Predominant factors limiting the recovery of the eastern oyster (*Crassostrea virginica*) in western Mississippi Sound, USA

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ARTICLE INFO

ABSTRACT

In addition to suitable substrate, successful oyster recruitment requires an adequate supply of planktonic larvae, followed by the subsequent survival and growth of early post-settlement stages. Substrate limitation, recruitment limitation and post-settlement limitation each played key roles in limiting the recovery of the eastern oyster, *Crassostrea virginica* (Gmelin 1791) within western Mississippi Sound between 2016 and 2019. Effects of a mortality event which eliminated the oyster spawning stock biomass from two major oyster reefs of western Mississippi Sound in 2016 carried over to the 2018 recruitment period. But the supply of larvae from outside the affected reefs was sufficient to provision spat to restored and reference sites within the 2018 study area. Thus, substrate limitation was inferred to be the primary limitation to early oyster recruitment in 2018. Moreover, variability in spat metrics from settlement samples implied potential post-settlement limitation in 2018. Spat density, mortality, size, and inferred growth varied on temporal, subregional, and microhabitat scales. In 2019, oyster spawning stocks were completely decimated throughout the western Mississippi Sound region by unprecedented freshwater discharge from the Bonnet Carré spillway. Consequently, early oyster recruitment was effectively eliminated, as evidenced by the lack of spat settlement across the entire oyster metapopulation of western Mississippi Sound. Within the course of a single year, the predominant deterrent to oyster recovery had shifted from substrate limitation to recruitment limitation.

1. Introduction

1.1. Background

Over the last 130 years, 85% of oyster reefs have been extirpated worldwide due to joint effects of overfishing, shell extraction, eutrophication, habitat degradation, and disease (Beck et al., 2011; Grabowski et al., 2012; Brown et al., 2014; George et al., 2015). In addition to fishery yields, oyster reefs provide multiple ecosystem services, including improved water quality through filtration and nutrient removal, improved nutrient cycling, enhanced secondary production of associated fauna, and increased protection of nearby shorelines (Beck et al., 2011; Coen and Humphries, 2017). Many ecological benefits of oyster reefs depend on positive feedback effects of the oyster as a biogenic foundation species (Schulte et al., 2010). Thus, much importance has been placed on restoring oyster reef habitats to reestablish ecosystem services (Coen et al., 2007; Baggett et al., 2015).

Unfortunately, deleterious effects of multiple stressors impede the recovery of oyster reefs at a time when their services are most critical. Accordingly, extensive efforts to restore oyster reef habitats in different regions have been met with erratic success (Mann et al., 1991; Mann and Powell, 2007; Lipcius et al., 2015). Clearly, there is a pressing need to understand how to facilitate oyster reef restoration on a regional level.

In addition to hard substrate for larval settlement, successful oyster restoration requires adequate delivery of planktonic larvae, followed by settlement, survival, and growth of early post settlement stages (i.e., spat) (Brumbaugh and Coen, 2009). Recruitment limitation occurs when the size of the spawning stock is constrained by the supply of larvae (Chesson, 1998). While larval supply would ordinarily be non-limiting for healthy self-replenishing oyster reefs containing viable adult oyster stocks (Lipcius et al., 2008), the need for oyster restoration implies that recruitment limitation is important especially when adult oyster stocks are diminished (Lipcius et al., 2015). In addition to producing larvae, adult oysters produce chemical cues which larvae home in on during

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settlement (Kennedy et al., 1996). Deprived of live adult oysters, substrate subsidies provided by dead oysters (i.e., boxes) quickly disappear through dissolution (Pace et al., 2020a), and early recruitment can be greatly reduced where living adult oysters are deficient (Pace et al., 2020b). Nevertheless, restored reefs may overcome recruitment limitation via connectivity to the larger oyster metapopulation (Peterson and Lipcius, 2003). As such, the supply of early recruits would originate from outside source areas.

Diminishing adult oyster stocks can impose the double jeopardy of substrate limitation and recruitment limitation on restoration success (Soniat and Burton, 2005; Brumbaugh and Coen, 2009). Both kinds of limitation are important considerations for the selection of restoration sites by managers. Thus, the proximity of living adult oysters as a source of early recruits is an important criterion for the selection of restoration sites (Coen and Humphries, 2017), as densities of adults often correlate positively with abundances of settlers (Atwood and Grizzle, 2020). However, the proximity of adult stocks as sources of larvae can vary over a wide range of spatial scales, spanning from tens to tens of thousands of meters (Bushek, 1988; Quayle, 1988; Pineda et al., 2010; Atwood and Grizzle, 2020). Because larval oysters remain planktonic for two to three weeks, they can be transported long distances by currents (Kim et al., 2010). Thus, the determinants of spatial relationships between adult oyster stocks and early recruits is a primary concern. The capacity for an oyster reef to serve as a larval source or a sink depends on its spatial context relative to other reefs from which larvae can be transported during the planktonic period.

Recruitment limitation is primarily envisaged for populations that are regulated by density-independent processes operating at low levels of larval supply (Doherty, 1981; Connell, 1985). In contrast, post-settlement limitation entails the regulation of early recruitment through density-dependent processes (Steele, 1997), including competition, predation, and growth. The transition from post-settlement limitation to recruitment limitation presumably occurs at some threshold level of larval supply below which density-dependent regulation of post-settlement stages is negligible (Knights et al., 2012). However, both density-independent and density-dependent processes can operate concurrently at low levels of larval supply (Holm, 1990). Conversely, early recruitment is never totally independent of the larval supply rate (Caley et al., 1996; Chesson, 1998). Notwithstanding substrate limitation, it is the relative importance of recruitment limitation versus post-settlement limitation that accounts for variability in early recruitment (Bushek, 1988; Knights et al., 2012).

The demise of oyster reefs extends throughout the Gulf of Mexico due to various pressures (La Peyre et al., 2014). In the western Mississippi Sound, oyster landings have fluctuated erratically and declined steadily since 1939 (Posadas, 2018), in response to multiple stressors largely in connection with excessive freshwater discharge. The history of oyster decline reflects the construction and use of the Bonnet Carré spillway since 1931 (Bonnet Carré Spillway Overview, 2019; Swenson, 2019), because the spillway diverts excess discharge from the Mississippi River towards the Mississippi Sound. Oyster yields have been particularly low since 2000, and especially since 2011, in connection with the increased frequency of Bonnet Carré opening (Renfro, 2019). The spillway has been opened six times in past 11 years compared to a total of eight times during the first 70 years of its existence. Accordingly, oyster harvests have fluctuated between 2% and 15% of pre-Hurricane Katrina (2004–05) levels of ~500,000 sacks (i.e., ~8.1 × 10^6 kg) of live oysters.

In late summer 2016, the spawning stock biomass of oysters was virtually eliminated within the core reef areas of Mississippi Sound by flooding induced hypoxia (Pace and Powell, 2019; Pace et al. 2020a, 2020b, 2020b). Consequently, these core reefs were precluded from self-replenishing or contributing oyster larvae to other reefs. In 2019, an even more extensive mass mortality event occurred in response to unprecedented sustained freshwater discharge from the Bonnet Carré spillway. The spillway was opened over two successive periods (Renfro, 2019; Swenson, 2019), the first for 43 d starting in late February 2019

and the second for 80 d starting in mid-May 2019 (Gledhill et al., 2020; Pace et al., 2020b). Consequently, more than 90 percent of adult oysters perished throughout western Mississippi Sound (Pace and Powell, 2019, Bonnet Carré Spillway Overview, 2019). In addition to excessive low salinity, other exacerbating stressors included high temperatures, harmful algal blooms, and low dissolved oxygen (Gledhill et al., 2020). Consequently, the entire oyster metapopulation of western Mississippi Sound was completely decimated (Pace et al., 2020b, Bonnet Carré Spillway Overview, 2019).

1.2. Objectives

The overarching objective of this study was to examine how early recruitment of oysters was limited within western Mississippi Sound during the 2018–2019 study period. Specific objectives include: (1) whether outside sources of oyster larvae were sufficient to support early recruitment in the absence of local sources; (2) how spatiotemporal variability in early oyster recruitment could potentially limit oyster reef restoration; and (3) whether oyster recovery was impeded predominantly by substrate limitation, recruitment limitation, or post-settlement limitation during the study.

2. Materials and methods

2.1. Study area

The study area encompassed the western Mississippi Sound (Fig. 1), a shallow estuary partially closed off from the Gulf of Mexico by several barrier islands (Chigbu et al., 2004). Mississippi Sound is often well mixed and turbid due to winds and tides (Eleuterius, 1978). Within the Sound, saltwater influx is regularly mixed with freshwater discharge originating from seven coastal drainages, primarily the Pearl River at the western end and the Pascagoula River at the eastern end of the Sound. Excessive discharge also periodically flows into the Sound from the Mississippi and Mobile Rivers (Orlando et al., 1993). Mean annual salinity and temperature varies between 9 and 21 ppt, and between 14.5° and 19.4 °C, respectively (Chigbu et al., 2004). Salinity and water temperature also vary considerably during the oyster recruitment season (Ogle, 1979). The western Mississippi Sound historically harbored some of the most productive oyster stocks in the Southeastern US (Gore, 1992).

