Environ Monit Assess (2022) 194: 96
https://doi.org/10.1007/s10661-021-09489-x

Effects of freshwater release on oyster reef density, reproduction, and disease in a highly modified estuary

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Received: 24 February 2021 / Accepted: 22 September 2021 / Published online: 14 January 2022

Abstract Few estuaries remain unaffected by water management and altered freshwater deliveries. The Caloosahatchee River Estuary is a perfect case study for assessing the impact of altered hydrology on natural oyster reef (*Crassostrea virginica*) populations. The watershed has been highly modified and greatly enlarged by an artificial connection to Lake Okeechobee. Accordingly, to generate data to support water management recommendations, this study monitored various oyster biometrics over 15 years along the primary salinity gradient. Oyster reef densities were significantly affected by both prolonged high volume freshwater releases creating hyposaline conditions at upstream sites and by a lack of freshwater input creating hypersaline conditions at downstream sites. Low freshwater input led to an increase in disease caused by *Perkinsus marinus* and predation. Moderate (< 2000 cfs) and properly timed (winter/spring) freshets benefited oysters with increased

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10661-021-09489-x.

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gametogenesis, good larval mixing, and a reprieve from disease. If high volume freshets occurred in the late summer, extensive mortality occurred at the upstream site due to low salinity. These findings suggest freshwater releases in the late summer, when reproductive stress is at its peak and pelagic larvae are most vulnerable, should be limited to < 2000 cfs, but that longer freshets (1–3 weeks) in the winter and early spring (e.g., December–April) benefit oysters by reducing salinity and lessening disease intensity. Similar strategies can be employed in other managed systems, and patterns regarding the timing of high volume flows are applicable to all estuaries where the management of healthy oyster reefs is a priority.

Keywords Water management · Source-sink dynamics · Salinity · Growth · Predation · Disease

Introduction

The most recognized definition of an estuary is "a semi-enclosed coastal body of water, which has a free connection with the open sea, and within which sea water is measurably diluted with fresh water derived from land drainage" by Pritchard (1967) that came out of the 1964 Estuaries symposium (Lauff, 1967). While Caspers (1967) stressed the importance of tidal forcing, most would agree that freshwater inflow from land drainage is one of the most influential drivers affecting community structure (Sklar & Browder, 1998). Despite this recognition, few estuaries today remain unaffected by water management (Alber, 2002) and Montagna et al. (2002) stress the need for indicators that aid adaptive management and to better understand these systems.

The Caloosahatchee River Estuary (CRE) is located on the southwest coast of Florida (Fig. 1). Alterations began in the late 1800s, when it was artificially connected to Lake Okeechobee through a large channel (the Okeechobee Canal). The river has also been straightened, deepened, and dammed by the construction of three water control structures along its length (Barnes, 2005). The W. P. Franklin Lock and Dam (designated S-79), the furthest downstream structure, acts as a salinity barrier and marks the beginning of the estuary (Doering & Chamberlain, 1999). As a result of these alterations to the hydrology of the Caloosahatchee system, as much as 39–49% of the water entering the estuary comes from the lake (Armstrong et al., 2019; Liu et al., 2009; Rumbold & Doering, 2020). This contribution to the CRE comes mainly as regulatory releases intended to maintain lake water level (Scarlatos, 1988). Urbanization within the 1100 km^2 watershed has led to increased impervious surfaces, reduced retention of runoff, and reduced groundwater recharge. Storm water is not retained but runs off quickly at higher peak flows, and, because there is low watershed retention, base flows are low or nil during the dry season (Volety et al., 2009). The combination of over-draining and the addition of S-79 as a salinity barrier results in a truncated and highly variable salinity gradient in the estuary (Volety et al., 2009). During periods of low freshwater discharge, typically during the dry season, saltwater regularly intrudes all the way to the S-79 structure, where salinities often exceed 10 in the upper reaches of the estuary. In contrast, high freshwater discharge can cause salinity to drop below 5 in the lower estuary and often results in salinities near 0 at the river mouth (Volety et al., 2009).

The transition between hyper- and hyposaline conditions can be rapid, sometimes less than a week (Volety et al., 2009), leaving sessile organisms and those with a small home range vulnerable to osmotic stress and, in some cases, may exceed the salinity tolerances of oligohaline and marine species. In southwest Florida estuaries, salinity has shown to be a good predictor of community assemblages on oyster reefs (Tolley et al., 2005). Dramatic reductions in salinity due to increased freshwater flow can cause relocation of reef resident species, decrease survival of larval fishes and crabs within the estuary, and reduce reproductive output and recruitment of reef residents (Tolley et al., 2013a, 2013b) and oyster reef mortality which leads to losses in microhabitat for these species, thereby altering community structure (Sklar & Browder, 1998; Tolley et al., 2006).

The eastern oyster *Crassostrea virginica* is prevalent in Florida estuaries, as natural reefs, and is a prominent feature of the CRE. Although not locally harvested for consumption, oyster reefs are important ecosystem engineers and valued as a keystone species (Volety et al., 2014; Wasno et al., 2020). As filter feeders, oysters act as a biofilter for the estuary, removing suspended particles and, thereby increasing light penetration and supporting healthy seagrass beds (Grabowski & Peterson, 2007; Grizzle

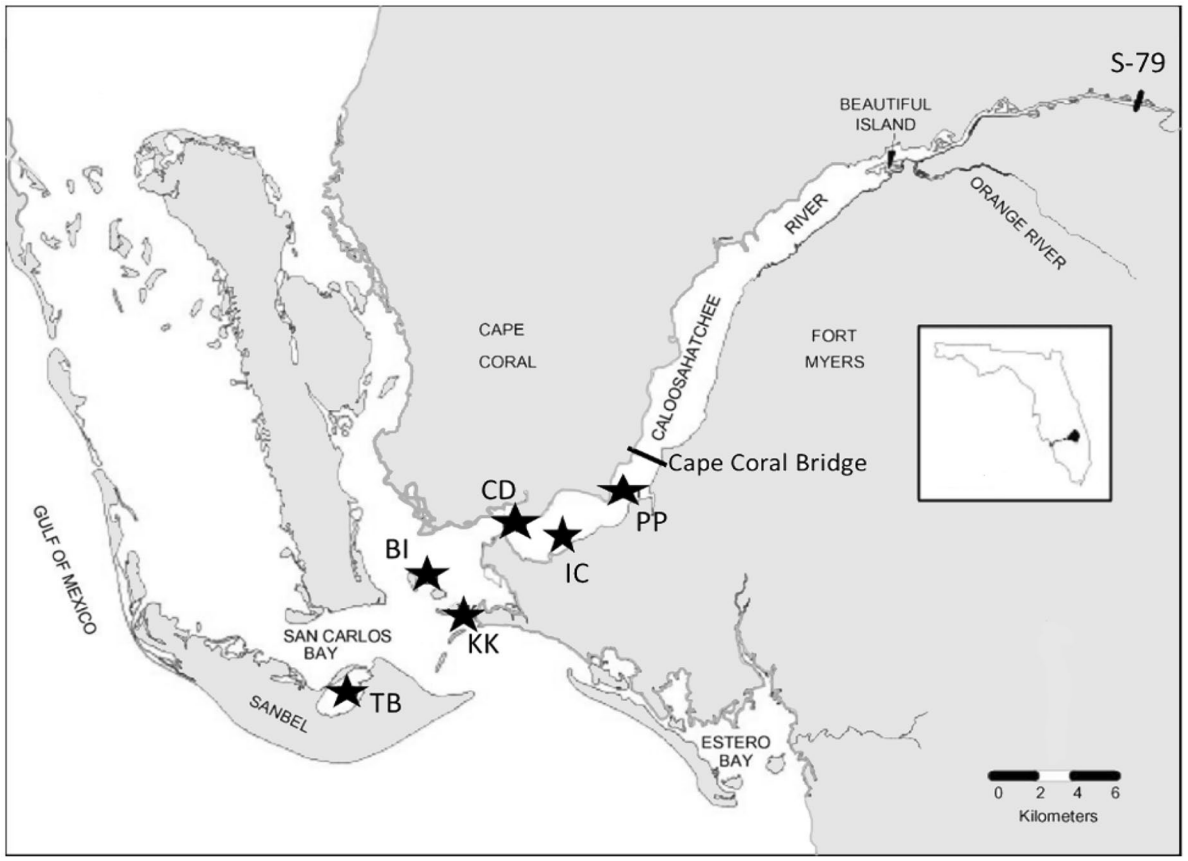

Fig. 1 Map of the study sites within the Caloosahatche River Estuary in Southwest Florida. Study sites from upstream to downstream include Peppertree Point (PP), Iona Cove (IC), Cattle Dock (CD), Bird Island (BI), Kitchel Key (KK), and Tarpon Bay (TB). The Cape Coral Bridge was the source of continuous salinity measurements and is located approximately 4.2 km upstream of the Peppertree Point sampling location. The W. P. Franklin Lock and Dam (S-79) is the site of continuous flow measurements and is located upstream of all sampling sites. This is the most downstream structure controlling freshwater flow from Lake Okeechobee and the watershed between S-79 and the lake into the CRE

et al., 2008). Oysters also ameliorate the effects of eutrophication by cycling nutrients from the water column to the benthos through their filter-feeding nature (Newell, 2004). Their three-dimensional reef structure creates habitat for many ecologically and economically important species, increasing the benefits of using the estuary as a nursery for marine species and as a permanent home for small estuarine species (Tolley & Volety, 2005; Wells, 1961). Increased oyster reef density and coverage have been shown to affect species richness significantly (Bergquist et al., 2006). Additionally, the vertical structure of reefs acts as a natural breakwater, attenuating wind and wave action and aiding in the stabilization of sediments (Piazza et al., 2005). Oysters themselves are sessile organisms that lack the ability to move once settled. Their sensitivity to environmental change, ability to manifest the cause of change directly, availability for continuous monitoring over a wide range of stressors, and the relative cost-effectiveness of such monitoring makes them an ideal indicator species (see Carignan & Villard, 2002). As the environment changes over time or large-scale acute stressors are introduced, the presence and condition of oyster reefs can serve as an indicator of ecosystem health. Oyster reefs have been one of several indicators used in the CRE to assess the impacts of water management.

Oysters are osmoconformers and therefore are sensitive to extreme alterations in salinity due to

freshwater input (Pierce, 1982; Shumway, 1977a). The optimal salinity range is reported to fall between 14 and 28 (Shumway, 1996), but regions with high seasonal freshwater input see healthy oyster populations in the salinity range of 9–13 (La Peyre et al., 2013), and oysters are capable of tolerating salinities as low as 5 for prolonged periods of time and as high as 40 (Galtsoff, 1964). Seclusion from undesirable conditions via valve closure can mitigate the effects of osmotic stress; however, prolonged exposure to extremes can be lethal (La Peyre et al., 2013). Water management of the CRE, either retaining water during droughts or prolonged releases during very wet years, drives salinity changes that can result in such osmotic stress events. When a large magnitude freshwater release occurs over a short timescale, the tolerance range is constricted compared to a gradual change during which cellular processes have time to adjust and allow for expanded tolerance. For example, acute salinity changes ≥ 15 cause increased mortality compared to gradual changes over several days that allow the animals to acclimate and therefore survive more extreme salinity exposure (McFarland et al., 2014; Volety et al., 2016). Additionally, the timing of large magnitude changes can further limit or enhance survival during exposure, making the timing of freshwater release just as critical as the magnitude and duration (La Peyre et al., 2009, 2013). Extreme decreases in salinity can have a greater impact, even for short durations, when they occur during periods in which oysters normally have maximal gametogenic activity (Andrews et al., 1959; La Peyre et al., 2003, 2013; Loosanoff, 1952) or if they occur during typical south Florida summer high temperatures, as increased metabolic stress can compound the effects of osmotic stress, amplifying the negative response (La Peyre et al., 2016; Rybovich et al., 2016). By contrast, prolonged high salinity events resulting from a lack of sufficient freshwater input can also be stressful to oysters. In addition to hyperosmotic stress, the most devastating oyster pathogen in the Gulf of Mexico, *Perkinsus marinus*, thrives at moderate to high salinities (>12) (Chu & Volety, 1997; La Peyre et al., 2009; Reece & Dungan, 2006) which can cause extensive mortality events (Southworth et al., 2010). Previous work suggests that winter/spring freshets (i.e., timed to avoid the reproductive season) allow oysters a reprieve from disease and predation pressures (La Peyre et al., 2009, 2013; Wilber, 1992). Thus, management actions must consider the physiological state of biota when estimating the potential effects of prolonged freshwater releases or the withholding and storage of freshwater.

This paper describes changes documented in the CRE oyster populations over a 15-year monitoring period (September 2000–April 2016). Both the positive and negative impacts of freshwater inflow in a highly altered system are estimated using biological data on reef density, juvenile recruitment, gametogenesis, juvenile growth, disease, and predation in relation to salinity patterns and freshwater inflows. It is anticipated that these results will serve as a tool to resource managers to assist in determining the timing and duration of managed freshwater releases.

Methods

Study site

Sites were initially selected based on the distribution of natural oyster reefs along a salinity gradient in the CRE. Five sites were chosen for the collection and measurement of adult oysters beginning in September 2000: Iona Cove (IC), Cattle Dock (CD), Bird Island (BI), Kitchel Key (KK), and Tarpon Bay (TB) (Fig. 1). In June 2004, an additional site, Peppertree Point (PP), was added for monitoring juvenile recruitment for a total of six sites. Peppertree Point was used only for recruitment and outplanted juvenile growth bags due to a lack of adults upstream of IC. During sampling in July–December 2005, November 2008, and August 2013–January 2014, no living oysters were present at IC and thus could not be collected for analysis of gametogenesis, condition index, or *P. marinus* infection.

