year allowed us to observe the effects of successive disturbance on RE in Florida Bay.

2. MATERIALS AND METHODS

2.1. Area of study

Florida Bay is located at the southern tip of Florida, bordered on the north by the Everglades, on the south and east by the Florida Keys, and to the west by the Gulf of Mexico. Florida Bay consists of approximately 50 shallow basins (0.5 to 3 m deep) separated by a network of shallower mud banks (<0.5 m, Fourqurean & Robblee 1999, Lee et al. 2016). Most of the mud banks are tidally exposed to air or nearly so (tidal range 1.0−1.5 m with a 14 d period), resulting in long hydrological residence times (6−12 mo) and limited exchange among basins (Boyer et al. 1999, Lee et al. 2016). Western basins tend to have higher phosphorus concentrations, deeper sediments, and denser seagrasses with lower leaf N:P ratios than eastern basins (Fourqurean et al. 2003). Humans have drastically altered freshwater flow through the Everglades during the past century, resulting in substantially less freshwater entering Florida Bay (Fourqurean et al. 2003). Together with high evaporative potential, Florida Bay now behaves as a reverse estuary, with annual periods of hypersalinity in the west-central portion of the system (Fourqurean & Robblee 1999, Nuttle et al. 2000).

During times of regional drought, hypersalinity can become particularly severe and has been associated with several events of mass *Thalassia testudinum* mortality (Robblee et al. 1991, Schmidt 2002, Hall et al. 2016). Schmidt (2002) reported that *T. testudinum* beds in western Florida Bay, which were exposed to salinities >60 for several months, exhibited a 'die-off' during the 1974−1975 drought. A regional drought in 1987−1991 preceded mass mortality of *T. testudinum* over large portions of central and western Florida Bay (Robblee et al. 1991). During this event, salinity steadily rose and for the years 1989−1990 often

exceeded 50 (Fourqurean & Robblee 1999). It was estimated that during the course of 4 yr, 230 km^2 of *T. testudinum* were negatively affected and 40 km^2 were completely lost (Robblee et al. 1991). For nearly a decade following the die-off, phytoplankton blooms and sediment re-suspension halted seagrass recovery and caused secondary mortality due to light limitation (Fourqurean & Robblee 1999, Durako et al. 2002). When turbid conditions subsided, re-colonization and recovery of seagrasses took another 7−10 yr before reaching pre-die-off conditions (Hall et al. 2016).

The most recent widespread *T. testudinum* die-off began in Florida Bay in the summer of 2015 (Hall et al. 2016). Initial water quality sampling determined that the water column in die-off regions was highly stratified with warm (>36°C), hypersaline (>60) bottom water, exceeding known temperature (Zieman & Wood 1975) and salinity (Koch et al. 2007a,b) limits for *T. testudinum.* Between 6 and 22 July 2015, hourly monitoring at the Everglades National Park (ENP) marine monitoring network (SFNRC 2019) Buoy Key (BK) platform did not record salinities below 60. The subsequent die-off ranged from small patches of dead *T. testudinum* in otherwise healthy-appearing beds to the near loss of entire basins. The location, initiation rate, and physiognomy of the 2015 die-off were similar to the 1987 event, suggesting similar causal factors: hypersalinity, high water temperature, low dissolved oxygen, and sulfide intrusion in areas with very dense *T. testudinum*, high sediment organic content, and high porewater sulfide (Hall et al. 2016, Johnson et al. 2018).

2.2. Sampling design

All study data were collected in Florida Bay (Fig. 1) as part of the annual spring sampling of the south Florida Fisheries Habitat Assessment Program (FHAP). FHAP is a long-term, status and trends assessment program, which began in 1995 in response to the large-scale die-off of *T. testudinum* in Florida Bay in the late 1980s and early 1990s (Robblee et al. 1991, Durako et al. 2002). FHAP is one of several multi-agency coordinated monitoring programs implemented in the south Florida region to detect changes in regional-scale seagrass distribution and abundance (Fourqurean et al. 2002). Since 2006, FHAP sampling has been conducted annually during May in 17 basins (Fig. 1). The sampled basins are representative of the range of physicochemical conditions and gradients across
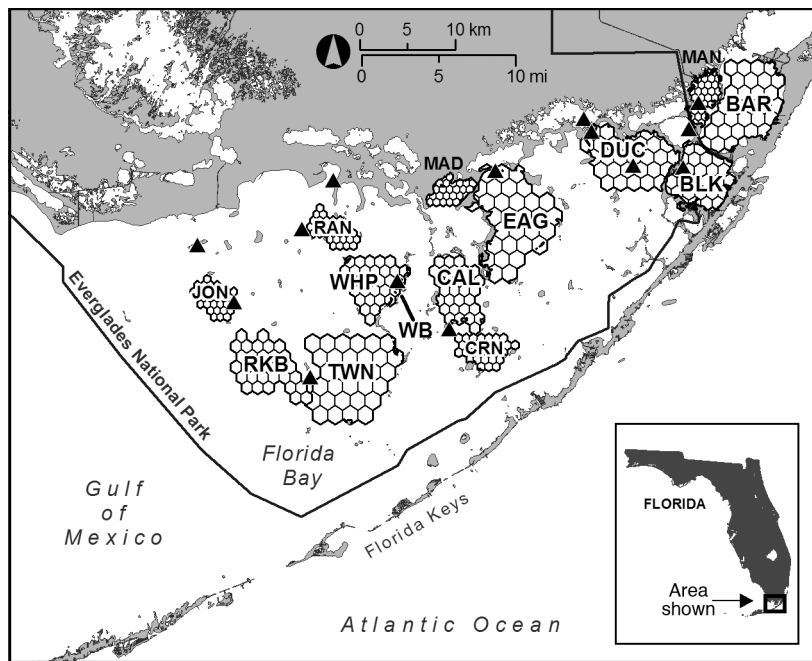
Fig. 1. Florida Bay, Florida, USA, showing the 13 of 17 basins sampled by the Fisheries Habitat Assessment Program (FHAP) that contained *Thalassia testudinum* during the current study (2006–2019). Tessellated hexagonal grid cells were sized to allow for 29–31 hexagons per basin. Black triangles denote Everglades National Park marine monitoring network stations. Whipray Bay (WB) was used to characterize bottom-water temperatures for the bay. See Table 1 for basin names