Diminishing yields of the eastern oyster *Crassostrea virginica* (Gmelin 1791) have prompted programmatic efforts to restore oyster reefs in Mississippi, often through substrate augmentation (Mississippi Oyster Council, 2015). After the 2010 Deepwater Horizon oil spill, a large-scale oyster restoration program was undertaken within Mississippi coastal waters, supported by the Natural Resource Damage Assessment (NRDA) and the RESTORE Act. Accordingly, in summer 2017 the substrate was augmented by relic shell or limestone gravel at six sites within the Pass Christian and Pass Marianne reefs (Fig. 1). To investigate variability in early oyster recruitment during the 2018 period, larval density and spat settlement were sampled at a total of eight sites. Four restored sites within the Pass Christian reef, two of each by limestone gravel or relic shell, as well as two comparable unrestored reference sites were designated for study by MBRACE investigators. For a broader spatial context, two additional sites within the Pass Marianne reef were also sampled in 2018, including one unrestored site and one site that had been previously augmented with limestone gravel in 2014. To focus on the effects of the 2019 Bonnet Carré Spillway opening on early recruitment, oyster spat were sampled from seven unrestored sites more widely dispersed among the major reefs throughout western Mississippi Sound during the 2019 oyster recruitment period (Fig. 1). Field sampling was conducted under the auspices of MDMR Permit # SRP-015-18 and # SRP-015-19.
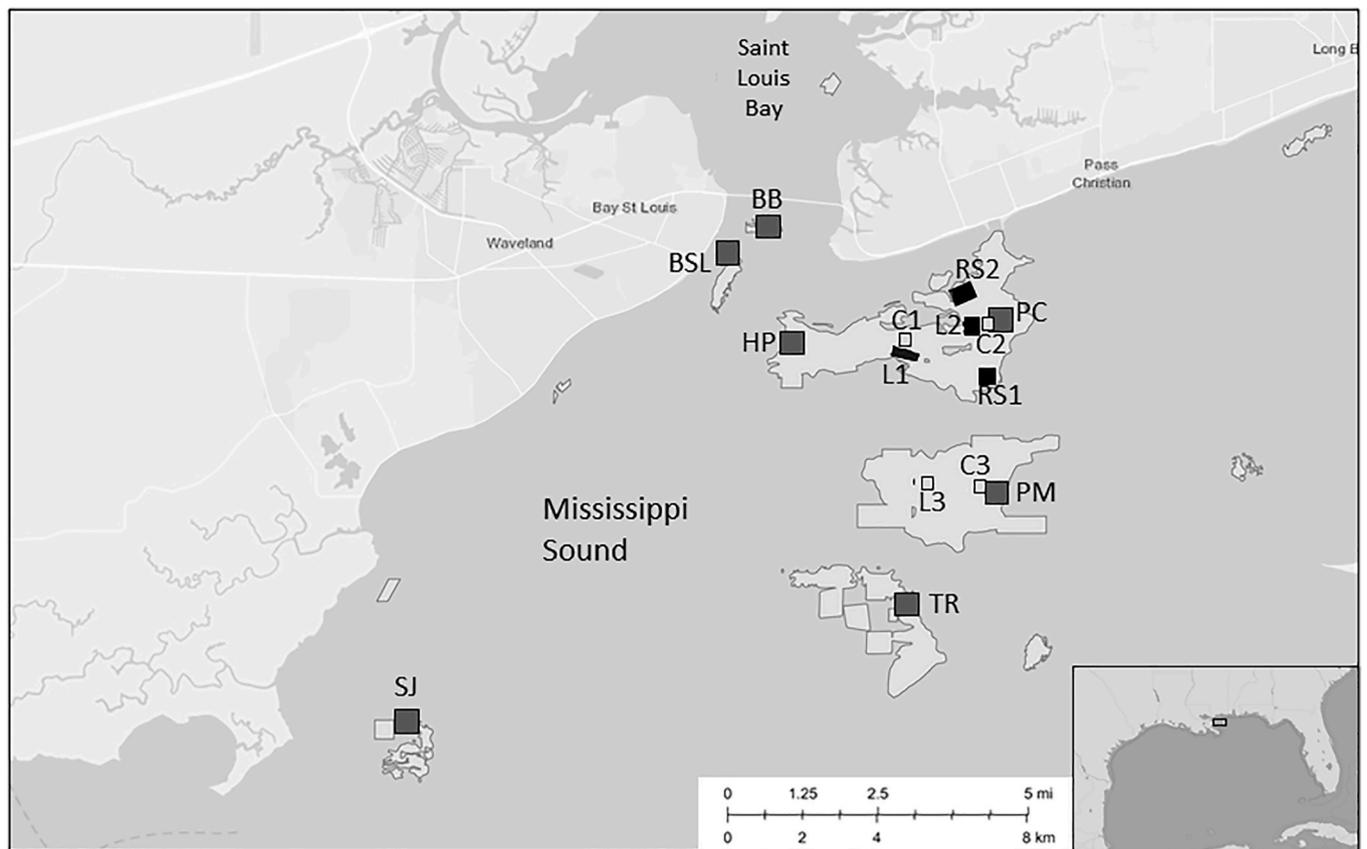
Fig. 1. Map of the study area including sites that were sampled for oyster larvae and spat in 2018: two recently restored relic shell sites (RS1, RS2) and two recently restored limestone sites (L1, L2) within the Pass Christian reef (small dark boxes), as well as two reference sites (C1, C2) within the Pass Christian reef and one reference site (C3) and one formerly restored limestone site (L3) within the Pass Marianne reef (small open boxes). In 2019, spat were sampled from major reefs (medium shade boxes) located throughout western Mississippi Sound: BB = Between Bridges; BSL = Bay Saint Louis; HP = Henderson Point; PC = Pass Christian; PM = Pass Marianne; TR = Telegraph Reef; SJ = Saint Joe (see Appendix A for geographical site coordinates).

2.2. Field sampling

Oyster larvae were sampled monthly from June through September in 2018 at the eight sites. At each site, two 3-min horizontal tows were made using a 64-μm mesh conical plankton net. The center of the net opening was suspended at a depth of 1 m within the water column by attaching a dive weight to the base of the net frame and bullet float and line to the top. An Oceanics™ Model 2030R flowmeter estimated the volume of water filtered during tows. After each tow, the net was rinsed with filtered seawater using a WORX® 20V Hydroshot portable power washer; net contents were filtered through 35-μm mesh nitex and preserved with 5% formalin.

In 2018, four settlement samplers were set out at each of the eight sites over two monthly periods starting in mid-July and ending on 10 September (Appendix A.1.a). One sampler was lost from the first sample period and five were lost among two sites during the second sample period (i.e., 3 from one relic shell site and 2 from the Pass Marianne limestone site). In 2019, four settlement samplers were set out at each of the seven sites located at major reefs spread throughout western Mississippi Sound to assess the impact of the Bonnet Carré opening on early recruitment (Appendix A.1.b). Exclusion cages were not used in 2019, as predation was not a focal point. Settlement samplers were consecutively deployed over three monthly periods starting in early July and ending in mid-October. Samples from the July monthly period were not processed due to the apparent lack of spat settlement while the spillway was still open; however, samples from the latter two monthly periods were processed. A total of five samplers were lost during the latter two sample periods in 2019, four from three of the sites during the August period and one from the September period.

Settlement samplers consisted of concrete cinderblocks (39.7 cm L × 19.4 cm W × 19.4 H cm) to which paired ceramic tiles (i.e., settlement plates) measuring 15.2 cm on a side (i.e., 0.023 m²) were securely fastened using zip ties (Fig. 2). To facilitate spat settlement under plates in a predation-restricted condition, a wide zip tie elevated the underside of each settlement plate 3 mm above the cinderblock surface. The upper surfaces of plates were smooth, and lower surfaces were unfinished (i.e., rougher texture). In 2018, the upper surface of one of the two settlement plates on each sampler was covered by a 4-mm stainless-steel mesh cage to exclude macropredators (e.g., oyster drills and mud crabs). Cages were affixed to plates using zip ties.