Environmental variables

Freshwater flow (cubic feet per second, cfs, equivalent to 0.0283 m^3 per sec) at the W. P. Franklin lock and dam (S-79) and salinity at the Cape Coral Bridge were provided by the South Florida Water Management District (SFWMD). The estuary also receives inflow from tributaries to the tidal portion; however, several studies have revealed that flows at S-79 are the major driver explaining variation in salinity

and other constituents in the estuary (Doering & Chamberlain, 1999; Rumbold & Doering, 2020). Moreover, while the flow is not monitored at these tributaries on a routine basis, modeling estimates they only contribute 15.4 to 24% of the flow to the estuary (Armstrong et al., 2019). Hourly salinity and freshwater flow were averaged monthly for the characterization of year types according to criteria derived from performance measures for the CRE (Volety et al., 2014). Mean salinity values of <16, 16–28, and >28 at the Cape Coral Bridge represented wet ($n=7$), moderate ($n=5$), and dry ($n=4$) year types, respectively (Table S1) based on previous work assessing oyster populations in the CRE (Volety et al., 2009, 2014). Salinity and temperature were also recorded monthly at each site during the collection of adult oysters using a handheld YSI 6600. Salinity data from SFWMD were not available for 2001–2002, so monthly salinity data collected during on-site sampling from IC were used to define year type for that year. Data were collected over a continuous 15-year period (September 2000–April 2016) and are reported and averaged based on water year. Water years were defined by SFWMD to include a full wet and dry season in each year and began May 1 of the calendar year, the beginning of the wet season, and ended April 30 of the following calendar year, the end of the dry season. For example, the first month of water year 2006 is May 2005 and the last month is April 2006.

Density of living oysters

The density of living oysters was assessed biannually during the early spring and late summer each year (approximately February–March and July–September, respectively) from 2003 to 2016. During each visit, four 0.25 m^2 quadrats were randomly sampled during mean low tide at approximately the same tidal height on the reef and all living oysters (juvenile and adult) were counted within each quadrat. Beginning in 2010, the oyster shell lengths (longest axis) of 50 oysters (juvenile and adult) were randomly measured in the field from each quadrat to the nearest 0.1 mm using dial calipers to compare oyster size among sites and year types.

Juvenile recruitment

Oyster recruitment was monitored using shell stringers made of oyster shell to provide substrate for spat settlement. Each stringer consisted of 12 clean shells oriented inner surface down with holes drilled through the center and strung together using weighted galvanized wire (Haven & Fritz, 1985). Three stringers were deployed $1-2$ feet apart per site at monthly intervals by hanging them from PVC T's to keep the shells suspended in the water column ($10-15$ cm above bottom). Shell stringers were collected monthly and replaced with new clean stringers for continuous monitoring. Oyster spat on the underside of each shell were counted in the laboratory and expressed as the number of spat per shell per month.

Assessment of reproductive state

Adult oysters ($n=15$) were collected monthly by hand from reefs at each site for assessment of gametogenesis. Adult oysters were targeted at each site based on size, but size varied among sites ($L=58.5\pm0.2$ mm; mean $\pm$ SE; Table S1); when mortality events occurred, the sample size was reduced to only the number of adults that could be collected. In the late summer/early fall of water years 2006, 2009, and 2014, low salinity events resulted in mortality events that prevented the collection of adult oysters for gametogenesis for several months. Gametogenesis was measured to track reproduction using the standard histological technique developed by Fisher et al. (1996) and the International Mussel Watch Program (National Research Council, 1980). Whole tissue was dissected from the shell and cut cross-sectionally to include mantle, gonad, gill, and digestive tract. Tissue sections were fixed in Davidson's fixative (Fisher et al., 1996), washed in ethanol, run through a ThermoShandon Citadel 1000 automatic processor (Global Medical Instruments Inc., Ramsey, MN), and embedded in wax blocks. Tissue sections were later cut to 5-µm thickness on a HM 325 Rotary Microtome (Thermo Fisher Scientific™, Waltham, MA), mounted on slides, and stained with Harris' hematoxylin and eosin. Slides were then examined microscopically to determine the oyster gametogenic stage. Gametogenesis was ranked on a gonadal index (GI) scale of 1–5 (Volety et al., 2009), with a score of 1 indicating inactive stages with no gametogenesis

occurring, 5 when the oyster is fully reproductive and ready to spawn, and stages 2–4 indicating progressively developing gametes. GI decreases back to 1 as gametes are shed during spawning and gonads become depleted. Thus, the annual mean GI is higher when larger proportions of the population are reproductive at the same time or with a longer duration of the reproductive season. In either case, higher GI should lead to increased reproductive output (Volety et al., 2009). Spawning period duration was estimated annually for each site by summing the number of months in which gonadal index was ≥ 3.

Assessment of disease and condition index

Adult oysters ($n = 15$–20) were collected monthly by hand from reefs at each site and cleaned of epiphytic growth for assessment of *Perkinsus marinus* infection and condition index. As mentioned previously, adult oysters were targeted at each site based on size which varied among sites (Table S1), and in water years 2006, 2009, and 2014, low salinity events resulted in mortality events that prevented the collection of adult oysters for several months. Oysters were assayed for the presence of *P. marinus* cells using Ray's fluid thioglycollate (Ray, 1954). Small digestive diverticulum sections were dissected and incubated in Ray's medium for 4–6 days. Tissue sections were then stained with Lugol's iodine to visualize *P. marinus* cells and analyzed microscopically. The prevalence was reported as the percentage of infected oysters at each site and included all oysters in which *P. marinus* cells were detected. The intensity was reported on a scale based on cell density within the tissue sample using a modified Mackin scale (Mackin, 1962) from 0 to 5, with 0 representing individuals with no infection and 5 representing individuals heavily infected.

Condition index (CI) was determined as a measure of the physiological condition according to Lucas and Beninger (1985). Whole oyster tissue was dissected out of the shell and both tissue and shell were dried at 60 °C for 48 h and weighed. Condition index was calculated as $[weight\ of\ dry\ tissue(g)/weight\ of\ dry\ shell(g)] \times 100$. Higher CI values indicate better physiological condition.

Juvenile oyster growth and survival

Beginning in water year 2003, wild juvenile oysters were collected from natural reefs within the CRE, separated from the reef to deploy as individuals, and measured for initial length. Collection of juveniles was completed in the early winter (December–February) representing juveniles from the fall recruitment class and the initial size of juvenile oysters ($L = 12$–30 mm) was dependent upon availability and varied among years. Oysters were randomly placed in 0.5 mm closed wire mesh bags (1 m^2, to prevent predation) for deployment at each site (50–250 individuals per bag). The initial number of juvenile oysters per bag varied among years, but was uniform across sites within each year. Water year was assigned to each bag deployment based on summer period during which the bags were monitored for growth, so if bags were deployed January–December 2008, they would be categorized as water year 2009. This assignment was chosen because extreme low salinity due to high freshwater input in the summer months is the most frequent cause of large-scale mortality events in the CRE. Closed bags were used during the entire monitoring period of water years 2003–2016. Open bags were added alongside closed bags in water years 2009–2016 and were identical to closed bags except that they did not have a top covering, leaving them exposed to predation. Comparison of the two bag types allowed for an estimation of mortality due to predation versus mortality due to environment (both water quality and disease). At the initiation of the monitoring period (water year 2003), only one replicate bag was deployed at each site, but beginning in water year 2012, three replicate bags per type were deployed at each site. Bags were monitored monthly, and all live oysters within the bag were counted to assess survival rates. Fifty juveniles from each bag were randomly selected for shell length measurement to the nearest 0.1 mm using dial calipers to assess growth rate, then returned to the bag. The duration of the monitoring period for each deployment was approximately 12 months, however varied from 10 to 14 months among years based on juvenile survival rates.

Statistical analysis

Power regressions were performed in Sigma Plot 14 (Systat Software, Inc.) to determine relationships between inflows from S-79 (monthly means) and monthly salinities measured at various sampling locations. Adult oyster density was examined using a three-way analysis of variance (ANOVA) to determine the effects of the three factors (site, season, and year type). Gonadal index, *P. marinus* prevalence, and recruitment data failed to meet the assumptions of equal variance and normality, and therefore, the non-parametric Kruskal–Wallis test was used to test for significance among sites and year type. Pairwise comparisons were then used to identify significant differences between groups. Linear mixed models were used to determine the effects of sampling location, month, and year type as fixed effects on oyster responses (*P. marinus* intensity, *P. marinus* prevalence, and condition index). When significant differences were detected in the response means, a Tukey's HSD multiple comparison test was used to identify significant differences among treatments. Spearman rank correlations tested for relationships between biological parameters and environmental conditions (salinity and temperature) for each biological parameter separately. All data were analyzed in SPSS 22 (IBM SPSS® software). The statistical analysis of growth bag data, monthly survival, and growth rates was limited due to a lack of cage replication during the first half of the monitoring period. Generalized additive models (GAMs) allow for nonparametric parameters robust to missing data and were therefore used to test for significance in the growth data. GAMs were run with a smoothing parameter on the salinity by year type and the salinity by site factors using the gmcv package (Wood, 2011) in R Studio (version 4.0.2) to test for significant trends in juvenile growth and survival in response to salinity by site and year type using the restricted maximum likelihood (REML) method. When less than 25 oysters remained in the growth bag, the replicate was removed from growth rate analysis to avoid artificial inflation due to size-related mortality. For all measured parameters, results were deemed significant at $p < 0.05$. Results are presented as means ± standard errors. Time series plots for all biological parameters with reference to daily freshwater flow rate are included in the Supplemental Material (Supp. Figs. S1-S7).

Results

Environmental conditions

Temperature varied significantly by month ($F_{11,\ 995} = 89.029$; $p < 0.001$), site ($F_{5,\ 995} = 62.279$; $p < 0.01$), and year type ($F_{2,\ 995} = 13.940$; $p < 0.001$), but among-site comparisons were only significant at those sites monitored for only a portion of the 15-year monitoring period (PP and TB; 2004–2015 and 2001–2010, respectively). When these sites were excluded and statistical analysis included only those sites monitored during the whole 15-year period, no significant difference was detected among sites. Because seasonal variation in temperature had the greatest effect on biological parameters, data were grouped by season (winter, summer) to assess seasonality in relation to biological trends. Winter temperatures (December–February) averaged 19–20 °C with minimum values of 10.7–12.3 °C; however, these lows occurred during a particularly cold winter (January 2010), and temperatures ≥ 15 °C were more common. During summer months (June–August), average temperatures reached 30–31 °C at all sites, with a maximum of 36 °C recorded at KK in August 2010 (Fig. 2A).

Annual mean salinity was highly variable among years with sites tracking an upstream–downstream salinity gradient in all year types (wet, moderate, and dry), and the greatest difference observed during wet years (Fig. 2B). Salinity varied significantly with a site by year type interaction ($F_{10,\ 995} = 2.592$; $p < 0.01$) and a month by year type interaction ($F_{22,\ 995} = 4.491$; $p < 0.001$). Salinity differed markedly among sites, resulting in a strong gradient in which mean wet year salinity at the lower-estuary site Tarpon Bay (28.8 ± 7.1) was greater than mean dry year salinity at the upper-estuary site, Iona Cove (27.7 ± 7.7).

Salinity was significantly correlated with temperature $r_s = (\rho = -0.144$; $p < 0.001)$ at all sites, with salinity maxima in January when both rainfall and freshwater flow from S-79 were low and a salinity minimum in September after several months of increased freshwater flow (Figs. 2B, 3). During dry seasons, salinity at all sites frequently became hypersaline, exceeding 35, and occasionally exceeded 40 (Table 1). Although all sites experienced the lowest salinity levels in the summer rainy season when freshwater input was high, only TB remained above 10 throughout the monitoring period