2.3. Plant parameters

Upon completion of the visual cover assessment at each sampling site, 10 short shoots of *T. testudinum* were collected at each site by breaking the shoot at the point of attachment to the rhizome. Successive shoots were collected haphazardly approximately 2 m apart if *T. testudinum* was abundant. If not, shoots were collected from individual patches within a 10 m radius of the anchored vessel. If no shoots were found within the 10 m radius, the site was considered to have no *T. testudinum* present. Before being placed on ice in labeled plastic bags, each shoot was visually assessed for the presence of reproductive structures (peduncles, male floral buds, male flowers, female floral buds, female flowers, or fruits). *T. testudinum* is dioecious, with male and female flowers exhibiting significantly different temporal patterns of development (Durako & Moffler 1987); male flowers are distinguishable earlier and disappear first. Both sexes temporally overlap during anthesis, but post-anthesis, females persist longer because of fruit formation and maturation. Although the sex of flower

Florida Bay and the Everglades. *T. testudinum* occurs in 13 of the FHAP basins (Fig. 1, see Table 1 for basin abbreviations and names). FHAP utilizes a systematic random sampling design. Each basin was partitioned into 29 to 31 tessellated, hexagonal grid cells (392 hexagons total, Table 1) using algorithms developed by the US Environmental Protection Agency (EPA) Environmental Monitoring and Assessment Program (EMAP). One sampling site per grid cell was randomly chosen each year and field-located using handheld GPS. At each site, benthic cover or abundance of all macrophytes, by either species or taxonomic/functional group, was visually estimated using a modified Braun-Blanquet cover/abundance scale (Braun-Blanquet 1932, Kenworthy et al. 1993) within 0.25 m^2 quadrats (n = 8), haphazardly distributed within a 10 m radius of the anchored vessel (Furman et al. 2018). Water depth, temperature, salinity, pH, Secchi depth, and sediment depth—determined by inserting a graduated metal pole into the sediments—were also recorded at each site.

Table 1. Basin names, abbreviations, number of hexagonal grid cells in each basin, and number of hexes with *Thalassia testudinum* short shoots collected during all sampling events for the 13 Florida Bay basins sampled by the Fisheries Habitat Assessment Program for *T. testudinum* reproductive effort

Basin	Abbreviation	Sample hexes	Hexes with complete time series
Barnes Sound	BAR	30	21
Blackwater Sound	BLK	29	16
Calusa Key Basin	CAL	39	26
Crane Key Basin	CRN	30	25
Duck Key Basin	DUC	30	27
Eagle Key Basin	EAG	31	30
Johnson Key Basin	JON	39	13
Madeira Bay	MAD	30	22
Manatee Bay	MAN	30	20
Rankin Lake	RAN	30	13
Rabbit Key Basin	RKB	31	29
Twin Key Basin	TWN	31	23
Whipray Bay	WHP	30	23

buds is easily distinguished by the presence of a simple inferior ovary in pistillate flowers (Orpurt & Boral 1964), the sex of shoots possessing only peduncles cannot be determined in the field because the numbers of flowers per shoot overlap between male (range 1–5) and female short shoots (range 1–3, Durako & Moffler 1985a). Therefore, to assess site-level *T. testudinum* RE (proportion of short shoots in flower) we pooled sexes, regardless of reproductive stage, and only report counts or percentages of short shoots that exhibited reproductive structures. We recognize that RE as defined here is not equivalent to reproductive output, fecundity, or success, but rather reflects the relative degree to which carbon resources were shifted from storage or somatic growth toward reproduction, consistent with RE as calculated by Cabaço & Santos (2012). To obtain a rough estimate of possible sexual reproductive output, we report the proportion of shoots that had female reproductive structures (flower buds, anthesis, or developing fruits). To quantify bay-wide RE and relate it to annual thermal regime, we calculated the proportion of sites that had flowered in a given year (i.e. at least 1 short shoot with a sexual structure at a site, with all sites pooled across the bay).

2.4. Bottom-water temperature

Because flowering phenology in *T. testudinum* is thought to be a temperature-mediated process (Moffler et al. 1981, Phillips et al. 1981), we attempted to correlate inter-annual variability in bay-wide *T. testudinum* RE to thermal regimes. Bottom-water temperatures were obtained from the ENP marine monitoring network (Stabenau & Kotun 2012, SFNRC 2019) for 31 May 2005 to 1 May 2019. We selected 10 ENP platforms distributed throughout the bay (designated BA, BK, BS, DK, JK, LM LR, MB, TC, and WB). Of these, only Whipray Basin (WB) had a complete time-series of daily mean bottom-water temperature. The mean (±SE) absolute difference in daily temperature among the 10 platforms was 1.12 ± 0.01°C; thus, although the 10 platforms did not cover the spatial extent of the 13 sampled basins, we considered the WB station to be representative of the bay as a whole, and reasoned that basins with offset thermal regimes would still co-vary inter-annually (i.e. a colder basin or a basin that takes longer to warm to summer temperatures would still be warmer in a warm year as measured by the WB station). To summarize annual thermal regimes, we defined year for any given RE estimate as 31 May the previous year to

1 May immediately prior to FHAP sampling. Based on published thermal tolerance and productivity data (Phillips 1960, Glynn 1968, Zieman 1975, Zieman et al. 1989, Zieman & Wood 1975), we calculated 5 measures of thermal exposure: (1) heat accumulation following the winter low (identified after passing 30 d running mean filter), (2) number of days in the optimal range of 28–32°C, (3) number of days above 32°C, (4) number of days below 28°C, and (5) total annual heat accumulation (the numeric sum of daily mean temperatures during each annual interval).