At each site, four settlement sample units were deployed near predetermined GPS positions, typically at the corners of designated subsample areas (Appendix A.1.a). Samplers were deployed by gently

Fig. 2. Spat settlement collector comprising concrete cinderblock, ceramic settlement plates, 4 mm mesh stainless steel exclusion cage, and spacer for elevating settlement plates to form restricted spaces. Exclusion cages were used in 2018, but not in 2019.

lowering them to the seabed with buoy lines in an upright position (i.e., plates facing up) at depths ranging between 3.0 and 5.5 m. Prior to deployment, the bottom was probed using a PVC pole to ensure placement onto hard substrate, and GPS waypoints of deployment locations were taken. Upon deployment and retrieval, salinity, temperature (°C), dissolved oxygen (DO; mg l^{-1}), and percent DO saturation were measured at the surface and near bottom using a YSI® model 85 handheld meter. Depth and Secchi depth (m) were measured. Recovered settlement plates were carefully removed, double bagged with Ziplock® freezer bags, labeled, and placed on ice. When damaged, exclusion cages were mended using stainless steel locking wire, and cages were cleaned before fastening new settlement plates for redeployment. Upon returning to the laboratory, settlement plates were stored in chest freezers until processed and archived.

2.3. Sample processing

Plankton samples containing large amounts of phytoplankton and detrital material were subsampled using a Folsom splitter while maintaining a target abundance of at least 50 larvae per sample (Morgan, 2019). Plankton was sorted and quality controlled using a Nikon SMZ1500 stereoscope. D-stage larvae (i.e., straight-hinge) and veliger (i. e., developing umbo) morphotypes were quantified separately to distinguish earlier and later stages of the planktonic developmental period. Although D-stages of bivalves are difficult to identify to species, we made every effort to distinguish D-stages of oysters using (1) a reference series of larval stages of oysters; (2) reference photos shared by other colleagues, (3) key literature sources with detailed descriptions of larval morphology for all stages of various bivalve species, and (4) thorough examination of the larvae from each plankton sample. However, we prudently refer to D-stages generically, while maintaining they accurately reflect the amplitudes of D-stage oyster larvae. Abundances were normalized as the number of larvae per cubic meter separately for D-stage and veliger larvae (Morgan, 2019).

Settlement plate surfaces were examined at 20× using a Nikon SMZ1500 stereoscope to identify and quantify spat (i.e., post-settlement stages of oyster). Spat abundances were validated when two consecutive counts agreed within 5%, upon which the mean was recorded. Lengths of up to twenty-five individual spat were measured to the nearest mm from within randomly selected cells of 1.5 cm on a side defined by an overlaid transparent grid of 100 cells. All spat wholly or partially contained within each selected grid cell were measured. Any additional spat above the 25-count target remaining within the last selected grid cell were also measured. Percent cover was estimated separately for spat and barnacles using the point-grid method by overlaying a transparent 100 cell grid marked by cell center points. Percent cover by sediment was also visually estimated when accumulation was substantial, before irrigating and examining plates. Mobile organisms associated with settlement plates (e.g., oyster drill, mud crabs, etc.) were noted, counted, and measured (mm). The presence of other fouling organisms such as mussels was also noted. After processing both surfaces, plates were placed into labeled ziplock bags and archived by refreezing.

2.4. Post-settlement metrics

From each settlement plate surface, recently settled early spat (i.e., ≤2 mm in length; c.f. 'settlers' in Newell et al., 2000), and large spat (>2 mm in length) were quantified separately. Densities (i.e., number per 0.023 m^2) of early and large spat were expressed in terms of soak time in days. Mean size was estimated as the geometric mean length of the randomly selected spat and maximum size (mm) as the length of the largest spat present. Implicit specific growth was estimated as: $\log_{10}$ (mm maximum size/1 mm)/# days deployed (Morgan, 2019). Also, a spat scar index was calculated from the number of spat scars: (# Spat Scars + 1)/((# Spat Scars + # Large Spat) + 1) (Morgan, 2019). Finally, the percent cover was estimated based on the point-grid method for both spat and barnacles.

2.5. Statistical analysis

Responses were analyzed with the Linear Mixed Models (LMM) procedure using restricted maximum likelihood (REML) estimation and F-tests to determine significance levels in SPSS version 25. Larval densities were log transformed (log N + 1) before analysis. The larval LMM design entailed a repeated measures model in which sample period served as a repeated within-subjects factor to account for non-independence of residual errors and unequal variance. Heterogeneity of variance across sample periods was fitted using a diagonal covariance structure and confirmed by a Wald Z test. The larval LMM model also contained an intercept term, site as a fixed between-subject factor, and the interaction between sample period and site. Individual plankton tows were designated as subjects. A set of four custom and interaction contrast statements focused on temporal patterns and interactions between temporal and site groups as inferred from profile plots of larval abundances. Contrast statements were incorporated within the LMM syntax using Reverse Helmert coding and included distributed terms for all implicit interaction effects. Statistical significance of contrast statements was based on t-tests.

Settlement metrics from 2018 samples were analyzed using a Linear Mixed Model (LMM) containing four fixed factors: month (2 levels), site (8 levels), plate surface (2 levels, upper vs. lower), and exclusion (2 levels, caged vs. exposed). The full LMM consisted of terms for a general intercept, the four fixed factors, and all higher-order interactions among the fixed factors. Individual settlement samplers served as subjects and were specified as a random factor (i.e., variable intercepts) for which the Variance Components covariance structure was specified. The random factor designation was omitted from models for the spat scar index and the implicit specific growth index, due to a redundant variance term.

A set of seven *a priori* custom contrast statements addressed differences between specific factor levels and selected groups of interest: (1) early vs. later sample period; (2) offshore sites vs. inshore sites; (3) reference vs. relic-shell restored sites; (4) reference vs. limestone restored sites; (5) reference vs. all restored sites; (6) excluded upper surfaces vs. exposed upper surfaces of settlement plates; and (7) restricted lower surfaces vs. exposed upper surfaces of settlement plates. Contrast statements were incorporated within the LMM syntax using Reverse Helmert coding and included distributed terms for all implicit lower- and higher-order interaction effects. Statistical significance of contrast statements was based on t-tests. Settlement samples from 2019 were examined using descriptive statistics.

3. Results

3.1. Synopsis

Larval abundances varied widely among the 64 plankton samples taken at the eight sites over the four sample periods in 2018. Densities (i. e., mean ± 1 se) of D-stage larvae averaged 649.0 m^{-3} ± 510.7 and reached as high as 32,574 m^{-3}, and densities of veliger larvae averaged 56.4 ± 8.6 se m^{-3} and reached as high as 323 m^{-3}. Corresponding physical data for plankton samples are presented in Appendix A.2.a.

Settlement metrics from 232 plate surfaces (i.e., 116 plates) varied widely among the eight sites over the two sample periods in 2018 (Table 1). Abundances of early and large spat ranged from 0 to 365 and from 0 to 421 per 0.023 m^2, respectively. And the number of spat scars ranged from 0 to 178 per 0.023 m^2 (i.e., area of plate surface). The geometric mean spat size ranged from 1.03 to 9.95 mm, while the maximum spat size ranged from 2 to 32 mm. Percent spat cover reached 79%, while the percent barnacle cover reached as high as 99%. Corresponding physical data for the 2018 spat samples are presented in Appendix Table A2. b.

A total of 5 spat were recovered in 2019 from 102 settlement plates

Table 1

Descriptive statistics for seven variables from 232 settlement plate surfaces (i.e., 116 settlement plates), each of 0.023 m² in area, deployed at eight sites during two consecutive monthly periods in 2018. Mean spat size and maximum spat size in mm.

Variable	N	Range	Minimum	Maximum	Mean	Std. Dev.
Number Large Spat	232	421	0	421	53.60	61.76
Number Early Spat	232	365	0	365	27.59	47.00
Percent Spat Cover	232	79	0	79	16.86	15.77
Number Spat Scars	232	178	0	178	7.56	17.31
Mean Spat Size	232	8.82	1.03	9.95	4.34	2.07
Maximum Spat Size	232	30	2	32	12.50	5.41
Percent Barnacle Cover	232	99	0	99	27.61	31.99

(i.e., 204 plate surfaces representing 4.74 m² total area) obtained and processed from the seven major reefs in western Mississippi Sound over the latter two sample periods when salinities were extremely low (Appendix Table A2. c). All five spat were larger than 2 mm and the largest was 32 mm. Three spat were recovered from the Pass Marianne reef during the August sample period and two spat were recovered from Telegraph reef during the September sample period. Both sites are located relatively offshore where salinities were not as low. Salinities were extremely low at the nearshore reefs (Appendix Table A2. c). Even barnacle cover (i.e., mean ± 1 se) was notably lower at four nearshore reef sites (3.36% ± 0.72 se) than at the Pass Marianne, Telegraph and Saint Joe reefs (11.89% ± 1.89 se).