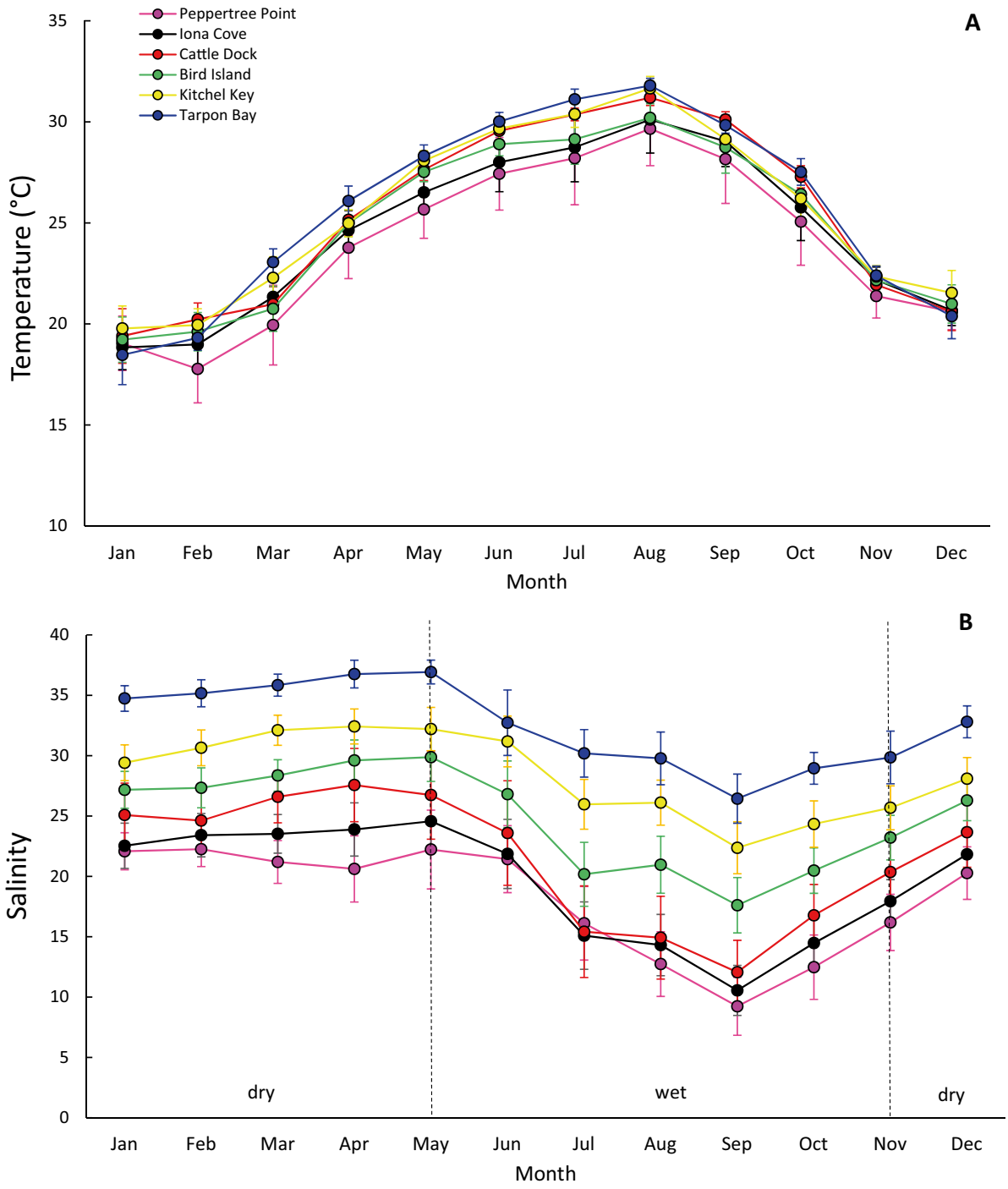

Fig. 2 Seasonal changes in temperature (**A**) and salinity (**B**) at each site averaged by month across all years. Dashed lines distinguish months included in wet and dry seasons. Error bars represent standard error

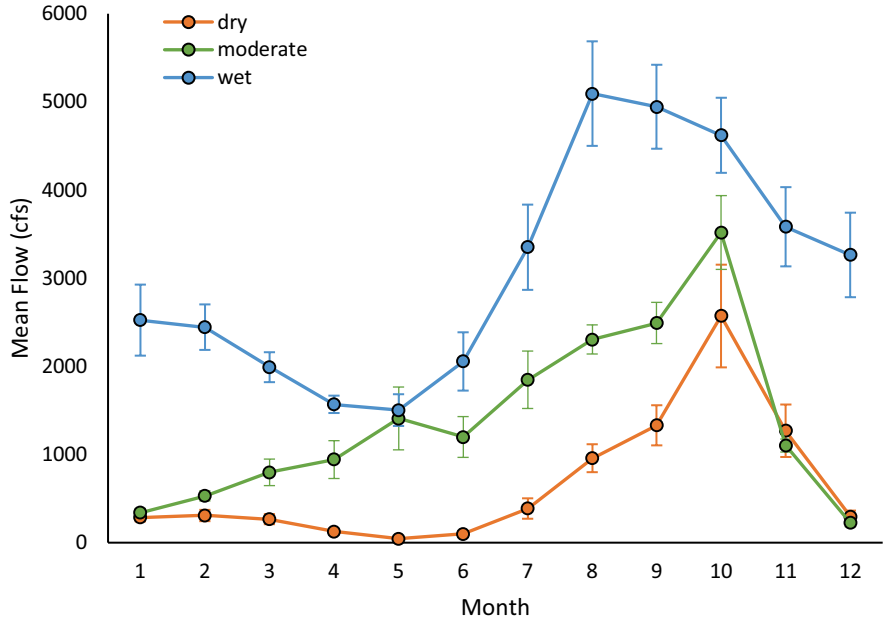

Fig. 3 Mean monthly freshwater flow measured at S-79 during wet, moderate, and dry year types. Error bars represent standard errors. The numbers on the *x*-axis represent months in the calendar year from January to December

(Table 1). All other sites experienced salinities below 5 (Table 1), and on several occasions, the most upstream sites experienced salinities of 0. When examined by the percentage of days at extremes, a clear salinity gradient along the study sites was observed with a dependence on year type (Table 1). During dry years, salinity at all sites remained above 5 (except PP). In contrast, during wet years, salinity at all sites remained below 40. A negative correlation was observed between salinity and freshwater flow at S-79 when data from all sites were pooled ($r_s = -0.613$, $p < 0.01$). Average maximum flow rates occurred late summer through early

Table 1 Percentage of months (averaged by year type) with salinity values below or above designated criteria at each site, organized from upstream (Peppertree Point) to downstream (Tarpon Bay). Salinity values were chosen to represent the duration of exposure to salinities outside the reported optimal range (16–28; Shumway, 1996). Data includes the entire 15-year monitoring period

	Peppertree Point	Iona Cove	Cattle Dock	Bird Island	Kitchel Key	Tarpon Bay
< 5						
Dry	3%	0	0	0	0	0
Moderate	8%	7%	5%	0	0	0
Wet	27%	20%	19%	8%	1%	0
< 10						
Dry	8%	5%	0	0	0	0
Moderate	25%	14%	14%	5%	3%	0
Wet	48%	31%	31%	16%	9%	0
> 35						
Dry	6%	19%	31%	32%	52%	59%
Moderate	0	2%	2%	3%	14%	38%
Wet	0	0	0	0	4%	21%
> 40						
Dry	0	2%	3%	7%	9%	22%
Moderate	0	0	0	2%	2%	4%
Wet	0	0	0	0	0	0
Total time at 15–28						
	48%	47%	40%	48%	35%	21%

fall (August–October) for all year types, but wet years had the longest and greatest peak flows (Fig. 3). During wet years, average freshwater flow rates in the late summer to early fall were nearly twice as high as those observed in moderate or dry years (Fig. 3).

Regression analysis of salinity and freshwater flow indicate a significant relationship for all sites individually, with the strongest relationship at the most upstream site (PP, Fig. 4). Flows < 3700 cfs created hypersaline conditions at the most downstream site (TB) resulting in salinities > 28 (nonshaded region, Fig. 4), while flows > 2200 cfs resulted in hyposaline conditions at upstream sites PP and IC (Fig. 4).

Living reef density

Live oyster density varied significantly among sites and year types, and between seasons (interaction effect: $F_{8,\ 399} = 2.946$; $p < 0.01$). Overall, salinity and freshwater flow did not have an effect on density, but when analyzed by site density at IC was positively correlated with salinity ($r_s = 0.359$; $p < 0.001$), while density at BI and TB were negatively correlated with salinity ($r_s = -0.210$; $p = 0.036$ and $r_s = -0.360$; $p < 0.01$, respectively). Density at IC was also negatively correlated with flow ($r_s = -0.332$; $p < 0.01$), but no relationship between density and flow was detected for other sites. All sites frequently had densities exceeding 1000 individuals m^{-2} except CD, which had low densities regardless of year type (< 700 individuals m^{-2}) (Fig. 5). BI consistently had the greatest densities across all sites and CD the lowest (Table 2; Tukey's HSD; $p < 0.05$), but no significant difference among year type was detected at these sites (Fig. 5). The highest density observed during the monitoring period was at TB in 2004 (6151 ± 336 individuals m^{-2}), but live oyster densities dropped significantly across the whole estuary during the wet season of 2005, likely as a result of prolonged high-volume freshwater flow. Flow rates during the

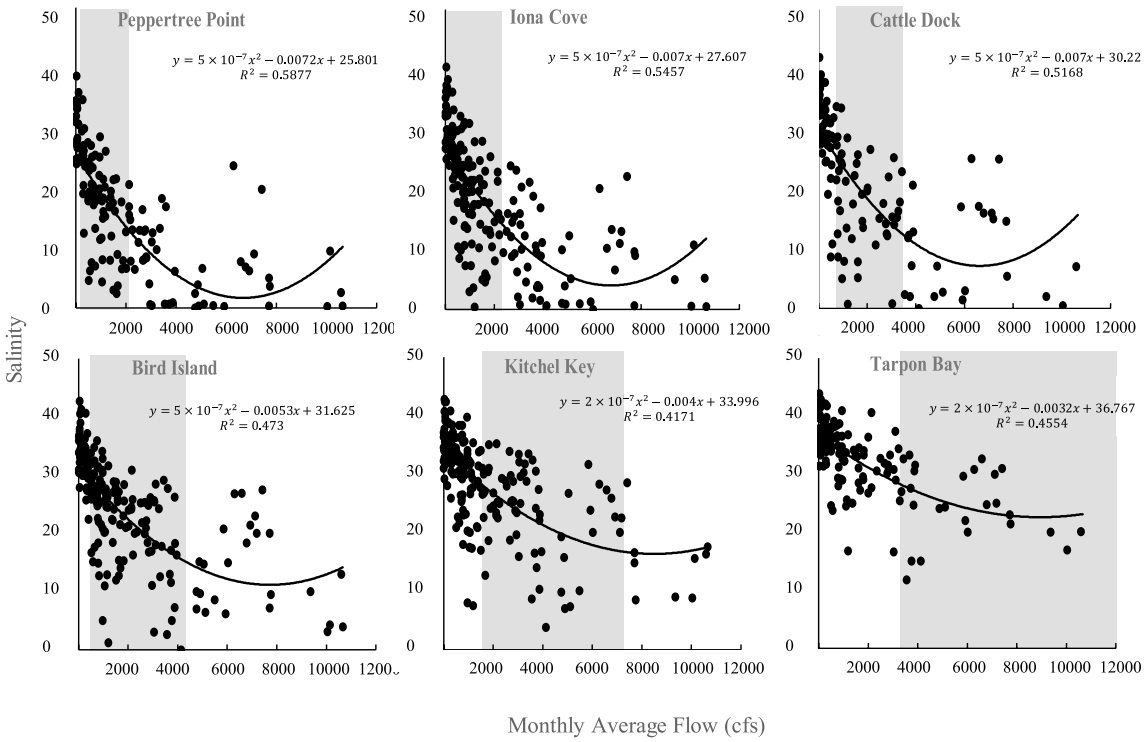

Fig. 4 Significant relationships ($p < 0.001$) were found at all sites between monthly average freshwater flow at S-79 and salinity at each site. Each regression follows a power relationship: $y = ax^2 + bx + y_0$, where y is monthly salinity and x is monthly average flow. Regression parameters for each site are shown in figure insets. Shading indicates monthly average flow needed to attain optimal salinities at each site for oyster growth, survival, and reproduction (14–28, Volety et al., 2009)

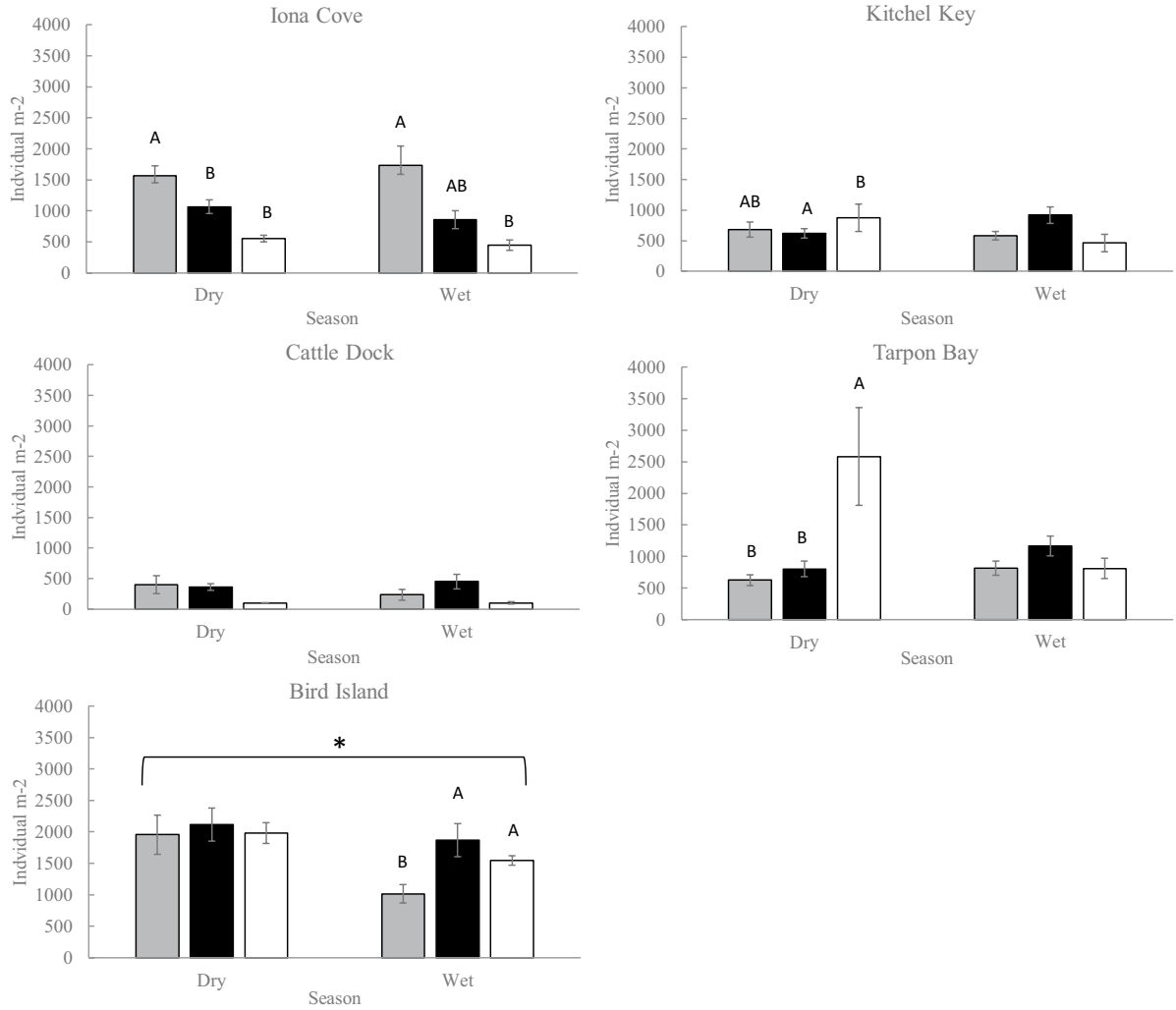

Fig. 5 Living densities at each sampling location averaged by year type: moderate (black), wet (white), and dry (gray) for each season (dry and wet). Error bars represent standard errors. Bars with different letters represent significant differences between year types (colored bars) within each season (wet or dry) and * represents significant differences between season for each site. If no letters or * are present, this indicates no significant difference among sites (letters) or between seasons (*)

summer (June–October) of 2005 exceeded 5000 cfs during 69% of the days of this 5-month period, and July flows averaged 11,593 (±351) cfs. Although all sites showed a rebound in oyster density by 2006, during the remainder of the monitoring period (2005 − 2017) oyster density did not exceed 3000 individuals m^{-2} for any site. The greatest difference in density among year types was observed at IC, with the lowest densities found during wet years (551 ± 53 individuals m^{-2}) and highest densities in dry years (1650 ± 172 individuals m^{-2}; Tukey's HSD; $p < 0.01$). In 2014, low salinity left no living oysters during the wet season at IC, but populations showed recovery by the following spring.