2.5. Statistical analyses

Statistical analyses were completed using SAS for Windows version 9.4 (SAS Institute) and R version 3.2.4 (R Development Core Team). To compare temporal variation in RE of *T. testudinum* among basins within Florida Bay, we assessed data on samples collected only from hexes in which *T. testudinum* shoots were present for the complete time series (288 hexagons, Table 1; see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m647p065_supp. pdf). This allowed for unbiased among-year comparisons of basin- and bay-scale RE. Within this dataset, most samples had short shoots with no apparent reproductive structures. To account for the high incidence of zeros and overdispersion, we used a log-linear regression model fit to zero-inflated negative binomial (ZINB) distributions (Table S1, Ridout et al. 2001). Differences in RE (dependent variable) among basins and years (independent explanatory variables) were assessed by comparing least-squares mean differences in parameter estimates calculated using a log-linear ZINB regression model implemented by the SAS GENMOD procedure. Spearman's rank correlation was used to determine whether basin-level RE was correlated with *T. testudinum* Braun-Blanquet cover and whether aspects of the annual thermal regime were related to bay-wide RE. Statistical significance was assessed at $\alpha = 0.05$.

3. RESULTS

A total of 5302 sites were sampled in the 392 hexagons within 13 basins over the 14 yr period from 2006 to 2019 (a total of 52 798 *Thalassia testudinum* short shoots collected); 4018 of the sites (75%) were within the 288 hexes that had *T. testudinum* present every year (40 041 short shoots, Table 1). Most of the sites lacked shoots with reproductive structures, resulting

in a frequency distribution that was highly zero inflated (Fig. 2). Overall, 4.7% of the collected short shoots had floral structures. However, on one occasion each, either 90% (2013, RAN Site 26) or 80% (2011, RKB Site 42) of short shoots were reproductive (Table 2). Flowering short shoots were observed in all 13 basins, with mean RE at the basin level for the 14 yr period ranging from 1.74 ± 0.23% in BLK to 15.31 ± 0.92% in RAN (mean ± SE; Table 2; Fig. S1). At the shoot level, RE was slightly female biased, with 51–68% of the reproductive shoots within all 13 basins having either female buds, flowers, or developing fruits (Table 2). RE over the study period exhibited multiyear cyclicity of peaks from 2006–2013 and then declined to below 4% from 2014–2019 (Fig. 3a). A very similar temporal pattern was observed for the proportion of sites with flowers (Fig. 3b), indicating that during years of higher RE, flowering was also more widespread, and during years with lower RE, flowering was more spatially restricted. RE ranged from 0 to >30% at the basin level, and, along with *T. testudinum* cover, RE was generally highest and most temporally variable in the western basins (Fig. 4). Basin-level Spearman correlation analyses indicated that RE was weakly (coefficients ranging from −0.11 to 0.30), but significantly correlated with *T. testudinum* cover in 5 of the 13 basins: positively correlated in 4 basins (BLK = 0.12, JON = 0.19, MAD = 0.12, and RAN = 0.30) and negatively correlated in DUC (−0.11). The 2 western basins (JON and RAN) with the highest correlation coefficients also exhib-

Table 2. Summary of reproductive effort (RE) of *Thalassia testudinum* in 13 Florida Bay basins during May from 2006 to 2019 (SS: short shoots). Basin abbreviations as in Table 1

Basin	N	Reproductive SS (%)		Female SS (%)		Max RE
		Mean	SE	Mean	SE	(per 10 SS)
BAR	409	2.29	0.33	1.34	0.22	7
BLK	378	1.74	0.23	1.03	0.18	3
CAL	416	3.89	0.37	2.12	0.26	4
CRN	412	1.89	0.27	1.10	0.19	5
DUC	417	3.40	0.33	2.27	0.27	5
EAG	433	3.42	0.31	1.94	0.23	4
JON	390	6.34	0.62	3.46	0.39	7
MAD	405	5.73	0.47	3.94	0.40	5
MAN	406	2.61	0.32	1.82	0.28	6
RAN	367	15.31	0.92	8.84	0.67	9
RKB	432	5.83	0.52	3.60	0.41	8
TWN	426	5.09	0.45	2.85	0.33	6
WHP	411	7.10	0.58	3.93	0.44	7

ited the largest declines in cover following the 2015 die-off with associated declines in RE to near zero (Fig. 4).