3.2. Temporal variation

The highest densities of D-stage larvae occurred in September compared to the three earlier sample events in June, July, and August of 2018 (t = 6.59; P < 0.001). Additionally, D-stage densities were higher in the earlier June period than in the middle two periods in July and August (t = −6.74; P < 0.001) (Fig. 3a). Moreover, densities of D-stage larvae were higher in June and alternately lower in September at the two further offshore Pass Marianne reef sites compared to the six Pass Christian reef sites (t = −4.98; P = 0.001). Densities of veliger larvae were higher during the latter half of the 2018 study period, in August and September, than during the first half of the study period, in June and July (t = 5.92; P < 0.001) (Fig. 3b).

Temporal differences (i.e., mean ± 1 se) in 2018 were marked by significantly higher values in the early months (i.e., June and July) than in the later months (i.e., August vs. September) for the density of large spat (134.35 ± 13.04 vs. 44.78 ± 3.91 per m² d⁻¹; P < 0.001), implicit growth rate (0.044 ± 0.001 vs. 0.033 ± 0.001 mm d⁻¹; P < 0.001), mean spat size (5.19 ± 0.15 mm vs. 3.38 ± 0.19 mm; P < 0.001), spat cover (0.78 ± 0.07 vs. 0.47 ± 0.04% d⁻¹; P < 0.001) and barnacle cover (1.28 ± 0.12 vs. 0.77 ± 0.10% d⁻¹; P = 0.033); while temporal differences (i.e., mean ± 1 se) were marked by significantly higher values in the later than the earlier sample period for the density of early spat (11.30 ± 1.30 vs. 70.87 ± 7.83 per m² d⁻¹; P < 0.001) and maximum spat size (11.18 ± 0.36 mm vs. 14.02 ± 0.61 mm; P < 0.001) (Appendix Table A4) (Fig. 4). Thus, densities of early and large spat varied inversely between early and later sample periods. Mean spat size varied widely among sites within each sample period. Larger mean sizes of spat in the early sample period possibly reflected corresponding lower densities of early spat. The maximum spat size was larger and more variable in the later sample period, at least partly due to greater soak time (i.e., 32.7 d late vs. 22.8 d early). Nevertheless, the implicit specific growth rate was higher during the early sample period (Fig. 4d).

3.3. Sub-regional variation

In 2018, three settlement metrics differed between groups formed by the two offshore sites (within the Pass Marianne reef) versus the three closest nearshore sites within the Pass Christian reef (Appendix Table A4). The density (i.e., mean ± 1 se) of large spat (90.43 ± 12.61 vs. 62.17 ± 7.83 per m² d⁻¹; P = 0.008), and the spat scar index (0.31 ± 0.03 vs. 0.16 ± 0.02; P < 0.001) were higher at the offshore sites, whereas barnacle cover was ten-fold higher at the nearshore sites (1.45 ± 0.14 vs. 0.14 ± 0.04% d⁻¹; P < 0.001).
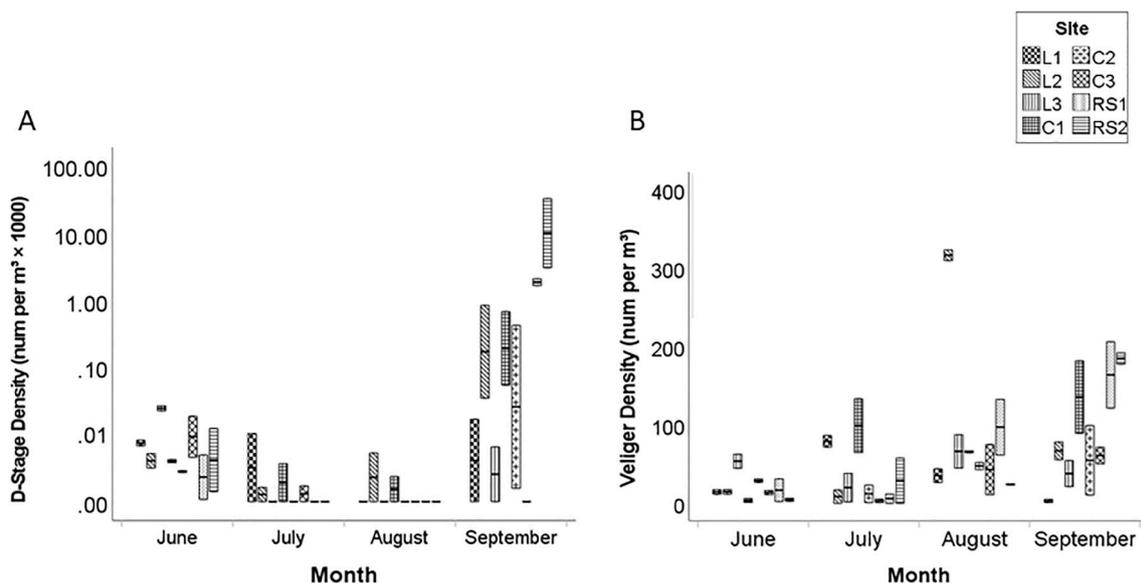
Fig. 3. Bar plots depicting distributions (median, interquartile, range) for densities of D-stage and veliger larvae sampled over four monthly periods during summer 2018. Legends: L1, L2 - recently restored limestone Pass Christian sites; L3 - formerly restored limestone Pass Marianne site; C1, C2 –reference Pass Christian sites; C3 - reference Pass Marianne site; RS1, RS2- recently restored relic shell Pass Christian sites. The highest densities of D-stage larvae occurred in September compared to the three earlier periods (t = 6.59; P < 0.001), and densities of D-stage larvae were higher in June than in July and August (t = −6.74; P < 0.001) Densities of veliger larvae were higher in August and September than during June and July (t = 5.92; P < 0.001).
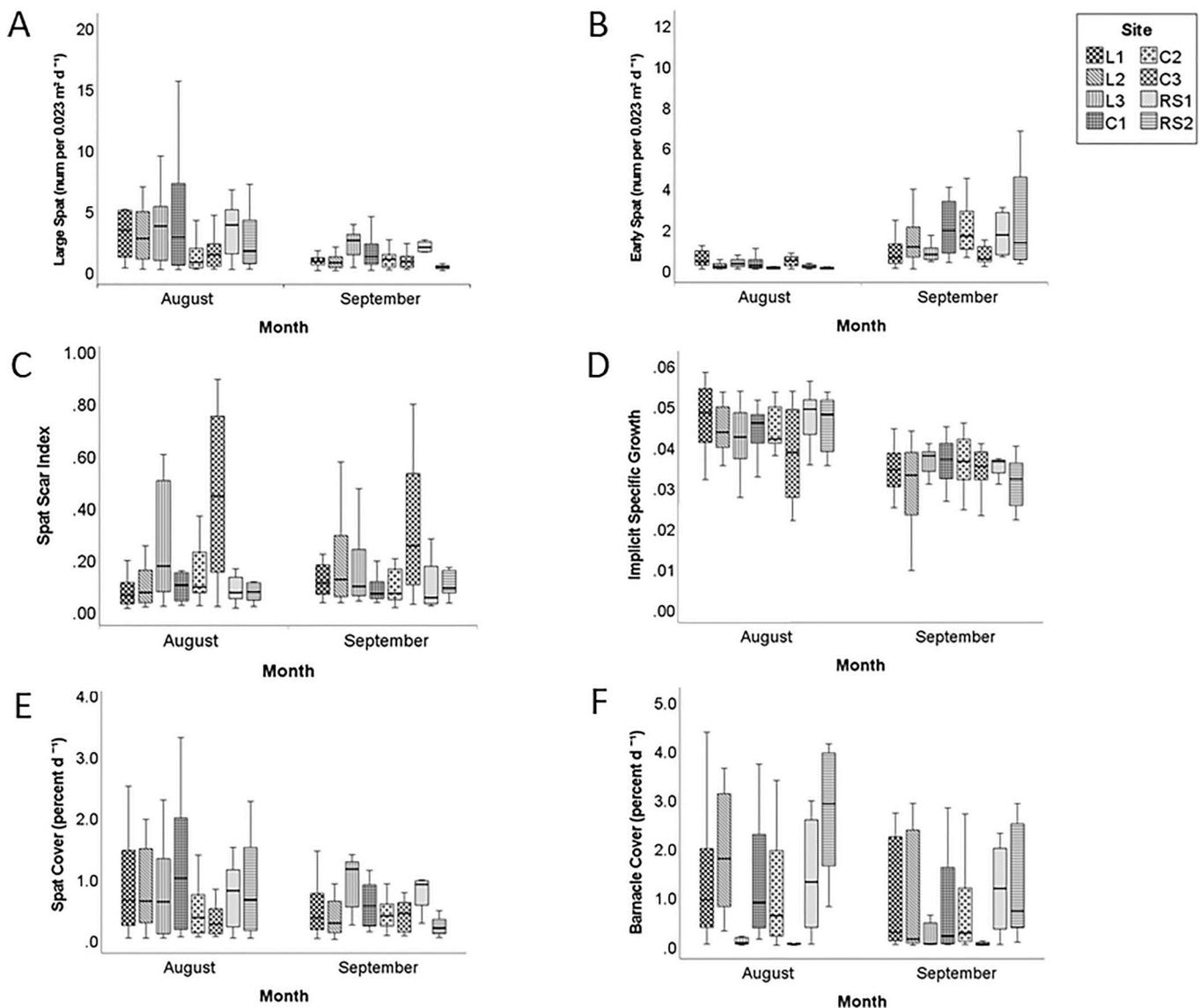
Fig. 4. Bar plots depicting distributions (median, interquartile, range) for six settlement metrics varying among the eight study sites between the two consecutive sample periods in 2018. A: Large spat abundance per 0.023 m² per day; B: Early spat abundance per 0.023 m² per day; C: Spat scar index; D: Implicit specific growth rate; E: Percent spat cover per day; F: Percent barnacle cover per day. Legends: L1, L2 - recently restored limestone sites at Pass Christian; L3 - formerly restored limestone site at Pass Marianne; C1, C2 –reference sites at Pass Christian; C3 - reference site at Pass Marianne; RS1, RS2- recently restored relic shell sites at Pass Christian. All metrics were different between sample periods at the P < 0.001 level, except for barnacle cover/soak time (P = 0.033).