Mean shell length measured from density quadrants varied significantly with an interaction between site and year type ($F_{6, 286} = 319.946$; $p < 0.001$). The largest oysters were observed at TB (45.9 ± 1.4 mm) and next largest at IC (34.3 ± 1.7 mm). Oysters at BI were on average small (26.3 ± 0.5 mm), but this site maintained the highest densities no matter the year type, consistently exceeding 1000 individuals m^{-2} (except 2008) and on several occasions exceeding 2000 individuals m^{-2} (Figs. 5, S1). KK had

Table 2 Results of the post hoc analysis for each biological parameter. Means (± standard error) are reported for each site by year type. Superscript letter represent significance between year types within each site. Bold letters to the right of means by year type indicate significance among sites. Tukey's HSD was used for density, *P. marinus* intensity, and condition index. Non-parametric pairwise comparisons were used for *P. marinus* prevalence, recruitment, and gonad index. Significance is reported at $p < 0.05$ for all tests

Site	Year type	Density		*P. marinus* prevalence		*P. marinus* intensity		Condition Index		Recruitment		Gonad Index	
Peppertree point	Wet									0.9 (±0.1)^{A}			
	Moderate									1.4 (±0.1)^{A}	**B**		
	Dry									1.9 (±0.1)^{A}			
Iona Cove	Wet	630 (±73)^{C}		51.3 (±3.8)^{A}		0.63 (±0.02)^{B}		2.90 (±0.05)^{A}		1.7 (±0.1)^{A}		3.3 (±0.1) ^{A}	
	Moderate	986 (±90)^{B}	**BC**	54.1 (±4.5)^{B}	**B**	0.84 (±0.05)^{AB}	**C**	2.81 (±0.04)^{A}	**B**	2.1 (±0.1)^{B}	**B**	2.8 (±0.1)^{B}	**A**
	Dry	1650 (±172)^{A}		67.0 (±3.5)^{B}		0.98 (±0.05)^{A}		2.91 (±0.04)^{A}		2.5 (±0.2)^{C}		2.6 (±0.1) ^{C}	
Cattle Dock	Wet	101 (±9)^{B}		43.3 (±4.3)^{A}		0.70 (±0.05)^{C}		2.77 (±0.06)^{A}		3.1 (±0.2)^{A}		2.9 (±0.1)^{A}	
	Moderate	406 (±64)^{A}	**D**	55.0 (±4.6)^{A}	**B**	0.84 (±0.05)^{B}	**BC**	2.39 (±0.04)^{B}	**C**	4.7 (±0.3)^{B}	**B**	2.6 (±0.1)^{B}	**B**
	Dry	316 (±86)^{AB}		71.9 (±3.4)^{B}		1.14 (±0.06)^{A}		2.33 (±0.05)^{B}		1.9 (±0.2)^{A}		1.9 (±0.1)^{C}	
Bird Island	Wet	1803 (±92)^{AB}		60.5 (±2.8)^{AB}		0.85 (±0.05)^{B}		2.37 (±0.03)^{A}		10.9 (±0.3)^{C}		2.1 (±0.1)^{A}	
	Moderate	1993 (±183)^{A}	**A**	68.8 (±4.2)^{A}	**A**	1.31 (±0.03)^{A}	**A**	2.41 (±0.03)^{A}	**D**	9.6 (±0.5)^{B}	**A**	2.3 (±0.1)^{B}	**D**
	Dry	1485 (±194)^{B}		72.5 (±3.5)^{B}		1.52 (±0.06)^{A}		2.35 (±0.04)^{A}		4.2 (±0.3)^{A}		1.9 (±0.1^{B}	
Kitchel Key	Wet	632 (±121)^{A}		59.7 (±2.9)^{A}		0.89 (±0.03)^{A}		2.99 (±0.03)^{B}		13.0 (±0.4)^{B}		2.4 (±0.1)^{A}	
	Moderate	771 (±81)^{A}	**C**	59.0 (±4.3)^{AB}	**AB**	0.96 (±0.04)^{A}	**B**	3.11 (±0.05)^{A}	**B**	7.2 (±0.3)^{A}	**A**	2.4 (±0.1)^{B}	**C**
	Dry	630 (±70)^{A}		68.4 (±3.3)^{B}		1.01 (±0.05)^{B}		3.22 (±0.05)^{A}		5.0 (±0.3)^{A}		2.2 (±0.1)^{B}	
Tarpon Bay	Wet	1874 (±501)^{A}		60.8 (±4.1)^{A}		1.27 (±0.05)^{A}		3.43 (±0.06)^{A}		17.1 (±0.8)^{C}		2.5 (±0.1)^{A}	
	Moderate	986 (±104)^{AB}	**B**	57.3 (±4.2)^{A}	**AB**	1.02 (±0.07)^{A}	**A**	2.99 (±0.04)^{B}	**A**	11.5 (±0.1)^{B}	**A**	2.4 (±0.1)^{A}	**BC**
	Dry	722 (±73)^{B}		63.9 (±4.0)^{A}		1.02 (±0.05)^{A}		3.37 (±0.05)^{A}		4.7 (±0.3)^{A}		2.3 (±0.1)^{A}	

the smallest oysters observed during the monitoring period (15.0 ± 0.7 mm; Tukey's HSD; $p < 0.01$). When averaged across the estuary, significantly larger oysters (Tukey's HSD; $p < 0.01$) were observed during moderate and dry years compared to wet years.

Juvenile recruitment

Recruitment showed clear seasonal patterns, with no juvenile recruits (on average < 0.5 spat per shell observed during this period) in the winter months (December–March) and peaks in recruitment numbers throughout the spring, summer, and early fall (Fig. 6). Kruskal–Wallis analysis showed a significant effect of site ($\chi^2 = 2239.669$; df = 5; $p < 0.001$), year type ($\chi^2 = 178.937$; df = 2; $p < 0.001$), and month ($\chi^2 = 12{,}720.395$; df = 11; $p < 0.001$) on recruitment density. Pairwise monthly comparisons further identified the highest seasonal peaks as occurring in August, September, and October, followed by June and July ($p < 0.01$). Overall, wet years had significantly higher recruitment than moderate or dry years ($p < 0.01$). Pairwise site comparisons showed no significant difference in recruitment at downstream sites (BI, KK, and TB), but recruitment at downstream sites was significantly greater than at upstream sites ($p < 0.001$). High interannual variation in recruitment was observed during the monitoring period, with maximum values occurring at downstream sites TB (206 spat shell^{-1}, wet year 2004), BI (142 spat shell^{-1}, moderate year 2011), and KK (120 spat shell^{-1}, wet year 2014). The lowest recruitment numbers were observed at upstream sites IC and PP (Fig. 6), with PP having the lowest recruitment of all sites ($p < 0.01$). Much of the variation in recruitment can be explained by year type which influenced both the intensity and timing of peak spat fall (Fig. 6). Although downstream sites maintained high recruitment during all year types, the highest peaks were observed in wet years when flow from S-79 was high. In contrast, upstream sites had the lowest overall recruitment during wet years and highest recruitment during dry years (Fig. 6). Average monthly recruitment, pooled across the estuary, ranged from 24 to 37 spat per shell during the spawning season (approximately May–October) and was significantly correlated with freshwater flow ($r_s = 0.308$; $p < 0.001$) and gonad index ($r_s = 0.517$; $p < 0.001$), but not with living reef density ($r_s = 0.085$; $p = 0.085$). Recruitment also showed strong seasonal trends, with correlations between salinity ($r_s = -0.130$; $p < 0.001$) and temperature ($r_s = 0.427$; $p < 0.001$) peaking in summer months when temperature is elevated.

Gametogenesis

Gametogenesis differed significantly among sites ($\chi^2 = 230.24$; df = 4; $p < 0.001$), year types ($\chi^2 = 64.53$; df = 2; $p < 0.001$), and months ($\chi^2 = 3{,}349.57$; df = 11, $p < 0.001$). The highest mean gonad index rankings (averaged over the year) were observed at IC during all year types and are partially attributed to a longer active period compared to downstream sites, averaging 6–9 months per year with a gonad index ranking ≥ 2 (Fig. 7). All other sites had average spawning periods between 2 and 7 months per year, with shortest periods (2 months) reported during dry years. Spawning period at the most downstream site (TB) was not affected by year type, with an average 4-month spawning period during all year types. BI had the lowest gonad index rankings (Fig. 7) and a short active period (3.0 ± 0.4 months). Although duration of the spawning period varied among years, peaks were consistently observed during July–October across all sites and years (Fig. 7). Gonad index showed a negative relationship with salinity ($r_s = -0.272$; $p < 0.001$), but appeared biphasic. The observed seasonal variation was better explained by the positive correlation with temperature ($r_s = 0.563$; $p < 0.001$). Resting periods, in which no active gametogenesis was evident, were observed in the winter months (December–February) when temperature was low (≤ 21 °C), with active gametogenesis occurring during warmer months (spring–fall). During the spawning period (July–November), females dominated the population, while males dominated the population post spawning (December); however, the percentage of individuals with active gametogenesis in December was low.

Condition Index

Condition index varied seasonally and had a significant 3-way interaction effect between site, year type, and month ($F_{88,\ 10513} = 6.36$; $p < 0.001$). At all sites, a seasonal low was observed in late summer to early fall (June–October), and a peak was observed in the spring of each year (Fig. 7). The

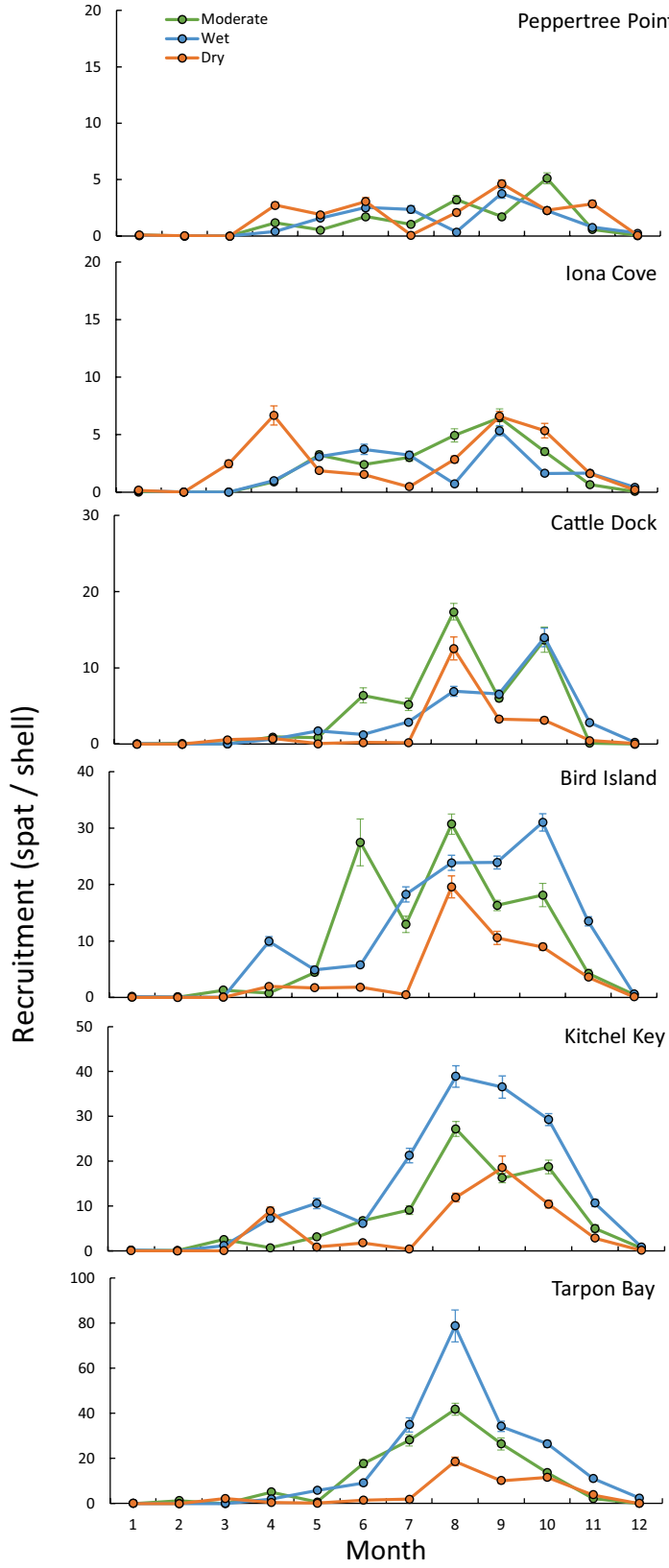

Fig. 6 Seasonal variation in mean recruitment by year type at each site. The numbers on the *x*-axis represent months in the calendar year from January to December