Fig. 3. (a) Bay-scale reproductive effort (mean ± SE) and (b) proportion of sites with reproductive shoots of *Thalassia testudinum* during May in 13 Florida Bay basins from 2006 through 2019. Gray bars indicate approximate times of the 2015 seagrass die-off and passage of Hurricane Irma in 2017
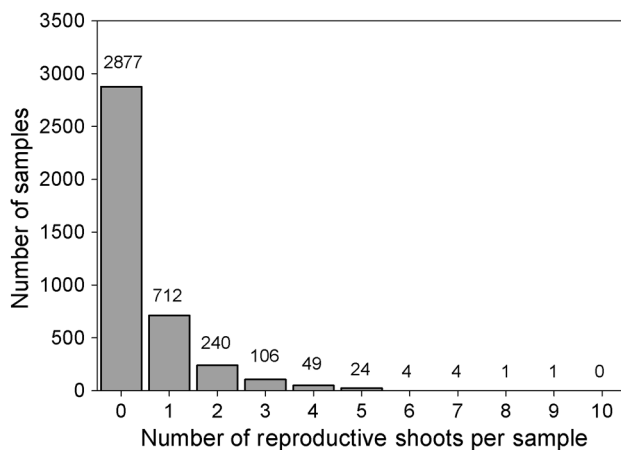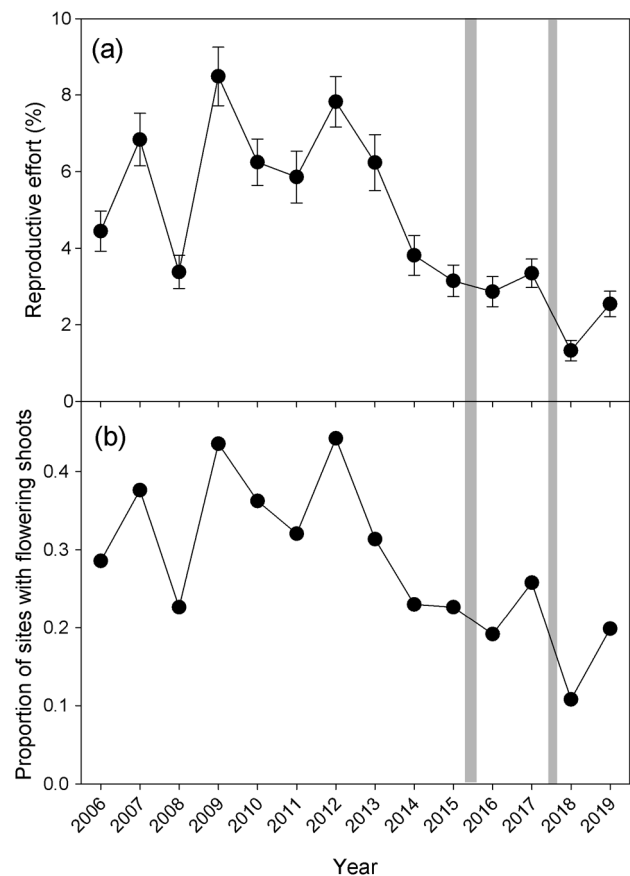

Fig. 2. Frequency distribution of sexual reproductive effort of 10 short-shoot samples of *Thalassia testudinum* collected from 13 Florida Bay basins each May from 2006 through 2019
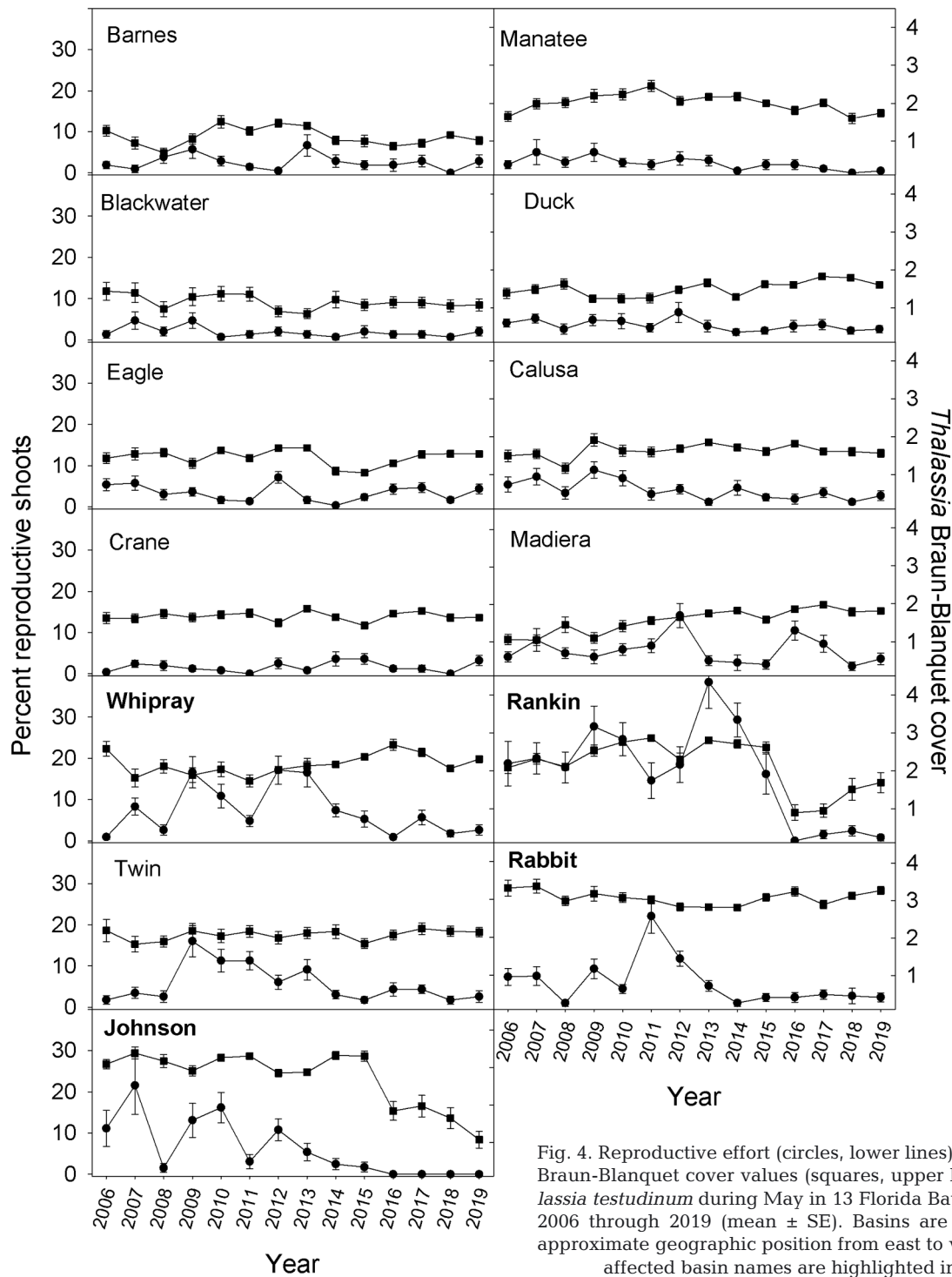
Fig. 4. Reproductive effort (circles, lower lines) and average Braun-Blanquet cover values (squares, upper lines) of *Thalassia testudinum* during May in 13 Florida Bay basins from 2006 through 2019 (mean ± SE). Basins are arranged by approximate geographic position from east to west. Die-off-affected basin names are highlighted in **bold**