3.4. Reef type

Although settlement metrics were not measured directly from the reef substrate, results still might reflect processes related to reef type. Percent barnacle cover (i.e., mean ± 1 se) was higher at restored relic shell sites than at both restored limestone sites (1.76 ± 0.18 vs. 1.30 ± 0.16% d⁻¹; P = 0.024) or comparable reference sites (1.76 ± 0.18 vs. 0.99 ± 0.14% d⁻¹; P < 0.001) (Appendix Table A4) (Fig. 4). Barnacle cover also trended higher at limestone sites than at comparable reference sites (1.30 ± 0.16 vs. 0.99 ± 0.14% d⁻¹; P = 0.051). Consequently, barnacle cover was also generally higher at both types of recently restored sites than at comparable reference sites (1.51 ± 0.12 vs. 0.99 ± 0.14% d⁻¹; P < 0.001).

3.5. Microhabitat

Densities of both large and early spat (i.e., mean ± 1 se) were much higher on caged surfaces than on exposed upper surfaces of settlement plates (52.61 ± 9.13 per m² d⁻¹ vs. 30.0 ± 5.22 per m² d⁻¹; P < 0.001; 42.17 ± 10.0 vs. 23.91 ± 5.65 per m² d⁻¹; P = 0.008, respectively), indicating higher early recruitment on caged surfaces (Appendix Table A4) (Fig. 5). Accordingly, the maximum spat size tended to be larger on caged surfaces than on exposed upper surfaces (9.60 ± 0.62 mm vs. 8.64 ± 0.43 mm; P = 0.037). In addition, percent spat cover was almost two-fold higher on caged surfaces (9.53 ± 1.49% vs. 5.26 ± 0.59%; P = 0.002), and inversely related to percent barnacle cover (34.64 ± 4.36% vs. 57.45 ± 4.46%; P < 0.001), possibly reflecting effects of hydrology or competition for space.

Densities of large spat and early spat were five-fold higher and two-fold higher (i.e., mean ± 1 se) on restricted lower surfaces of settlement plates than on exposed upper surfaces (150.43 ± 18.26 per m² d⁻¹ vs. 30.0 ± 5.22 per m² d⁻¹; P < 0.001; 48.70 ± 7.39 vs. 24.35 ± 5.65 per m² d⁻¹; P < 0.001, respectively), indicating higher early recruitment in restricted spaces (Appendix Table A4) (Fig. 6). Moreover, the spat scar index implied more than two-fold lower mortality on restricted than exposed surfaces (0.10 ± 0.01 vs. 0.26 ± 0.03; P < 0.001). Spat also
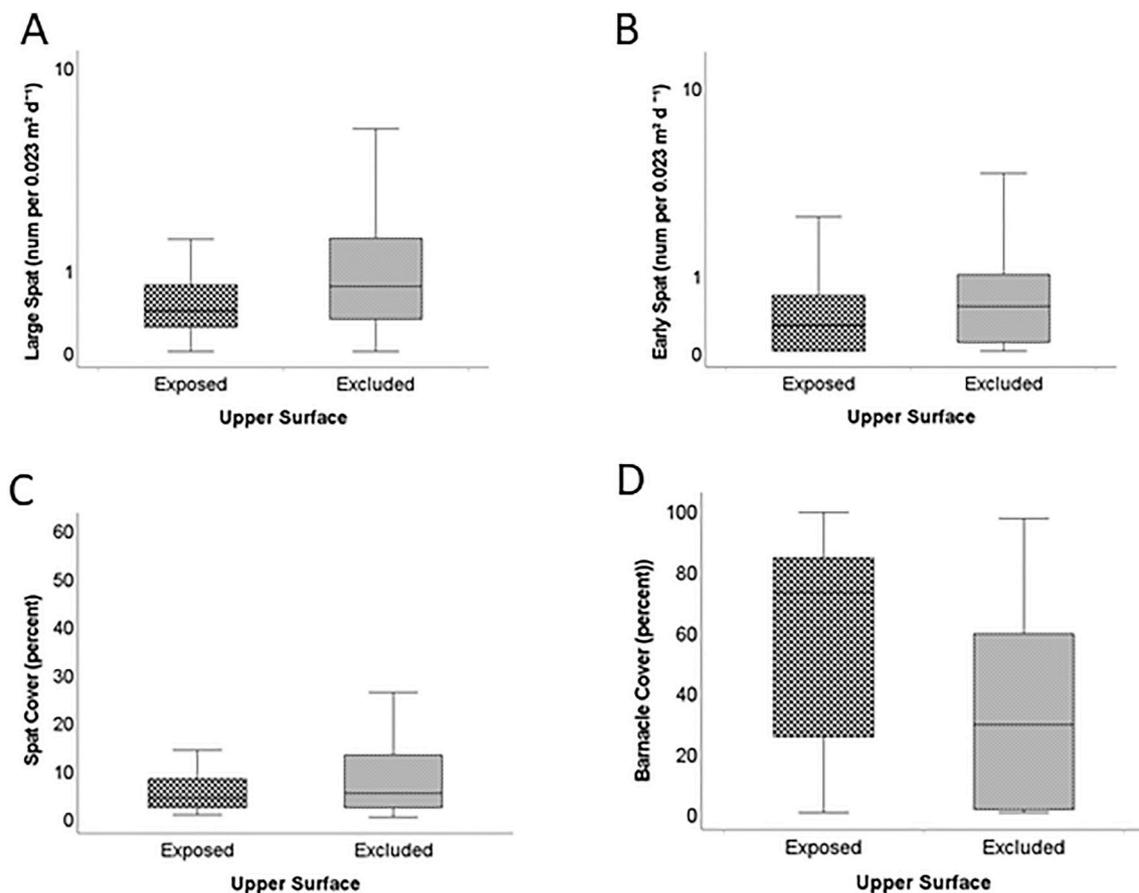
Fig. 5. Bar plots depicting distributions (median, interquartile, range) for four metrics showing differences between exposed vs. excluded upper surfaces of settlement plates. A: Large spat per 0.023 m^2 per day (P < 0.001); B: Early spat per 0.023 m^2 per day (P = 0.008); C: Percent spat cover (P = 0.002); D: Percent barnacle cover (P < 0.001).

reached larger sizes on restricted than exposed upper surfaces, as indicated by both the mean size (4.75 ± 0.26 mm vs. 3.74 ± 0.22 mm; P < 0.001) as well as the maximum size (15.71 ± 0.50 mm vs. 8.64 ± 0.43 mm; P < 0.001). Consequently, the implicit specific growth rate was also higher on restricted surfaces (0.044 ± 0.001 vs. 0.033 ± 0.001; P < 0.001). Finally, percent spat cover was five-fold higher on restricted surfaces (27.41 ± 1.98 vs. 5.26 ± 0.59%; P < 0.001), whereas conversely, percent barnacle cover was more than five-fold higher on exposed upper surfaces of plates (57.45 ± 4.46 vs. 10.09 ± 1.35%; P < 0.001) (Fig. 6). Percent spat cover reached as high as 80% on restricted surfaces, while reaching only 25% on upper exposed surfaces.

4. Discussion

4.1. Substrate limitation vs. recruitment limitation

Successful oyster recovery and restoration hinges on processes linking substrate availability, larval supply, and growth and survival of post-settlement stages (Steele, 1997; Brumbaugh and Coen, 2009; Lipcius et al., 2015; Coen and Humphries, 2017; Walles et al., 2016; Peters et al., 2017). Substrate augmentation cannot expedite early recruitment without a proper supply of larvae, and the recovery of oyster populations is impossible without successful growth and survival of post-settlement stages. Our study illustrates how interplay between all three major factors can limit the recovery of oyster stocks in western Mississippi Sound.