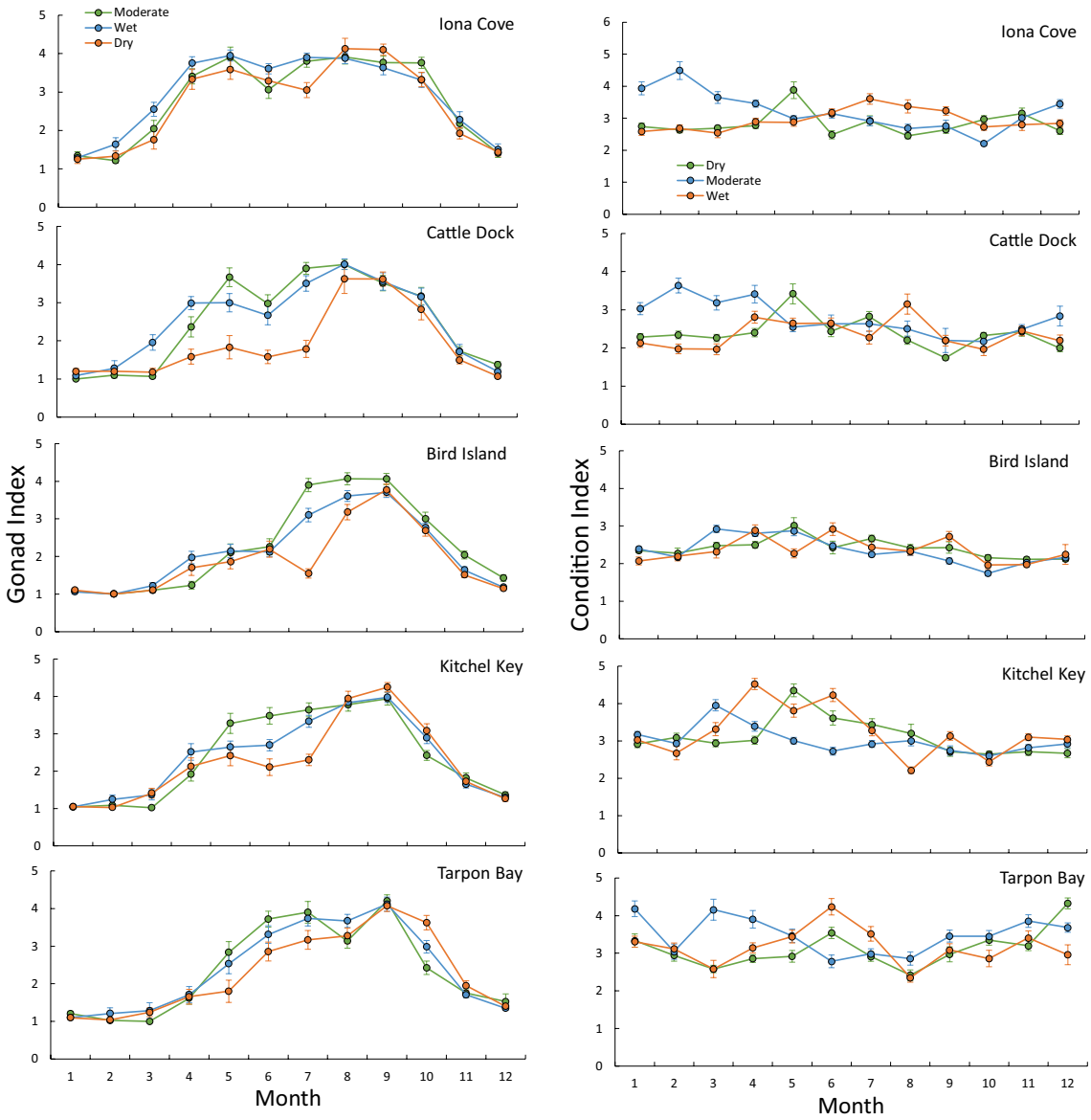

Fig. 7 Seasonal variation in mean gonad index (left panel) and mean condition index (right panel) over time for each site by year type. The numbers on the *x*-axis represent months in the calendar year from January to December

overall highest condition index rankings (annual mean CI values ≥ 3.0) were observed at KK and TB during all year types (Fig. 7). Oysters at BI and CD had the overall lowest condition index during all year types (Tukey's HSD, $p < 0.05$; Fig. 7), except during wet years at CD (Table 2). Average annual condition index was intermediate at IC (Fig. 7), with no significant difference among year types (Table 2). Oysters at IC exhibited a peak in condition index in February of wet years; however, high mortality preceded during three out of the eight wet years during this study period. This high mortality resulted in the absence of oysters in the months leading up to this collection; thus, condition index represents only the young of the year and individuals that did not experience low salinities observed in wet summers. Therefore, these data should be interpreted with caution. Condition index had a significant, but weak correlation with

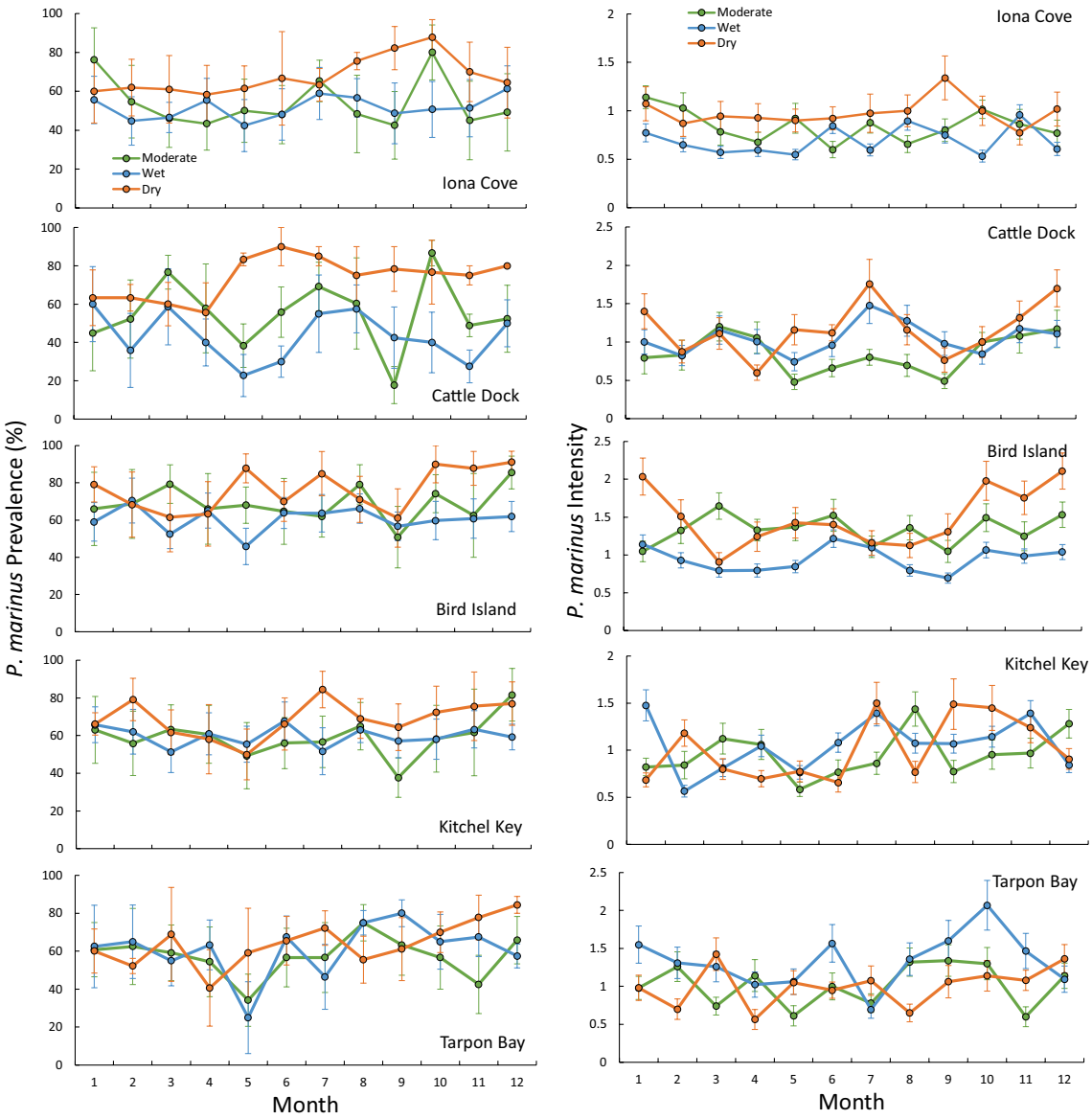

Fig. 8 Seasonal variation in mean *P. marinus* prevalence (left panel) and intensity (right panel) for each site by year type. Error bars represent standard errors. The numbers on the *x*-axis represent months in the calendar year from January to December.

salinity ($r_s = 0.138$; $p < 0.001$), but not with temperature. A significant, but weak positive correlation was observed between condition index and gonad index ($r_s = 0.166$; $p < 0.001$).

Perkinsus marinus infection prevalence and intensity

Infection prevalence of *P. marinus* varied widely in oysters throughout the monitoring period, ranging 0–100%; however, there were very few occasions with no infection reported. Year type ($\chi^2 = 28.41$; df = 2; $p < 0.001$) and site ($\chi^2 = 14.45$; df = 4; $p < 0.01$) had significant effects on infection prevalence. When averaged across all sites, the highest prevalence was observed during dry years (69.3 ± 1.6%; $p < 0.05$); there was no difference in prevalence between wet and moderate years (55.9 ± 1.6% and 59.1 ± 2.0%, respectively). All sites exhibited a trend of increasing prevalence with

increasing salinity (wet < moderate < dry). The overall highest infection prevalence was observed at the mid-estuary site BI during dry years (75.5% ± 3.5) and remained elevated during wet years (60.5 ± 2.8%). At the most downstream site (TB), no significant difference in infection prevalence was observed among year types, and annual means were 57–64% and typically higher than other sites (Fig. 8). Overall, the prevalence of infection was lowest (51.3 ± 3.8% and 43.3 ± 4.2%) at the upstream sites (IC and CD, respectively) during wet years (Fig. 8). During a period of three consecutive wet years (2003–2005), a prevalence of zero was observed on several occasions across all sites, concomitantly the average annual salinity at the Cape Coral Bridge was 3.8–10.7. However, throughout the remainder of the monitoring period, a zero prevalence was never again recorded. The infection prevalence peaked in 2007–2013, which were all moderate or dry years, with average annual infection rates of 56–87% across all sites, and, in several years, infection prevalence exceeded 90%. Infection prevalence was particularly high (> 75% at all sites) in 2012–2013, the end of a long dry to moderate salinity period, with annual average salinities ranging from 17–39 across all sites.

Similar to *P. marinus* infection prevalence, infection intensity varied widely over the study period ranging from 0 to 3.9; however, intensities > 3 were rare. Infection intensity was significantly affected by a 3-way interaction effect of site, year type, and month ($F_{88,\ 10513} = 6.36$; $p < 0.001$). Across all year types, lower intensities were observed at the most upstream site (IC) and highest intensities were observed at the mid-estuary site BI (Fig. 8). All sites, with the exception of TB, showed a significant trend of increasing *P. marinus* intensity under dry conditions (year type: Tukey's HSD; $p < 0.05$). Infection intensities at TB did not vary significantly among year types (Table 2). Although TB and KK had infection intensities exceeding 3.0 on several occasions, the highest annual average intensity (2.2 ± 0.1) was observed at mid-estuary site BI in 2012, and a moderate water year that was preceded by 6 years of moderate or dry years and annual average salinities ≥ 25. When infection intensity data were averaged by year type and site, BI had the highest infection intensities during dry years (1.7 ± 0.1), but intensity decreased significantly during wet years (Tukey's HSD; $p < 0.05$) with mean annual intensity dropping to 0.85 (± 0.03), while mean annual intensities at TB consistently remained ≥ 1.0 no matter the year type. The most upstream site (IC) had the lowest observed average infection intensities across the estuary, remaining < 1.0 throughout the monitoring period with the exception of two dry years, 2012 and 2013 (1.8 ± 0.09 and 1.5 ± 0.11, respectively).