Comparisons among least-squares mean differences of the maximum likelihood parameter estimates from ZINB regression analysis (Table S1) indicated that spatial and temporal variation in RE was significant among basins and years (Figs. 5 & 6). Confirming the trend shown in Fig. 4, the least-squares mean differences among basins also indicated that RE was higher in western basins (RAN, JON, WHP, RKB, and TWN, Fig. 5). RAN had the highest overall RE of any basin during the study period (Fig. 5), although this fell to zero in the year following the 2015 die-off and remained low
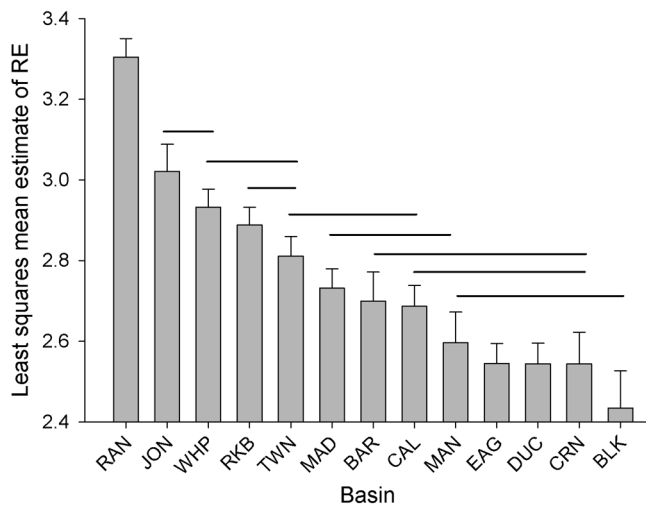
Fig. 5. Least-squares mean-difference comparisons among 13 basins in Florida Bay of maximum likelihood parameter estimates from zero inflated binomial regression analysis of log-transformed reproductive effort (RE) data for *Thalassia testudinum* during May from 2006 through 2019 (mean ± SE). Lines indicate basin least-squares mean differences, which are not significant at p < 0.05
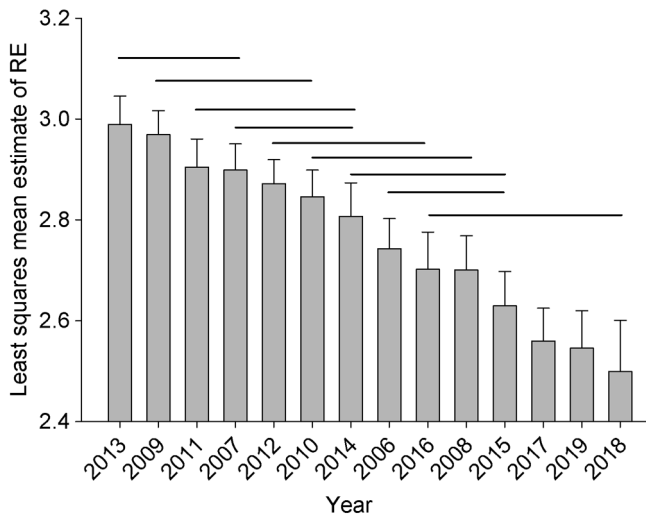

Fig. 6. Least-squares mean-difference comparisons among years of maximum likelihood parameter estimates from zero inflated binomial regression analysis of log transformed reproductive effort (RE) data for *Thalassia testudinum* during May from 13 basins in Florida Bay (mean ± SE). Lines indicate year least-squares mean differences, which are not significant at p < 0.05

through 2019 (Fig. 4). Following the active hurricane seasons of 2004 and 2005, overall RE increased from 2006 to 2007, dropped significantly in 2008, and then increased to its highest level in 2009 (Fig. 3). The increased RE from 2006 to 2007

occurred in both eastern and western basins (BLK, CAL, CRN, JON, MAD, MAN, RAN, TWN, and WHP; Fig. 4). RE was highest in years preceding the 2015 die-off and Hurricane Irma, with peaks in 2007, 2009, and 2012 (Fig. 3). RE declined to below 4% starting in 2014 and remained low following the 2 disturbances (Figs. 3 & 6). Of the 182 basin-level samplings over the 14 yr, there were only 7 occurrences (3.8%) where no reproductive shoots were observed within an entire basin (BAR: 2018, CRN: 2011 and 2018; JON: 2016, 2018, and 2019; RAN: 2016); 6 of these (86%) occurred after the 2015 seagrass die-off.

The Spearman's rank correlation matrix showed 4 significant correlations between either year or bay-wide RE and thermal regime (Fig. 7). The proportion of the bay that flowered each year was negatively correlated with total annual heat accumulation (r = −0.746, p < 0.002), and positively correlated with the number of days below 28°C (r = 0.573, p = 0.032). During the period of record (2006–2019), annual heat accumulation rose steadily (i.e. varied positively with year; r = 0.688, p < 0.007) and, accordingly, bay-wide RE declined (r = −0.638, p = 0.014).