Since the mid twentieth century, the productive oyster stock of western Mississippi Sound has been confronted by multiple stressors related to periodic freshwater discharge (Posadas 2018, 2019). Recent

mass mortality carried over to the virtual lack of oyster brood stock within our study area in 2018 (Pace et al., 2020b). Moreover, substrate limitation intensified due to the lack of input of live and recently dead oyster shell, as well as the rapid dissolution of dead shell (Pace and Powell, 2019; Pace et al., 2020b). Dead oyster shell has an estimated half-life of between 2.5 and 5 years within this system (Pace et al., 2020a). Notably low *in situ* spat settlement within the Pass Christian and Pass Marianne reefs in 2017 and 2018 (i.e., our 2018 study area) was largely attributed to the lack of ideal settlement sites in connection with limited natural substrate (Pace and Powell, 2019; Pace et al., 2020b). However, ample early recruitment potential was evidenced by our larval and spat samples in 2018. Thus, early oyster recruitment was impeded foremost by substrate limitation in 2018. Substrate augmentation can facilitate oyster recovery even where the availability of natural substrate and the local supply of larvae are lacking, provided there is an adequate supply of oyster larvae from outside sources (Peters et al., 2017). However, oyster recovery was ultimately precluded by recruitment limitation in 2019, following extensive oyster mortality in response to sustained excessive freshwater discharge from the Bonnet Carré spillway into western Mississippi Sound (Gledhill et al., 2020; Pace et al., 2020b). In 2019, the virtual lack of spat settlement throughout western Mississippi Sound revealed that both local and outside sources of larval supply were either completely cut off from the region or did not survive exposure to low salinity.

The supply of oyster larvae at any location originates from local retention or as influx from outside sources (Kim et al., 2010). Since the local oyster brood stock was lacking in the 2018 study area, the larval supply must have originated entirely from outside of our 2018 study area. Potential near-field sources of larvae might have involved other
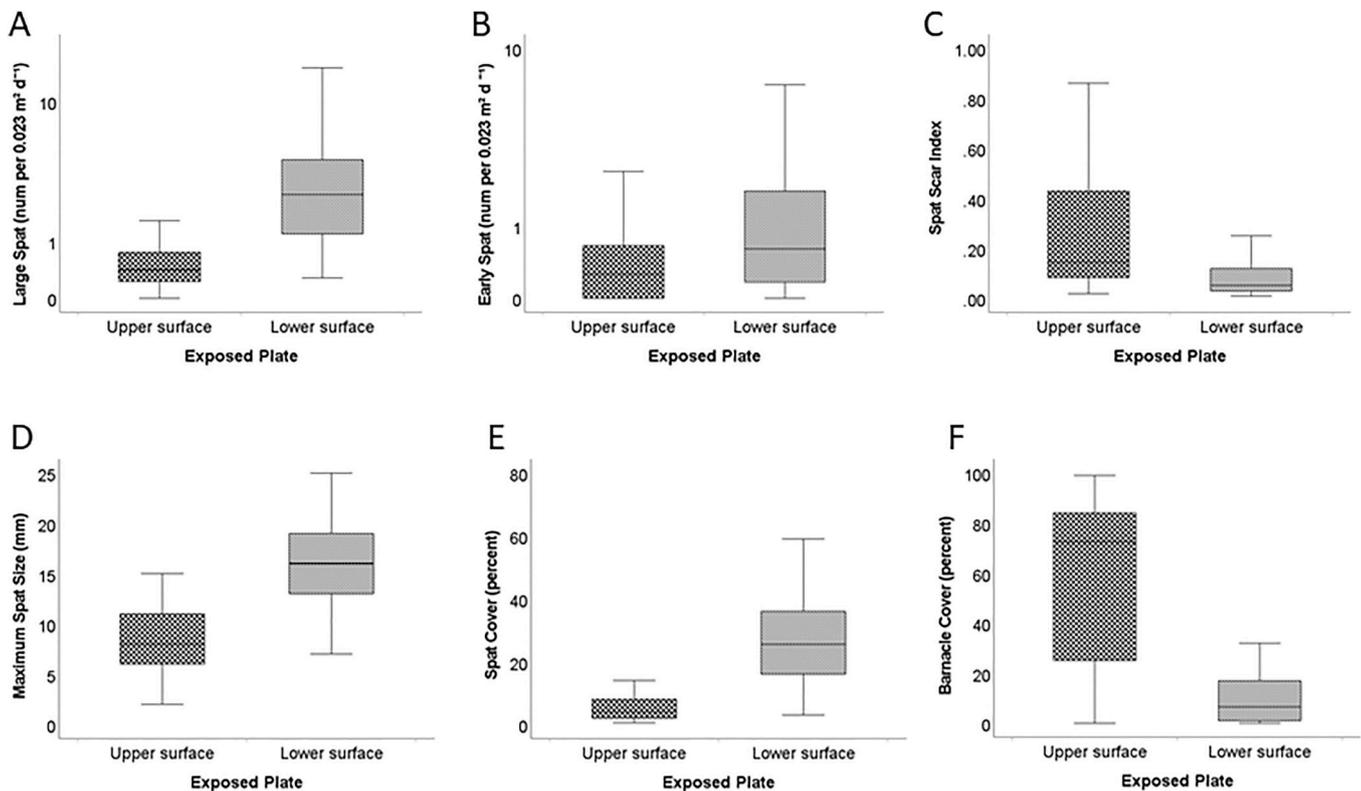
Fig. 6. Bar plots depicting distributions (median, interquartile, range) for six metrics showing differences between upper vs. lower surfaces of exposed settlement plates. A: Large spat per 0.023 m² per day (P < 0.001); B: Early spat per 0.023 m² per day (P < 0.001); C: Spat scar index (P < 0.001); D: Maximum spat size (P < 0.001); E: Percent spat cover (P < 0.001); F: Percent barnacle cover (P < 0.001).

reefs in western Mississippi Sound located from 3.0 to 9.5 km northwest of our 2018 sites, including Between Bridges, Bay Saint Louis and Henderson Point reefs. In summer 2018, Pace et al. (2020b) noted only immature oysters at the Henderson Point and Between Bridges reefs. However, the Bay Saint Louis reef was relatively unaffected by the 2016 mortality event and still harbored living adult oysters in 2018 (Pace et al., 2020b). Other possible nearfield sources of larvae included Telegraph and Saint Joe reefs, located from 3.0 to 19.0 km southwest of our 2018 study area. However, Telegraph reef was also affected by the 2016 mass mortality event (Pace and Powell, 2019), so it is unlikely that larvae originated from this area. Numerous artificial and historic reefs scattered along the Mississippi coast or within Biloxi Bay located about 40 km to the east possibly contributed to the larval pool in 2018. Potential far-field sources of larvae involved the Louisiana Marshes located between 20 and 40 km southwest, historic oyster reefs near the mouth of the Pascagoula River located between 61 and 69 km east, and the Mobile Bay area located 100 km east of our study area. The ability of larvae to reach an area from outside sources depends on the sizes of extant oyster stocks within source areas, as well as the directions and velocities of currents for transporting developing planktonic larvae relative to their survival and attainment of competency (Kim et al., 2010).

To understand connectivity of the oyster metapopulation within western Mississippi Sound, Wiggert et al. (2020) devised circulation models using a combination of CODAR arrays, drifter tracks, and larval suitability information for the 2017 oyster recruitment period. Their results demonstrated both concentrated local retention within western Mississippi Sound as well as considerable potential for connectivity to outside sources of larvae. Biloxi Bay was identified as a prominent potential source of larvae. Drifter tracks and habitat suitability also

revealed the feasibility of larval supply from the Louisiana Marshes. However, poor suitability during the trials made it unlikely larvae could make it from the Mobile Bay area. Salinity was the key stressor influencing suitability along larval transport pathways. The virtual lack of spat settlement that we observed in 2019 showed how disconnected from outside sources the metapopulation of western Mississippi Sound had become. For this region to have become so recruitment limited in a short, one-year period, either broodstocks had become extensively impaired within outside source areas or larvae from outside sources did not survive exposure to low salinities during transport.