Both infection prevalence and intensity were significantly correlated with salinity ($r_s = 0.085$; $p = 0.02$ and $r_s = 0.138$; $p < 0.01$, respectively), but only intensity was correlated with temperature ($r_s = -0.021$; $p = 0.04$). These correlations were, however, all weak and are likely affected by multiple environmental factors. The highest infection intensities were observed

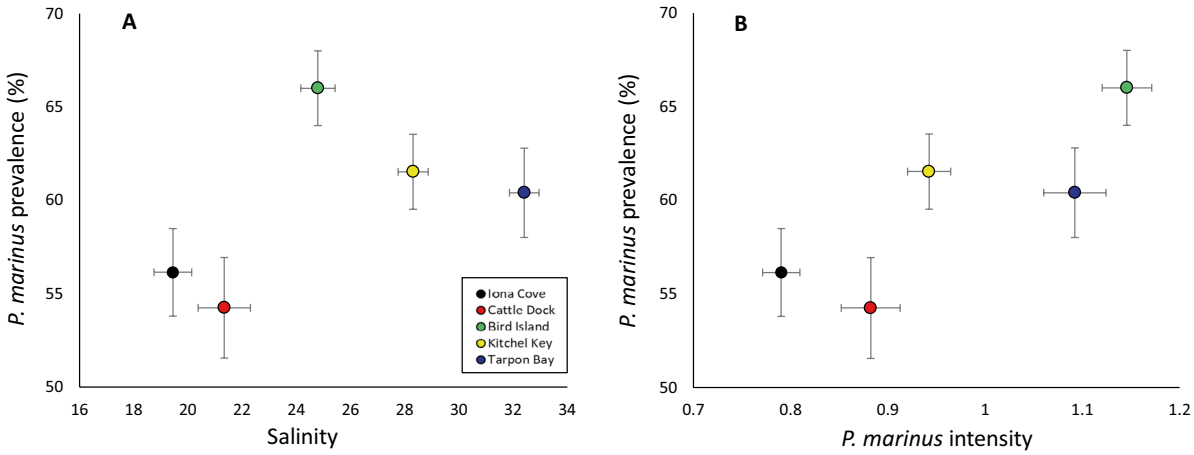

Fig. 9 Mean *P. marinus* prevalence in oysters during the study period from each sampling site as a function of average salinity (**A**) and average *P. marinus* infection intensity (**B**) at each site over the monitoring period. Sites are organized from upstream to downstream. Error bars represent standard errors

in the fall and early winter (September–December) of dry years following periods of both high temperature and high salinity; lowest intensities were observed in the winter and spring of wet years when both salinity and temperature were low (Fig. 8). In general, both intensity and prevalence were lower when salinities were less than 10 and elevated when they were above 10. Infection intensity closely tracked infection prevalence (Fig. 9B), with highest intensities observed during years in which prevalence also peaked ($r_s = 0.845; < 0.01$). There was also a significant correlation between *P. marinus* intensity and gonad index ($r_s = -0.148$, $p < 0.01$) among all sites, but the relationship was weak. Similarly, a weak negative correlation was observed between infection intensity and condition index ($r_s = -0.100$, $p < 0.01$).

Mean shell length of oysters collected for monthly analysis

Mean length of adult oysters collected for gametogenesis and disease characterization varied significantly by site and year type (interaction effect: $F_{8,\ 8309} = 13.71$; $p < 0.01$). The largest oysters were found at IC (71.57 ± 0.5 mm) and the smallest oysters at KK (47.19 ± 0.2 mm) (Table S3). Adults were targeted for the assessment of gametogenesis, yielding higher mean shell lengths than when quadrats were haphazardly sampled during density counts; however, the trend in mean shell length among sites remains the same.

Juvenile oyster growth and survival

Variation in the number of sites and lack of replication over much of the monitoring period, indicate a cautious interpretation of juvenile growth and survival data. First, triplicate cages were only deployed for 5 of the 14 years (water years 2009–2016) juvenile growth and survival were monitored, so replication is lacking for much of the monitoring period. During this time, only 1 year (water year 2012, a dry year) included all 6 sites, and from water years 2013–2016, only 4 sites remained in the monitoring plan. During the years in which both open and closed bags were deployed (water years 2009–2016), only 4 years included all 6 sites (water years 2009–2012) during which time only dry or moderate year types occurred. Lastly, the most upstream site PP was not added to the monitoring plan until water year 2006, so all comparisons during water years 2003–2005 excluded this site. Graphical trends, however, show similarity among years within sites and support data collected from field monitoring on reef density and trends in mean shell lengths. These data are therefore helpful to include as they benefit interpretation of the other biological parameters measured during monthly monitoring.

Generalized additive models (GAMs) for interval monthly survival explained 22% of the deviation with bag type (open vs. closed) being a strong predictor for survival ($t = -17.213$, $p < 0.05$) and salinity by site showed significance in the smoothed parameter at PP ($F = 2.621$, $p = 0.04$) and IC ($F = 3.000$, $p = 0.02$) (Figs. 10 and 11A). In order to identify trends in interval monthly survival among sites and year types resulting from primarily abiotic factors (closed bags) and predation (open bags), the data were parsed by bag type and analyzed separately. Closed bag GAMs explained only 10.5% of the deviation and no year type effect, but did identify site effects. The salinity by site smoothed parameter was significant for predicting survival at KK ($F = 2.782$, $p = 0.03$) and CD ($F = 3.519$, $p < 0.01$). KK generally had the lowest survival in closed bags among all sites except during wet years when CD had the lowest interval survival (Fig. 10A). Mean interval survival was generally higher for closed bags compared to open bags across all sites no matter the year type (Fig. 11). GAMs for open bags explained 29.4% of the deviation with site and year type as significant predictors, but there was no salinity effect for either the salinity by site or salinity by year type smoothing parameters. PP ($t = 5.666$, $p < 0.05$) and CD ($t = 6.066$, $p < 0.05$) had significant positive growth and both wet ($t = 2.233$, $p = 0.03$) and moderate ($t = 2.111$, $p = 0.04$) years also had a positive effect on interval survival. With the exception of extreme wet years, when mass mortality events occurred at upper-estuary sites, PP had the highest interval survival across all sites with the exception of CD. These mass mortality events typically occurred rapidly over a 1-month period and therefore may not be truly represented in the mean interval survival plots, but they can be observed in time series plots (Fig. S7). While CD also maintained high survival, open growth bags were only deployed at CD for 4 years (out of 9 total for other sites) and therefore

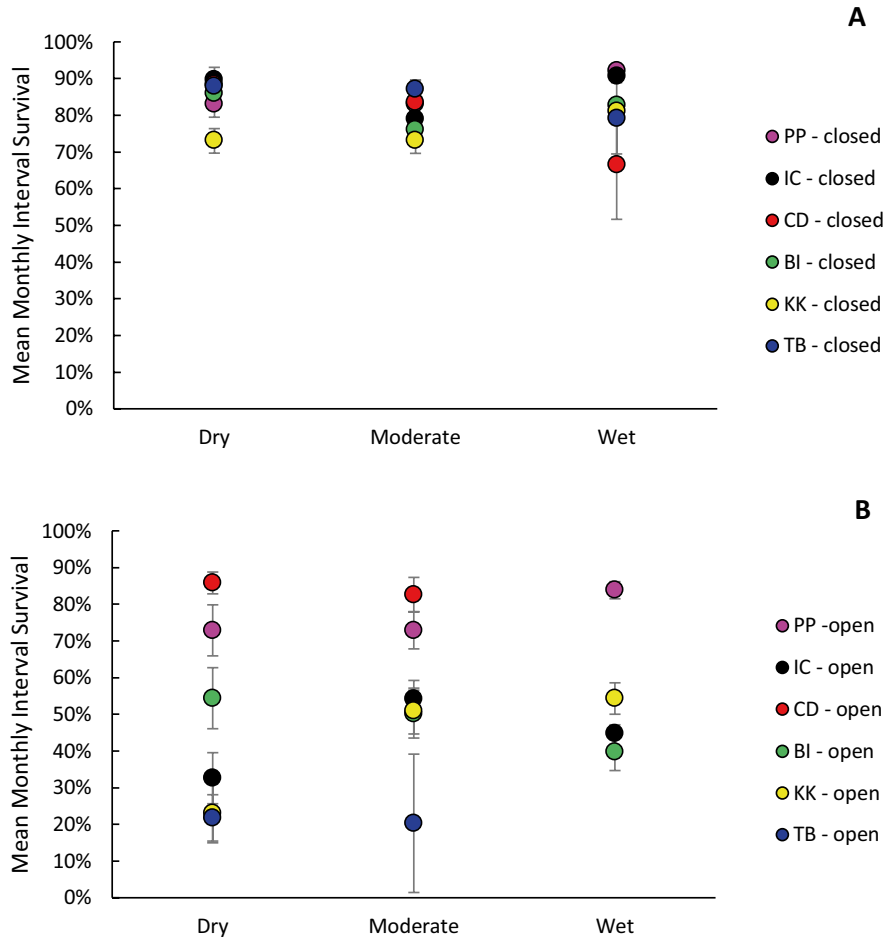

Fig. 10 Mean monthly interval survival of juvenile oysters for closed (**A**) and open (**B**) bags by year type for each site. Error bars represent standard error. There are no data for open bags at TB or CD during wet years because open bags were not added to the monitoring plan until water year 2009, and TB and CD were dropped from the monitoring plan in water years 2013 and 2012, respectively, resulting in only moderate and dry year observations for these sites

should be interpreted with caution. Downstream sites, KK and TB, had the lowest open bag survival among sites during dry years (Fig. 10B).

Monthly growth rate was only modeled for closed bags due to high mortality in the open bags leaving too few oysters for length measurements; growth rate is graphically represented for both bag types to show trends across sites (Fig. 11B). The GAM for growth rates explained 16.1% of the deviation and the smoothing parameter for salinity by year type was significant for wet years ($F=6.332$, $p<0.001$) reflecting the overall lower mean growth rate in wet years (Fig. 12). No salinity by site effect was observed, but site alone was a good predictor of growth rate with PP ($t=6.942$, $p<0.001$) and IC ($t=2.822$, $p<0.001$) on average, having higher growth rates than all other sites.

Discussion

This study details the effects, both positive and negative, of controlled freshwater input as it relates to site specific salinity across all life stages of the eastern oyster over a 15-year monitoring period. The results of this study highlight the balance and seasonal considerations that are needed to maintain healthy oyster reefs in highly managed coastal ecosystems and provide data that can help to inform management decisions. The observed biological response to low salinity events is not novel on its own and is well documented within the literature; however, the duration of the monitoring period and the breadth of the biological response variables measured provide a unique and comprehensive approach to under-

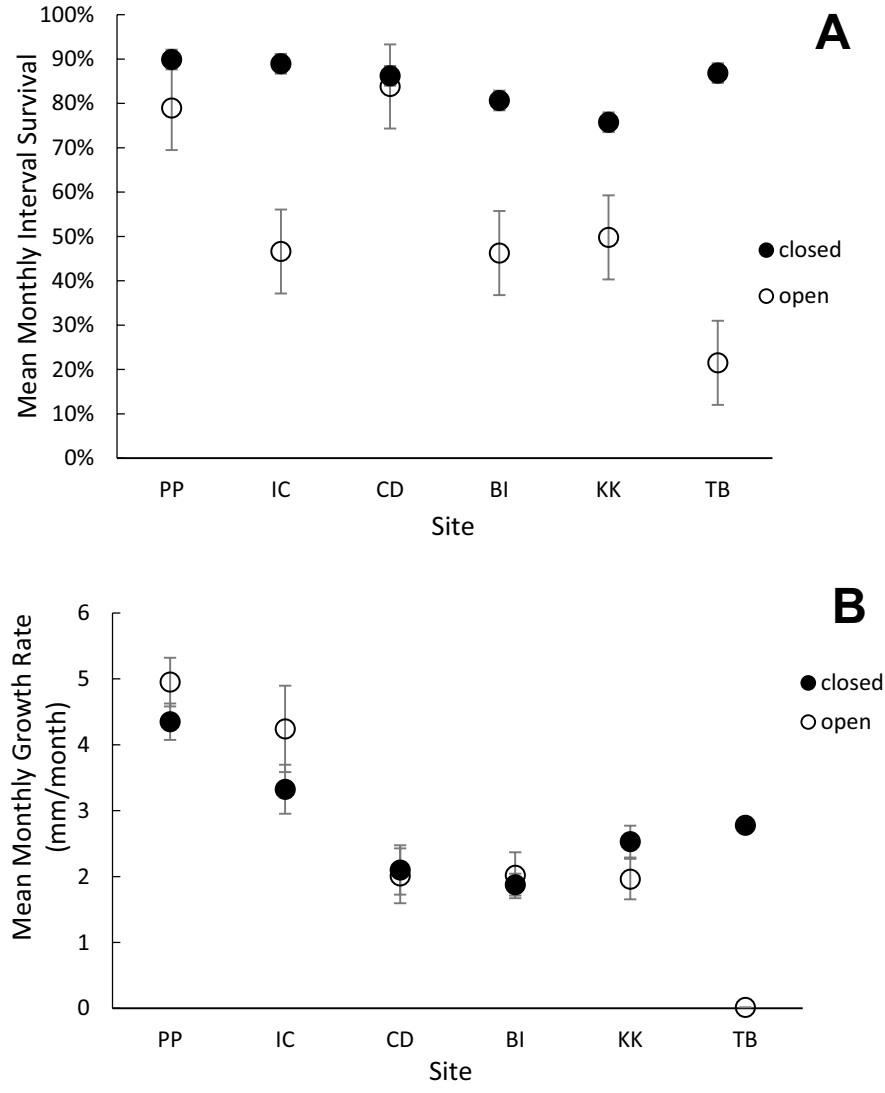

Fig. 11 Comparison of survival (**A**) and growth (**B**) between open and closed bags among monitoring sites; only years that included both open and closed bag types are included in analysis for direct comparisons (WY 2009–2015). Error bars represent standard error

standing how the timing, magnitude, and duration of controlled freshwater releases can effect oyster populations and their ability to rebound from extreme events. Although extreme wet years led to the disappearance of oysters from the most upstream site (IC), reduced salinity at downstream sites during wet years gave reprieve from hypersaline conditions resulting in lowered disease intensity and improved larval recruitment in the lower estuary (sites KK and TB). In contrast, prolonged dry periods, with little to no freshwater input, resulted in increased disease prevalence and intensities at all sites. Average oyster densities in the CRE were high (500–2700 oysters m^{-2}) compared to other systems, such as the St. Lucie Estuary, Florida (< 2–300 oysters m^{-2}, Parker et al., 2013), Apalachicola Bay, Florida (14.8–418 oysters m^{-2}, Livingston et al., 1999), and the James River, Virginia (300–500 oysters m^{-2}, Mann et al., 2009), and were similar to densities observed in the neighboring Estero Bay (1474 ± 624 oysters m^{-2}, Tolley et al., 2005). The information from this study can provide water managers of the CRE and other coastal estuaries where oysters are present guidance to make informed decisions on the best way to manage water releases to protect oyster populations. The discussion below details the direct and indirect effects of freshwater flow on oyster populations using the S-79 as the primary indicator of freshwater input to the estuary.