4. DISCUSSION

4.1. Disturbance and sexual RE

Over the 14 yr period from 2006−2019, we observed an overall RE of 4.7% for *Thalassia testudinum* short shoots collected during May at 13 basins across Florida Bay, with higher levels of RE (5.8−15.3%) in western basins (JON, RAN, RKB, and WHP) and lower levels (1.7−3.9%) in northeastern basins (BAR, BLK, CAL, CRN, DUC, EAG, and MAN). The levels of RE we observed were generally within the ranges of 3.9−17.8% reported for this species and other large-bodied seagrasses (Gallegos et al. 1992 and references therein). Reproductive shoots were slightly female biased, with 51−68% of the reproductive shoots having recognizably female floral structures (Table 2). Although this female bias may be an artifact reflecting earlier anthesis and the more ephemeral nature of male flowers in this species (Durako & Moffler 1987), our observations of developing fruits confirm the potential for seed production in all basins. The 4 western basins with the highest RE were greatly affected by both the 1987−1990 (Robblee et al. 1991) and 2015−2016 (Hall et al. 2016) seagrass mortality events, with RAN, the basin with the highest overall RE, also having a die-
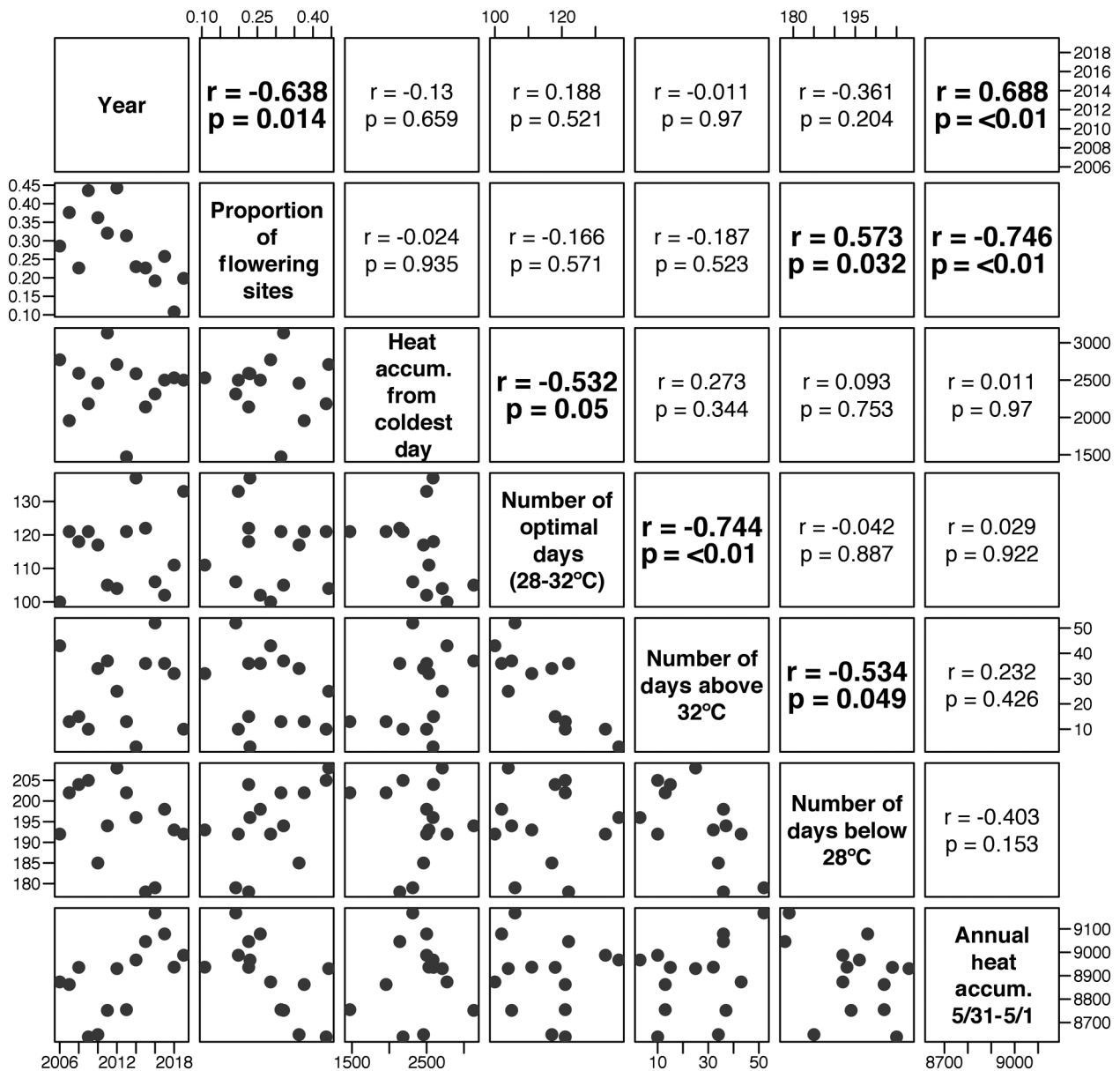
Fig. 7. Spearman's rank correlation matrix used to assess monotonic relationships between bay-wide reproductive effort (RE) of *Thalassia testudinum*, calculated as the proportion of sites that flowered during annual Fisheries Habitat Assessment Program (FHAP) sampling in May of each year, and various metrics of the thermal regime summarized between 1 May prior to FHAP and 31 May in the previous year. Significant correlations and their p-values are shown in bold

off in 1974–1975 (Schmidt 2002). Western basins are characterized as having the densest *T. testudinum* beds with the largest shoots and highest above- and below-ground biomass in the bay (Hackney & Durako 2004). The high RE there could be a product of relatively high belowground storage reserves (70–87% of total biomass is underground; Hackney & Durako 2004), which allows for more resources to be allocated to the production of physiologically costly reproductive structures (Durako & Moffler 1985b).