4.2. Post-settlement limitation

Despite sparse *in situ* spat settlement observed by others during the same season (Pace et al., 2020b), we observed spatio-temporal variability in post-settlement metrics on spat samplers in 2018. Bartol and Mann (1997) noted disparate densities of spat between natural reef material and oyster shell samplers due to substrate properties and detection efficiencies. *In situ* substrate limitation could also help explain the discrepancy. However, post-settlement potential was normal in 2018, as corroborated by total spat densities of 130.4 ± 8.3 per m² d⁻¹ (mean ± se) on settlement plates. Comparable spat densities of ~100 per m² d⁻¹ within the eastern Mississippi Sound near Mobile Bay were recorded from settlement plates deployed over a three-week period in 2006 (Kim et al., 2010). And spat densities from settlement plates across four sites within the Mobile Bay system in 1998 and 1999 (Saoud et al., 2000), were also comparable with our study. However, total spat density can reach much higher levels (i.e., 2293 per m² d⁻¹), as was seen over a 2-week period in September 1999 at one of the sites in Mobile Bay.

Within a wider geographic context, our observed spat densities also fit squarely within documented ranges for the Atlantic coast of South Carolina and Georgia (Kenny et al., 1990; O'Beirn et al., 1995). Thus, recruitment potential was ample within our 2018 study area, despite the lack of capacity for self-replenishment and substrate limitation.

Early recruitment is never completely independent of the abundance of newly settled spat as a reflection of the larval supply (Holm, 1990; Chesson, 1998). Small spat 2 mm or smaller in size are typically deemed newly settled, in contrast to early recruits which have survived more than 1–2 weeks post-settlement (Roegner and Mann, 1995; Newell et al., 2000). Because they can be subject to different mortality pressures, it is useful to consider early spat separately from late spat. Moreover, newly settled spat may be subject to heavy density-independent mortality (Roegner, 1991; Roegner and Mann, 1995). In contrast, later post-settlement stages are largely vulnerable to density-dependent mortality. Furthermore, Newell et al. (2000) showed how high mortality of newly settled spat was driven by density-dependent mortality in the guise of flatworm micro-predators. Micro-predators were not likely to be a critical source of mortality in our study, as they would have had free access to both caged upper and restricted lower surfaces which excluded macropredators and where early spat were two-fold more dense than on exposed upper surfaces.

Early recruitment processes appeared more dynamic at offshore sites than inshore where the recently restored sites were located. Despite more intense inferred predation (i.e., spat scars), densities of large spat were higher at the two Pass Marianne sites than at the three most inshore Pass Christian sites in 2018. A variety of oyster predators occurred throughout our study area in 2018, including oyster drill (*Stramonita haemastoma*), stone crabs (*Menippe adina*), mud crabs (Panopeidae), and blue crabs (*Callinectes* spp.) (pers. Obsv.). Ten-fold higher barnacle cover at inshore sites suggest spat might have been more space limited inshore. Some studies have found barnacles to compete with early oyster stages (Ortega, 1981), although spat are not necessarily deterred by barnacles (Osman et al., 1989; Barnes et al., 2010). The influence of spatially varying factors on post-settlement processes is an important consideration when selecting sites for oyster restoration.

Reef type contextual factors like predation pressure or settlement cues can influence post-settlement processes. For example, differential attraction of larvae to different substrate types could affect restoration success (Saoud et al., 2000). However, we saw no significant difference between relic shell and limestone sites in the density of spat. Oyster larvae are also attracted to cues released by adult oysters (Kennedy et al., 1996). Thus, the lack of a difference in spat settlement between recently restored and reference sites perhaps reflects the lack of adult oysters at our reference sites during the study period. Interstitial space promotes early oyster recruitment by providing refuge from predation and a less stressful habitat, which should also be considered when choosing substrate types for restoration (Bartol et al., 1999; Coen and Luckenbach, 2000; Nestlerode et al., 2007; Goelz et al., 2020). Although relic shell offers more interstitial space than limestone (Kuykendall et al., 2015), the effect of reef habitat complexity was not directly amenable to our sampling method.

Barnacle cover was greatest at recently restored relic shell reefs, and greater at recently restored limestone reefs than at reference reefs. Aggregated barnacles indicate gregarious settlement behavior (Bushek, 1988). Thus, newly augmented substrate may have been initially colonized by high numbers of barnacles, especially when relic shell was used. By comparison, preexisting substrate on reference reefs would have been limited and subject to fouling by sediment and other organisms, including algae, bryozoans and various epibionts (Osman et al., 1989; Bartol and Mann, 1997). All factors contributing to recruitment bottlenecks or substrate limitation should be considered when selecting

sites for oyster reef restoration (Brumbaugh and Coen, 2009).

Post-settlement metrics varied strongly at the microhabitat scale in our study. Compared to exposed upper surfaces, densities of early spat were higher on both caged upper surfaces and restricted lower surfaces of settlement plates, signaling greater settlement or survival within refuge microhabitats (Roegner, 1991; Newell et al., 2000). Caging can reveal spatial differences in spat settlement rates that are otherwise obscured by accumulated effects of predation (Knights et al., 2012). In our study, caging also yielded higher densities of large spat, larger maximum spat sizes, greater spat cover, and lower barnacle cover. Because oysters typically settle in low flow microhabitats, whereas barnacles settle in high flow microhabitats, these species often segregate based on hydrological differences (Bushek, 1988). Thus, cages likely promoted spat settlement by baffling flow around caged surfaces (Kim et al., 2010). Others have also found that early recruitment of oysters is enhanced by caging (Newell et al., 2000; Carroll et al., 2015). However, it is difficult to discern effects of caging on settlement from those on mortality.

Greater early recruitment within protected microhabitats was also inferred by higher settlement, lower mortality, and faster implicit growth on restricted lower surfaces of settlement plates. Late-stage oyster larvae prefer to settle on shaded sides of settlement plates (Cole and Knight-Jones, 1939; Nelson, 1953; Ritchie and Menzel, 1969; Kenny et al., 1990; Saoud et al., 2000), perhaps because larvae show negative phototactic behavior when seeking suitable sites for settlement (Wheeler et al., 2017). Indeed, Michener and Kenny (1991) found early spat density was five-fold higher on bottom surfaces of settlement samplers. Rough unfinished lower surfaces of settlement plates also differed texturally from smooth finished upper surfaces. Oyster settlement has also been shown to be greater on rougher surfaces. Indeed, larval oysters settle profusely in spaces exemplified by rough surfaces and low shear stress (Saoud et al., 2000; Whitman and Reidenbach, 2012). Thus, intrinsic qualitative properties of settlement plate surfaces could have contributed to differences between the upper and lower surfaces of settlement plates.

Restricted surfaces of our settlement plates resembled crevice and interstitial microhabitats where spat concentrate, grow, and survive best (Nestlerode et al., 2007). Spat dwelling in restricted microhabitats are known experience relatively low current flow, light levels, fouling, and predation (Abbe, 1986; Bushek, 1988; Michener and Kenny, 1991; O'Beirn et al., 1995; Bartol and Mann, 1997; Whitman and Reidenbach, 2012). Accordingly, we noted enhanced survival and growth of spat on lower surfaces of settlement plates, as indicated by the spat scar index and inferred specific growth metrics. Lower mortality implicates reduced predation pressure. Most macropredators (i.e., >3 mm) were excluded from restricted lower surfaces of settlement plates, including the southern oyster drill (*Stramonita haemastoma*) (Garton and Stickle, 1980), and many mobile crustaceans, like panopeid mud crabs, stone crabs (*Menippe mercenaria*) (Rindone and Eggleston, 2011), and blue crabs (*Callinectes sapidus*).

5. Conclusion

Depending on sources, sequences, and strengths of stressors imposed, the three major limiting factors of substrate availability, larval supply, and post-settlement processes may unfold and interact differentially to impede the recovery of oyster stocks. Moreover, primary limiting factors may preclude other limiting factors from being expressed, and the predominance of limiting factors may shift rapidly within stressed ecosystems. For instance, we showed that the lack of local larval supply can be counteracted by outside sources of larvae within our system. Furthermore, substrate limitation can prevent the manifestation of post-

settlement limitation by short-circuiting post larval settlement despite an adequate larval supply. As such, levels of spat settlement were normal when substrate was made available in the form of settlement samplers. Substrate and larval supply are prerequisites of post-settlement limitation. In 2018, spat density, mortality, size, and inferred growth metrics varied on several spatiotemporal scales. Thus, the restoration practice of substrate augmentation could have been effective in 2018. However, after the sustained opening of the Bonnet Carré spillway decimated the oyster spawning stock in 2019, early oyster recruitment was eliminated throughout the western Mississippi Sound. Hence, the primary impediment to oyster recovery shifted from substrate limitation to recruitment limitation within a single year. We conclude that compound effects of multiple stressors can elicit rapid shifts in the predominance of limiting factors – in our case, from substrate limitation to recruitment limitation. Oyster stock recovery and biogenic oyster habitat availability are currently faced with multiple impediments within this highly stressed ecosystem. Recovery of oyster stocks within the western Mississippi Sound will require concerted efforts aimed at restoring the connectivity and structure of oyster metapopulations. Ongoing and planned restoration efforts involve multifaceted projects described in the Mississippi Department of Marine Resources recovery plan (Spraggins et al., 2021). Strategies include habitat restoration through substrate augmentation, strategic siting and creation of oyster spawning reefs, oyster shell recycling, application of hatchery reared spat on shell, and water quality improvement. Experimental manipulations to examine the efficacy of different restoration practices will also help to inform management.