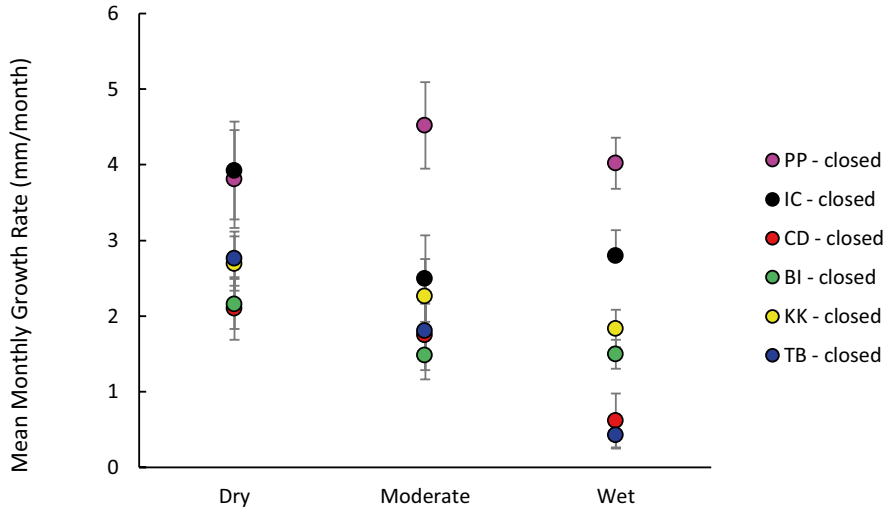

Fig. 12 Mean monthly growth rate for closed bags during the entire monitoring period (water years 2003–2016) by year type for each site. Only closed bags were used to monitor growth rates because high mortality in open bags often resulted in a low number of oysters for length measurements and could have resulted in size specific morality. Error bars represent standard error

Based on the results, we also suggest water management options for timing and magnitude of large freshwater releases to best protect oyster populations based on seasonal patterns in response variables measured.

Magnitude and timing of acute low salinity events

For oysters in the CRE, more important than the seasonal or annual mean salinity was the timing and magnitude of low salinity events. For example, in water year 2009 (dry year), mean flow rate was < 1000 cfs and mean salinity at IC was 27.7 ± 2.7. However, salinity at this site dropped from 24.9 to 9.5 within 1 month (August to September 2008) as a result of high freshwater releases, with flows frequently exceeding 5000 cfs and 2000 cfs (48% and 66% of the days, respectively; Fig. 13). During this time, temperature was also at an annual high (33 °C), and gametogenesis was at a peak (gonad index = 5), which likely exacerbated the impact of the low salinity event, resulting in extensive reef mortality at IC. In contrast, years in which a similar low salinity event occurred in the winter or spring did not result in as dramatic of a loss even when reported salinities were lower than that observed during late summer mortality events. For example, during April–July 2010, freshwater input exceeded 2000 cfs for 93% of the days over a 4-month period and salinity at IC reached a low of 1 in May 2010 (average salinity = 5.3), but no significant mortalities were noted. This difference in survival is likely linked to the elevated energy requirements for reproduction (Bayne et al., 1978; Tremblay et al., 1998) coupled with increased metabolic rates that accompany high summer temperatures (Bayne et al., 1978; Bougrier et al., 1995), both of which can leave oysters more vulnerable to environmental extremes. Andrews et al. (1959) found that if low salinity events occurred when metabolic rates were low (i.e., during winter months when temperature is low), oysters could survive salinities < 5, but if the low salinity event occurred after metabolic rates were stimulated by warming water temperatures, mortality becomes rapid. Likewise, Loosanoff (1952) reported high mortality rates when low salinity exposure occurred during advanced gametogenesis. Laboratory exposures to 3-week long freshets (salinity drop from 20 to 1 over 48 h), resulted in significant mortality of oysters collected and exposed in the summer (July) but not during winter or spring (December and April) (La Peyre et al., 2003). Similarly, McCarty et al. (2020) observed a doubling of mortality rate when 2-year old oysters were exposed to freshets (2.7) in the summer compared to spring. During extreme low salinity events, seclusion from the environment is a first response (Andrews et al., 1959; Loosanoff, 1952; Shumway, 1977b) and energy expenditure is often elevated to maintain cellular protection and osmotic homeostasis (Rivera-Ingraham & Lingot, 2017; Sokolova et al., 2012). When freshests occur in the summer, increased metabolic demands of reproduction and elevated metabolic rates due to increased temperature result in an elevated energetic demand to maintain homeostatic processes under environmental stress. In contrast, spring freshets allow oysters to

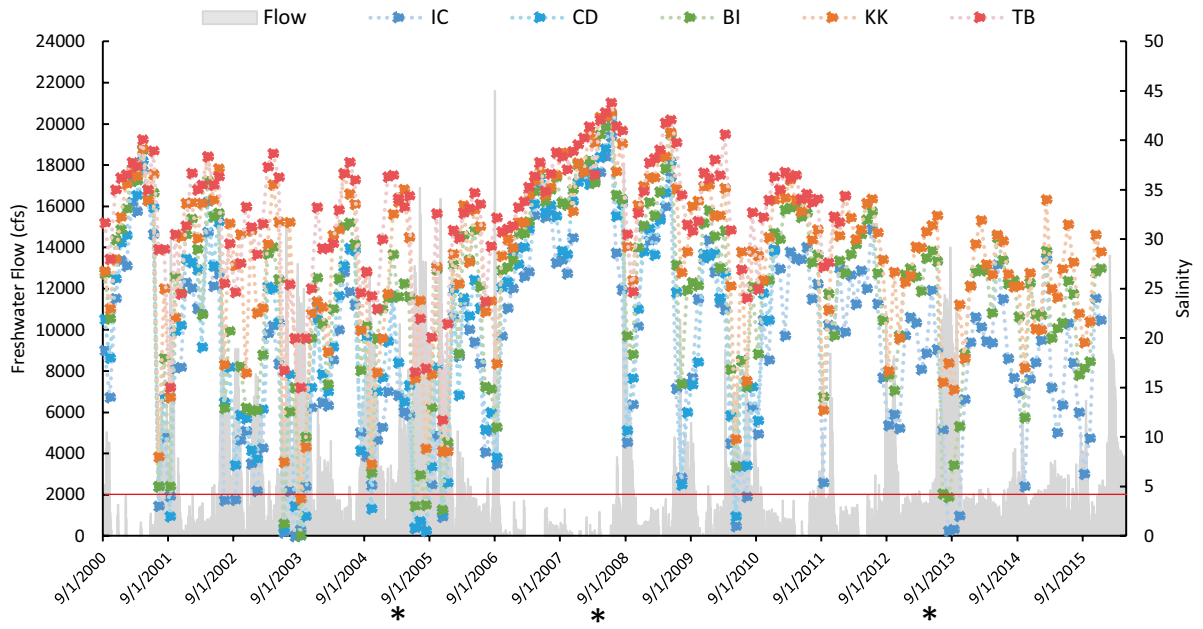

Fig. 13 Daily flow rate from S-79 and monthly salinity measurements for each site over the duration of the study period. Red line marks the maximum summer flow rate limit of 2000 cfs to highlight the frequency in which this is currently exceeded during the late summer spawning period (August–October) and the effect it has on salinity. Upper-estuary, low salinity sites (IC and CD) are marked in shades of light/dark blue and lower-estuary, and high salinity sites (KK and TB) are marked in shades of red/orange to highlight the impact on different regions of the estuary. Mid-estuary moderate salinity site BI is marked in green. Asterisks (*) mark years with significant mortality events at IC during which no oysters were alive to be collected for analysis

adjust osmotically before energetic demands increase with rising temperatures and advanced gametogenesis and subsequently allow for a greater salinity tolerance range. This highlights the importance of the timing of freshwater events as they relate to environmental temperature and physiological state of the oysters. In the case of the CRE, upstream reefs were most susceptible to the synergistic effects of temperature and salinity in the late summer when effects of environmental stress are further amplified because summer freshets coincide with periods during which oysters experience increased metabolic demands due to advanced gametogenesis and elevated temperatures.

Another important factor to consider is the magnitude of salinity change. As discussed, oysters can maintain valve closure as an avoidance mechanism, but are osmoconformers, and long-term survival depends on their ability to osmotically adjust their internal fluids to that of the external environment while maintaining cell volume control (Pierce, 1982; Shumway, 1977a). When the magnitude of salinity change is large (> 15) over a short time interval and persistent, oysters may experience osmotic shock, whereas gradual changes allow for slow adjustment of the internal fluids during which wider tolerances can be observed (La Peyre et al., 2009; McFarland et al., 2013, 2014; Volety et al., 2016). Valve closure serves as a protective first response to osmotic shock by isolating the animal from the undesirable external environment. During prolonged valve closure in response to longer-term extreme salinity events, the accumulation of waste products from anaerobic metabolism can result in cellular insult and death (Sokolova et al., 2012). Such rapid changes may occur following storm events (Phlips et al., 2020) or periods of heavy rainfall when water management structures remain open for extended periods (Volety et al., 2009). During many wet years, average salinity during summer months (June–August) was < 10, but because the change did not occur abruptly when temperature was also at an annual peak, upstream reefs were successful. The synergistic impact of high

temperature and low salinity is amplified in recently settled juvenile oysters and larvae, which experience higher mortality rates under these conditions compared to adult oysters (La Peyre et al., 2003; Volety et al., 2009, 2016). Therefore, acute exposure to extreme low salinity events in the late summer, when peaks in spawning and recruitment are observed, may not only inhibit gametogenesis and induce large scale mortality events in the breeding population but could also increase morality rates in free swimming larvae and newly settled juveniles. If such extreme events disrupt recruitment frequently enough, extirpation of the upstream portion of the metapopulation may be a consequence. Although reef structure is maintained during isolated or small-scale die offs, without new recruitment and shell growth old shells will eventually disarticulate, and the consequences of long-term lack of new growth could lead to a total loss in hard substrate and limit the ability of reefs to naturally repopulate once conditions improve (Grabowski & Peterson, 2007; Pace et al., 2020). As discussed later, this information has clear implications in terms of water management.

Importance of freshwater input for healthy downstream reefs

Although the most dramatic mortality events were observed as a result of extreme low salinity events, a lack of freshwater input can also lead to devastating effects on long-term reef success (Albright et al., 2007; Livingston et al., 2000; Wilber, 1992). In water year 2009, a dry year, salinity was 36–40 across all sites causing mortality of juvenile oysters deployed during this time in both open and closed bags (Fig. S7) showing that hypersaline conditions can result in significant mortality of juvenile oysters. Hypersaline events occurred more frequently at the lower-estuary sites and may partially explain low survival of juvenile oysters in closed bags compared to upper-estuary survival rates in moderate and dry years. Additionally, moderate to high salinity sites in the CRE also fall within the optimal salinity range for many oyster predators (Livingston et al., 2000; Roegner & Mann, 1995) and pathogens, including *P. marinus* (Reece & Dungan, 2006). The Gulf of Mexico oyster industry has experienced significant losses in oyster densities during drought years as a result of increased predation (Garland & Kimbro, 2015; Livingston et al., 2000) and disease (Petes et al., 2012) further highlighting the impact of freshwater input on estuarine ecosystems and the importance of proactive management in the face of a rapidly changing global climate.

In the CRE, the highest *P. marinus* infection prevalence and intensity were observed at the mid- (BI) and lower-estuary (KK and TB) sites where salinity remained high and was often hypersaline during dry years with low freshwater flow. During a period of several consecutive moderate and dry years (2007–2013), *P. marinus* prevalence and intensity were elevated across all sites, including the most upstream site (2012–2013; Figs. S5, S6), at levels which may result in mortality (intensity ≥ 2; Southworth et al., 2010). During spring 2012, no freshwater was released from S-79 for a total of 49 consecutive days (March 26, 2012–May 14, 2012) resulting in elevated summer salinities (Fig. 13) and ideal conditions for the spread and proliferation of *P. marinus*. The only remaining downstream site (KK) had the lowest infection intensity during this time, but this could be a result of mortality of heavily infected oysters (La Peyre et al., 2003, 2009) in addition to the fact that this site was dominated by young oysters (mean length = 25 mm; Table S2). An influx of juvenile oysters can keep mean infection low when adult densities are also low. Indeed, prior to this period, mean infection intensity at KK peaked in September 2011 (3.3) before falling dramatically to 1 in October 2011, suggesting mortality in heavily infected oysters. Following this prolonged dry period, densities of living oysters at KK dropped (67 oyster m^{-2}; Table S2) and failed to fully recover (< 250 oyster m^{-2}) during the remainder of the monitoring period even with good juvenile recruitment. In addition, lower juvenile growth rates at lower-estuary sites resulted in a smaller overall size of oysters compared to upper-estuary reefs where rapid growth rates were observed (Fig. S7). The smaller oysters at downstream sites were, therefore, likely a result of both disease infection limiting survival of adult oysters and slow growth rates in juvenile oysters.