Cabaço & Santos (2012) observed a significant positive relationship between rhizome diameter and compensatory RE among 7 small- and large-bodied seagrass species, suggesting that species with higher storage reserves had higher capacity to invest in sexual reproduction. However, *T. testudinum* was an outlier in this relationship, having the largest rhizome diameter but a relatively low capacity to increase RE in response to disturbance (Cabaço & Santos 2012).

The western basins also have the highest salinities, deepest sediments, and highest P availability in an otherwise P-limited system, resulting in potential N-limitation in *T. testudinum* (Fourqurean & Zieman 2002, Cole et al. 2018, Fredley et al. 2019). Darnell & Dunton (2017) reported that *T. testudinum* in Lower Laguna Madre produced more flowers under low N conditions. They also observed increased somatic growth (larger and leafier short shoots) in response to N enrichment. Thus, the relatively high levels of RE in the western basins may reflect characteristically dense beds with large, N-limited shoots. In contrast, the northeastern basins are characterized by lower salinities, sediment depths, and P availability, resulting in smaller *T. testudinum* shoots with lower areal cover and RE. At the bay scale, RE was positively correlated with *T. testudinum* cover (n = 4018, Spearman rank correlation coefficient = 0.122, p < 0.001); this was driven by 4 significant basin-scale positive correlations (BLK, JON, MAD, and RAN). Alternatively, increased RE in the western basins could also be a response to greater disturbance from repeated die-offs (McMahon et al. 2017). All 3 hypotheses are consistent with observations of increased RE or clonal richness in response to natural and anthropogenic perturbation (Keddy & Patriquin 1978, Plus et al. 2003, Greve et al. 2005, Lee et al. 2007, Kim et al. 2014, Darnell & Dunton 2017, Paulo et al. 2019), with the magnitude of the positive responses correlated to the level of disturbance (McMahon et al. 2017) or carbon storage capacity (Cabaço & Santos 2012). In our study, JON and RAN had high cover and RE and the highest correlations between the 2 over time. However, both metrics were greatly reduced following the 2015 die-off. This argues against a compensatory increase in RE in response to disturbance. Rather, prolonged reduced water clarity that followed each major disturbance, particularly Irma, likely reduced photosynthetic capacity, altered carbon storage patterns, and hindered RE. Possible evidence for this may be the differential, albeit slight, rebound in RE and *T. testudinum* cover in RAN, but not JON, following Hurricane Irma (Fig. 4); marginally better water clarity in RAN (Fig. S2) may have allowed for higher photosynthetic rates, which contributed to greater RE post disturbance.

There were significant inter-annual variations in RE of *T. testudinum* in many of the Florida Bay basins (Figs. 3, 4, & 6), with flowering peaks in 2007, 2009, and 2011−2013 followed by significantly reduced RE from 2014−2019. Durako & Moffler (1987) and Galle-

gos et al. (1992) suggested that large inter-annual variations in flowering intensity in *T. testudinum* were related to environmental perturbations. Gallegos et al. (1992) observed that *T. testudinum* RE increased steadily, from 3 to 11%, in the 3 yr following hurricane Gilbert in 1988 (but see van Tussenbroek 1994). In contrast, we observed cyclical bay-scale peaks in RE 2, 4, and 7 yr following the very active hurricane seasons of 2004 and 2005, with lower RE in the intervening years. The cyclical nature of RE may reflect the high resource costs associated with sexual reproduction, which may reduce the proportion of reserves available for flower production the following year (Durako & Moffler 1985b). Except for JON, highest RE was observed 4 or more years after the 2005 hurricanes both in western basins (RAN, RBK, TWN, and WHP) and in several northeastern basins (BAR, BLK, and CAL). Cole et al. (2018) observed that the passage of the 3 hurricanes in 2005 significantly perturbed both the water quality and macrophyte communities of Florida Bay for approximately a 2 yr period. We speculate that these perturbed conditions may explain the lagged RE response, although pre-disturbance data on RE would be needed to confirm this. Alternatively, time-lags in RE post hurricane disturbance could arise from storm-induced demographic changes, whereby losses of older shoots with greater size, biomass, and metabolic demand could have led to increased sexual (seedling) or asexual (new young shoots) recruitment with associated developmental delays in flowering, typically thought to be 1−5 yr for a given short shoot (van Tussenbroek 1994, Witz & Dawes 1995). Demographic responses may have contributed to the slight uptick in RE in RAN 2 yr post Hurricane Irma, when seedlings observed during early post die-off surveys (B. T. Furman pers. obs.) would have matured; however, the role of demography in compensatory RE remains a critical knowledge gap for any seagrass species. At the bay scale, 4 yr of the lowest RE (2016–2019) followed the 2015 die-off, and the lowest RE of the entire study period (2018; Figs. 4 & 7) directly followed the passage of Hurricane Irma in 2017.

In a review of information on the relationship between RE of seagrasses and disturbance, Cabaço & Santos (2012) reported that in 72% of the cases RE increased, in 25% it decreased, and in 3% of the cases no change was reported. These authors suggested that increased RE of seagrasses in response to disturbance or stress may enhance their resilience by increasing genetic variability among populations, facilitating adaptation to changing conditions. In an

experimental study, Hughes & Stachowicz (2004) argued that increased genotypic diversity also increases resistance to natural disturbances. Sexual reproduction allows dispersal to areas of lower physiological stress and to recently denuded areas, providing additional nodes of vegetative infilling. McMahon et al. (2017) reported that intermediate levels of physical disturbance resulted in increased genetic diversity in tropical seagrasses, but that both low and high levels of disturbance tended to depress genetic diversity. In Florida Bay, we observed the lowest RE in 4 western basins in Florida Bay for 4 yr following the 2015 die-off. Notably, this time period included die-off-related turbidity and algal blooms, direct and indirect impacts from Hurricane Irma, and resulting long-term degradation of water quality in the western basins. Taken together, this indicates that there may be limits to the resilience of *T. testudinum* in terms of compensatory increases in RE following repeated disturbance — particularly if associated with prolonged indirect effects on light availability. During times of chronic stress, *T. testudinum* may reduce allocation of storage reserves to sexual reproductive structures in an effort to preserve the long-lived genet.