Growth and survival patterns of juvenile oysters deployed in bags suggest that predation also plays an important role in shaping reef structure and population dynamics among sites within the CRE. Juvenile survival in closed bags was high compared to open

bags at all sites except PP and CD, suggesting predation-related mortality to be an important factor in shaping reef density. It should be noted that open bags were only included at CD during three of the years monitored, and therefore, this result should be interpreted with caution; however, high survival in open bags at PP, no matter the year type, suggests lower predation pressures in the upper-estuary. In contrast, the lowest survival in open growth bags was observed at the mid and lower-estuary sites (BI, KK, TB), suggesting heavy predation pressures in the lower-estuary where salinity remained high. Prolonged periods with little to no freshwater input also affected oyster reefs at the most upstream site (IC). Decreased survival in open juvenile growth bags at IC was observed in dry years when compared to closed bags suggesting an increase in predators moving up-estuary when salinity was elevated.

Previous work in the Gulf of Mexico has shown that oysters benefit from periods of low salinity in the winter and early spring when temperature is low (Andrews et al., 1959; La Peyre et al., 2003, 2013). Temporary decreases in salinity can reduce *P. marinus* infections (Ray, 1954) and could partially explain the overall larger oysters found at the low salinity site IC. Here, salinities < 12 are common and are likely to slow progression and spread of *P. marinus* (Burreson et al., 1994; McCoullough et al., 2007) and reduce the presence of predators (Livingston et al., 2000). This increased understanding of the interactive effects of disease and salinity on oysters will be discussed later in terms of improved water management.

Source sink dynamics

Although mean oyster density varied considerably during the 15-year monitoring period at all sites, when averaged across the estuary, no significant difference among year types was observed, indicating that the system as a whole is capable of maintaining a balance across the metapopulations. Extreme years resulted in mortality events, but annual recruitment offset this mortality and, based on variation in gametogenesis and recruitment at each site, extensive mixing likely occurred among sites within the CRE. In several years (e.g., water years 2006, 2009, 2014), reefs at IC experienced 100% mortality but rapidly recovered within 6 months due to upstream larval transport. Upstream transport is also indicated by the consistent observations of new juvenile recruitment at the most upstream site PP, where no living reefs exist. In late fall, oyster larvae pushed upstream may find moderate salinities at PP suitable for settlement, but when the summer rains and freshwater releases come, low salinity likely prevents long-term survival. PP also lacks the substrate necessary to support new reef growth, consisting of mainly soft bottom sediments that further limit reef development. The good growth and survival observed in the juvenile growth bags deployed at PP suggest that substrate replenishment and well-timed freshwater releases could produce self-sustaining reefs at PP. When flow rates are high during prolonged freshwater releases, upstream larval transport is inhibited; however, tidal influences and swimming behavior can account for some upstream larval transport, which would be consistent with predictive models (North et al., 2008; Shen et al., 1999), especially during periods of low freshwater flow from S-79. When flow rates are elevated, downstream larval transport fuels juvenile recruitment in the lower CRE and compensates for mortality at downstream reefs following dry years. However, high volume flows can flush out larvae, thus reducing recruitment across the estuary (Wilson et al., 2005); therefore, determination of seasonal flow rates should also consider strategies that optimize larval retention.

The lack of a significant relationship between reef density and recruitment in the CRE suggests that density is more a function of post-settlement survival and may be in part explained by disease and predation pressures coupled with salinity. Reefs at IC maintained high densities but consistently low recruitment, while downstream site KK typically had high recruitment but low densities. This suggests fewer new recruits survive to maturity at KK compared to IC. This is supported by the data from the growth bags. For both open and closed bags, survival and growth rates were higher at upper-estuary sites (IC and PP) compared to mid and lower estuary sites (BI, KK, and TB). Low juvenile growth rates observed in the growth bags were also reflected in the natural population at KK. Large oysters (> 50 mm) were difficult to find when targeting adults at KK (average length of 47.6 ± 0.2 mm; Table S3). By comparison, mean oyster length at low salinity site IC was consistently > 50 mm and averaged 72.0 ± 0.7 mm when adults were targeted, reflective of the higher growth rates observed in the juvenile growth bags deployed

at IC. Smaller oysters at downstream sites could also be a result of slowed growth due to *P. marinus* infection (Andrews, 1961; Paynter & Burreson, 1991), mortality of larger oysters with higher infection intensities (La Peyre et al., 2003, 2009) or a combination of both. Further, low densities at the downstream high-salinity sites may also be a result of increased predation pressures (Baker et al., 2006; Livingston et al., 2000; McFarland & Hare, 2018; Wilber, 1992).

Overall, CRE oysters exhibited long and somewhat continuous spawning periods from May through September/October across all sites; however, the long spawning period and high gonad index rankings observed at IC during all year types compared to all other sites suggests that it could be an important source population, supplying larval recruits to the CRE. Only the mid-estuary site (BI) maintained consistently high densities during all year types, as both upstream and downstream sites suffered losses during salinity extremes. BI had the lowest gonad index rankings and shortest spawning period but consistently maintained high annual recruitment and the highest living densities measured throughout the estuary, suggesting it to be a sink population. With high juvenile recruitment during all year types, BI likely received larvae from both upstream and downstream sites. Even in wet years, salinity at BI rarely fell below 5 and typically did not fall below 10 for more than one consecutive month. Larval stages and newly settled spat benefit from the moderate salinities at BI, while downstream sites experience hyperosmotic conditions (>30) during dry years and upstream sites experience hypoosmotic conditions (<5) during wet years, both of which can inhibit early developing larvae and recently metamorphosed spat (Scharping et al., 2019). Although salinities at BI remained more moderate, with less extreme hyper- and hyposaline events compared to other sites, this salinity range also created ideal conditions for *P. marinus* (Reece & Dungan, 2006) and was reflected by the high infection prevalence and intensity rates at this site. Infection of *P. marinus* has been shown to reduce condition index (Chu & Volety, 1997; Paynter & Burreson, 1991), growth (Paynter & Burreson, 1991), and reproduction (Barber, 1996; Dittman et al., 2001; Powell et al., 2003) in oysters, and weighted infection intensities as low as 2 can cause mortality (Southworth et al., 2010). Oysters at BI had the highest infection rates and the lowest gonad and condition indices, suggesting that infection of *P. marinus* has negative effects on the fitness of oysters at this site. It is thus likely that although BI serves as an important stepping-stone population, it may rely heavily on larval input from other sites within the CRE with little self-recruitment.

Although larvae are largely at the mercy of currents (and, thus, wind) and tidal transport due to their limited swimming behavior (Kennedy, 1996), they can be transported distances as great as 226 km (North et al., 2008). Habitat connectivity is therefore vital to maintaining populations across the estuary. Source–sink dynamics are likely to be fluid and shift between years (Lipcius et al., 2008) depending on hydrodynamics and salinity patterns, particularly during and following prolonged drought or flood conditions. These source–sink population dynamics are essential to maintain connectivity and allow for resilient oyster metapopulations in the CRE, even under extreme interannual variation in environmental parameters.

Management

This 15-year oyster monitoring program has resulted in an invaluable data set showing the dynamics of wild oyster populations in the CRE in relation to freshwater flows and variation in salinity regimes. When stages in Lake Okeechobee are low during dry years, little water is diverted to the estuary, resulting in hyperosmotic conditions. During wet years, when the lake levels are high going into the summer rainy season, often too much freshwater is released to the estuary to keep lake levels low for dam safety. Low salinity mortality events related to freshwater inflow elicit the most dramatic mortality events at upstream sites, but too little water can have equally as detrimental effects within the lower CRE, making the timing and duration of freshwater releases critical. It has previously been suggested that short-pulse freshets (1 to 3 weeks) to reduce salinity during periods in which oysters are less vulnerable to osmotic stress (winter and early spring) are effective at reducing disease burden (La Peyre et al., 2003, 2009). These freshets must be appropriately timed to prevent mortality associated with low prolonged salinity at upstream sites, which are believed to serve as vital sources of larval recruits to the CRE. To achieve salinities at the most upstream site (IC) of ≥ 5 would require limiting flows

at S-79 to < 5000 cfs but targeting a salinity of ≥ 16 would require flows be limited to < 2000 cfs (Fig. 4).

These two flow rates could represent maximum winter and summer flow rates, respectively; however, it is also critical to consider the duration of the freshet as well as the current state of the system. Continuous flows of 500 cfs in the summer months (June–October) are optimal for IC and maximum summer flows of 2000 cfs limited to short (< 1 week) pulses would both maintain optimal salinity regimes and reduce loss of larvae to the system. Short, repeated releases in the winter months would also allow for a reprieve from hypersaline conditions downstream as well as a reprieve from the disease at mid- and upper-estuary sites. As previously described, no significant mortality was observed during the winter of 2010 when freshwater flows were > 2000 cfs for nearly 4 months, even though salinity remained < 5. Prioritizing freshwater releases in the winter and spring is critical to maintaining healthy reefs across the estuary. In addition to management that includes annual winter/spring freshwater releases, adding minimum summer flow rates of 500 cfs will reduce hyperosmotic conditions in dry years and limit the spread of pathogens and predators into the upper estuary. Freshwater flow rates in this range (500–2000 cfs) will also protect submerged aquatic vegetation, another critical ecosystem engineer in the CRE (Doering et al., 2002), and ultimately protect species richness and diversity in the estuary (Peterson et al., 2003; Tolley & Volety, 2005).

Rapid fluctuations from periods with too much freshwater to drought conditions are predicted to be amplified under current climate change predictions (Vedwan et al., 2008), which further complicates the management of lake water levels and scheduled freshwater releases. Climate extremes are predicted to increase in frequency and intensity (Easterling et al., 2000; Smith, 2011) and are amplified during El Niño and La Niña events, which lead to increased rainfall or drought conditions, respectively, and cause significant changes in Lake Okeechobee water levels (Abtew & Trimble, 2010). Additionally, predictions suggest an intensification of El Niño Southern Oscillations (Fasullo et al., 2018) and hurricanes (Elsner et al., 2008), two factors that already significantly alter salinity in Florida estuaries (Gomez et al., 2019; Phlips et al., 2020). This further highlights a critical need to prioritize winter and spring releases to reduce hyperosmotic exposure during dry years and in preparation for the summer rainy season, particularly when El Niño events are predicted to bring higher than average rainfall.

Conclusions

Over a 15-year monitoring period, the effects of a highly managed water system on local oyster reefs were carefully documented. These results can be used to guide water management decisions based on timing, duration, and magnitude of freshwater releases to maintain healthy oyster reefs, and are applicable to other regions of Florida and where freshwater inflow may impact oyster farming and the aquaculture industry. Oysters on reefs in the CRE frequently experience both hypo- and hyperosmotic stress in response to seasonal patterns in salinity. The salinity regime across the estuary is associated with controlled freshwater releases, of which the timing, magnitude, and duration have significant effects on oyster populations. Due to the synergistic effects of thermal and osmotic stress, freshwater releases in the summer months, when oysters of all life stages are most vulnerable, can lead to extensive mortality at upriver sites. However, habitat connectivity and larval mixing between reefs allow for a rapid recovery and suggests fluid source-sink dynamics that respond to constantly changing, and sometimes extreme, environmental conditions. The interaction between salinity and *P. marinus*, in addition to the negative effects each can inflict individually, requires a dynamic approach to management strategies. Although low salinities in the late summer, when temperature and reproductive stress are at a maximum, were shown to be fatal, temporary decreases in salinity during winter and early spring may benefit oyster populations by reducing *P. marinus* infection intensities. Because oysters are a keystone species that provide habitat to hundreds of estuarine organisms (Wells, 1961), implementation of freshwater flow to maintain salinity regimes that support healthy oyster reefs will in turn improve habitat suitability for many other ecologically and economically important estuarine and marine species (Tolley et al., 2005). Using real-time data collection of freshwater flow from the S-79 lock and dam, salinity across the estuary can be estimated and used to maintain daily flows to preserve oyster populations across the estuary. This work demonstrates a balance required to maintain healthy oys-

ter populations in a significantly altered system and provides resource managers with biological response data linked to the timing and intensity of freshwater input.

Acknowledgements This project was funded through a grant from the RECOVER Program and the South Florida Water Management District (SFWMD; award #460000227). We are grateful to Ms. Darlene Marley for her assistance with data management. Flow data was generously provided by Ms. Kathy Haunert (SFWMD). Many people helped with the field and laboratory work associated with this project. First and foremost, we thank our laboratory managers, Ms. Erin Rasnake, Ms. Lacey Rains, Ms. Lindsay Castret, and Ms. Nicole Haas for their dedication, hard work, planning, and help with both field and laboratory tasks. Ms. Christal Niemeyer and Mr. Timothy Bryant were invaluable in providing logistical support. We are also indebted to many undergraduate and graduate students. Without their help and dedication, this project would not have been possible. Lastly, we would like to thank comments from three anonymous reviewers who provided valuable feedback during the review process.

Funding This project was funded through a grant from the RECOVER Program and the South Florida Water Management District (SFWMD; award #460000227).

Availability of data and material Data will be made publically available upon publication.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

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