4.2. Thermal effects on RE

Nested within the pulsed disturbances of die-offs and hurricanes (usually associated with a compensatory RE response), Florida Bay also has a long-term trend in rising bottom-water temperature. Florida Bay is characterized by periods of high water temperatures (>30°C) and highly variable salinities, with historic reductions in freshwater inflow leading to increased occurrence and severity of hypersalinity (Herbert et al. 2011). Over the past 35 yr, average water temperatures during the warmest months have increased by 1°C in western Florida Bay (Carlson et al. 2018), reflecting a 'tropicalization' of the system. Remote sensing data indicate that dense seagrass beds in the western bay also contribute to their own thermal stress because of their reduced albedo relative to bare white carbonate sediments (Carlson et al. 2018). Extremely high water temperatures (several days with temperatures >30°C and a maximum of 35.9°C) were associated with the 2015 seagrass die-off in Florida Bay (Hall et al. 2016, Fredley et al. 2019). The bay also exhibited an increasing trend in salinity from June 2012 to June 2015, peaking in western basins (maximum 66.3) immediately before die-off was observed in July 2015 (Fredley et al. 2019). Durako & Moffler (1987)

observed reduced flowering associated with temperature and salinity stress in *T. testudinum* in Tampa Bay, Florida; in addition, mature fruits and viable seeds were only observed in areas with near-seawater salinities. Similarly, RE in the 4 basins most affected by this die-off (JON, RAN, RKB, and WHP) all exhibited decreases in RE 1–3 yr prior to the 2015 mortality event (Fig. 4), suggesting that both high temperatures and/or high salinities can reduce flowering in this species. The passage of Hurricane Irma in the fall of 2017 reduced salinities, but also initiated water clarity issues across the bay, which continued for the balance of our observational period and contributed to light stress.

The warming trend results in greater heat accumulation between bud initiation and anthesis, and a reduction of cool-water days during historical periods of metabolic torpor (Fig. 7). Our correlative analyses suggest that this trend is associated with bay-wide declines in RE, resulting in less of the *T. testudinum* population contributing to RE in a given year. The precise mechanisms of this are not yet clear, but we hypothesize that increased metabolic demand during the winter could result in less carbon available to allocate to floral structures in the spring. Since climate predictions for South Florida include rising annual mean temperatures, over time, flowering and seedling recruitment might play a reduced role in the population dynamics of the species in this region. If sexual recruitment is important to recovery, then the loss of RE could have profound implications for the resilience of this important foundation species. Understanding the role of sexual recruitment in recovery is therefore a paramount concern.

4.3. RE, seedling recruitment, and resilience

A genotypic survey of 10 spatially separated *T. testudinum* populations across Florida Bay in 2005 indicated high genetic diversity within and among the populations, suggesting that effective seedling dispersal and sexual recruitment may be regular features of this system (Bricker et al. 2011). These data also showed directionality to gene flow, with seedlings originating in northeast basins (DUC and EAG) contributing to populations in the west and south (to JON, RAN, RKB, and WHP). This pattern runs counter to our observations of RE, where recipient subpopulations would have had higher RE than source subpopulations. Gene flow patterns are also at odds with the geologic history of the bay (Scholl et al.

1969). Based on the southwesterly slope of the underlying carbonate platform and resultant sequence of flooding, recruitment and migration would be expected from southwest to northeast. Nevertheless, the directionality of recruitment as inferred from Bricker et al. (2011) is consistent with patterns of wind-driven surface water movement within the bay, which has strong westward and southward components for much of the year (Smith 2005). Given that Bricker et al. (2011) obtained short shoots in the fall of 2005, their observations may reflect recruitment processes that occurred during recovery from the 1987 die-off. Thus, the relatively unaffected northeastern basins, which based on our data exhibited low but consistent RE, may have served as the source of recruits post 1987 die-off.

Low rhizome elongation and branching rates and lack of remnant *T. testudinum* patches in the most disturbance-affected basins also supports an important role for sexual reproduction and seedling recruitment in addition to clonal growth in seagrass bed maintenance and recovery (Darnell & Dunton 2017). Our observations following the most recent (2015) die-off are consistent with this, as haphazard shoot collections during the 2016 FHAP sampling in Rankin Lake and Garfield Bight, both severely impacted by the 2015 event, yielded high proportions of seedlings. These 1 to 2 yr old seedlings were previously unnoticed during FHAP sampling when canopy cover and shoot densities were higher.

Taken together, these findings provide additional insight into the plasticity in RE exhibited by *T. testudinum* and the role of sexual recruitment versus clonal growth, not only in meadow maintenance, but also in recovery from pulsed disturbances. The significant reduction in RE we observed for *T. testudinum* in Florida Bay following 2, temporally close, major disturbance events (2015 die-off and Hurricane Irma) indicates there may be limits to the resilience of this seagrass in terms of compensatory RE and may reflect increased allocation of resources towards vegetative expansion. Further, because these events were inset into a longer-term warming trend, we anticipate that bay-wide RE might continue to decline. Based on our time-series data, we suggest that warmer temperatures and more frequent severe tropical systems expected with climate change will lower not only basal RE, which is negatively correlated with temperature, but further reduce the population-level capacity for compensatory RE, resulting in lower genetic diversity, and thus reduced resistance of *T. testudinum* to future disturbance (Hughes & Stachowicz 2004).

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