CRediT author statement

Morgan: Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing- Original draft.Rakocinski: Conceptualization, Methodology, Formal analysis, Resources, Data Curation, Editing-Original draft, Revising, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A

Table A.1.a

Locations of the eight sites sampled for oyster larvae and spat in 2018. Samp Area = area within which stations for the deployment of settlement samplers were located; PC = Pass Christian; PM = Pass Marianne; Sqr = square shaped area; Rct = rectangular shaped area; Transct = transect within rectangular site area. (see Fig. 1)

Site	Site Area (acr)	Site Area Shape	Latitude	Longitude	Samp Area	Samp Area Shape
Relic Shell 1 - PC	50	Sqr	30.275	−89.250	13.3 acr	Sqr
Limestone 1 - PC	35	Rct	30.280	−89.273	0.5 km	Transct
Reference 1 - PC	20	Sqr	30.284	−89.273	5.2 acr	Sqr
Relic Shell 2 - PC	50	Sqr	30.295	−89.256	11.0 acr	Sqr
Limestone 2 - PC	35	Sqr	30.287	−89.254	8.5 acr	Sqr
Reference 2 - PC	20	Sqr	30.288	−89.250	5.2 acr	Sqr
Reference - PM	20	Sqr	30.248	−89.252	5.2 acr	Sqr
Restored - PM	20	Sqr	30.249	−89.266	5.2 acr	Sqr

Table A.1.b

Locations and areas for seven sites at oyster reefs located throughout western Mississippi Sound where spat settlement was sampled in 2019. Samp Area = area within which stations for the deployment of settlement samplers were located within larger site areas (i.e., Site Area). (see Fig. 1)

Reef	Site Area (acr)	Site Area Shape	Latitude	Longitude	Samp Area	Samp Area Shape
Between Bridges	20	Sqr	30.312	- 89.312	5.2 acr	Sqr
Bay Saint Louis	20	Sqr	30.305	- 89.323	5.2 acr	Sqr
Henderson Point	20	Sqr	30.283	- 89.304	5.2 acr	Sqr
Pass Christian	20	Sqr	30.289	- 89.246	5.2 acr	Sqr
Pass Marianne	20	Sqr	30.247	- 89.249	5.2 acr	Sqr
Telegraph	20	Sqr	30.220	- 89.273	5.2 acr	Sqr
Saint Joe	20	Sqr	30.192	- 89.412	5.2 acr	Sqr

Table A.2.a

Physical data recorded during four monthly sets of plankton samples taken at eight sites in 2018. Temperature in °C, DO in mg/l, depth and Secchi depth in m, and DO saturation as percent.

June 2018

Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
L1	30.6	29.3	15.0	15.8	7.93	7.05	112.2	100.1	5.2	NA
L2	31.0	29.5	16.0	16.6	9.25	7.26	135.2	98.3	4.5	0.75
L3	32.0	30.0	14.5	15.1	8.69	7.52	129.2	108.5	3.7	NA
C1	30.5	29.6	15.1	16.0	7.68	8.96	112.7	118.0	4.9	NA
C2	29.9	29.1	14.7	16.2	8.85	6.60	122.0	80.0	4.1	NA
C3	30.9	30.0	15.2	15.5	9.26	8.68	132.9	117.4	3.1	NA
RS1	31.4	30.1	16.3	16.7	9.86	7.37	144.4	117.6	2.9	NA
RS2	30.8	30.6	16.0	16.4	10.61	9.22	156.3	32.5	3.1	NA

July 2018

Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
L1	29.7	29.0	23.9	24.1	7.96	5.49	120.6	82.9	5.5	1.5
L2	29.9	29.7	23.0	25.2	9.31	7.79	137.1	119.4	6.0	1.0
L3	29.2	29.2	22.5	23.2	6.95	6.58	95.0	98.0	5.0	1.0
C1	29.7	29.4	24.0	24.2	7.58	7.01	114.0	104.7	6.0	1.5
C2	30.0	29.9	24.4	25.4	9.47	6.89	129.7	121.2	NA	NA
C3	29.8	29.5	24.5	24.4	6.47	5.89	99.4	82.5	3.5	1.0
RS1	30.0	29.8	25.2	25.2	8.75	8.17	133.2	123.6	5.5	1.0
RS2	30.2	30.0	24.4	24.9	9.73	6.90	149.9	123.0	4.5	NA

August 2018

Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
L1	29.7	29.6	24.3	24.8	5.27	5.37	82.3	79.6	4.5	1.2
L2	29.8	29.6	25.6	26.4	5.67	4.90	86.7	76.6	5.0	1.2
L3	30.4	30.1	25.9	26.0	5.79	5.04	92.9	76.1	4.0	1.5
C1	29.5	29.5	24.7	25.3	5.56	4.97	84.5	76.8	5.0	1.2
C2	30.0	29.7	26.3	26.9	6.50	5.45	98.2	84.4	6.0	1.2
C3	30.3	30.4	24.9	25.9	5.53	4.56	86.0	86.4	4.0	1.5
RS1	30.7	30.3	25.2	26.5	6.20	4.64	96.1	71.8	3.5	NA
RS2	30.4	30.6	25.3	25.8	5.81	2.87	89.4	46.8	5.0	NA

September 2018

Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
L1	29.1	28.1	22.1	22.2	6.81	5.71	99.5	76.1	5.0	1.1
L2	29.6	28.3	22.0	22.3	6.42	5.55	97.3	80.3	4.5	1.0
L3	27.7	27.5	22.9	23.0	6.04	5.86	88.3	84.4	4.0	0.75
C1	29.1	28.0	22.0	22.2	7.28	5.41	106.7	77.8	5.5	1.1
C2	29.1	28.3	22.2	22.4	7.03	5.76	102.3	83.6	5.5	1.0
C3	27.5	27.5	23.2	23.2	6.06	5.98	88.8	86.4	4.0	0.75
RS1	28.3	28.0	22.7	22.7	6.18	5.58	90.0	80.4	4.0	0.70
RS2	29.6	28.7	21.6	21.6	6.92	5.24	104.7	76.3	4.5	1.1

Table A.2.b
Physical data recorded at first deployment (Jul-18), second deployment/first retrieval (Aug-18), and final retrieval of settlement samples (Sept-18) at eight sites during the 2018 study period. Temperature in °C, DO in mg/l, depth and Secchi depth in m, and DO saturation as percent.

July 2018										
Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
L1	31.8	31.5	21.4	21.5	6.88	6.34	105.6	97.9	5.3	0.5
L2	30.9	30.9	21.1	21.0	6.23	6.38	95.3	97.1	4.2	0.5
L3	30.7	30.9	20.1	21.2	6.23	5.78	93.8	87.5	3.6	1.0
C1	31.5	31.4	21.1	21.7	6.73	5.92	102.6	90.7	5.3	0.5
C2	31.3	31.2	21.4	22.1	6.26	5.40	96.1	83.3	3.7	0.5
C3	30.6	30.6	21.2	21.3	6.04	5.30	92.3	88.3	3.2	0.5
RS1	31.5	31.3	22.3	22.7	6.77	6.12	106.4	99.6	3.4	0.5
RS2	31.4	31.4	21.4	21.4	6.59	6.55	101.5	99.6	3.1	0.5

August 2018										
Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
L1	31.3	30.5	25.6	25.8	5.84	5.33	90.5	82.0	5.0	2.5
L2	31.1	31.1	24.5	25.1	5.74	5.17	89.1	79.8	5.0	1.5
L3	30.7	30.7	25.2	25.2	5.71	5.30	88.6	84.1	3.9	2.5
C1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
C2	31.3	31.1	25.6	25.6	6.53	5.93	102.0	92.9	5.5	1.5
C3	30.5	30.3	23.3	24.8	6.44	6.53	98.7	99.7	3.75	3.0
RS1	31.8	31.1	25.8	26.2	6.67	7.03	107.1	108.6	3.2	3.0
RS2	32.0	31.4	24.5	24.8	6.03	3.88	94.6	65.1	4.5	1.0

September 2018										
Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
L1	30.2	29.6	20.7	21.4	7.24	6.15	110.7	89.6	5.5	2.0
L2	29.6	29.3	20.7	21.2	6.91	4.89	96.4	72.0	4.5	2.0
L3	28.9	29.0	20.3	21.4	6.45	5.66	96.4	90.6	4.0	2.0
C1	29.9	29.6	20.8	21.5	6.15	4.85	92.8	72.4	5.0	2.0
C2	29.7	20.9	20.9	21.3	6.39	5.91	94.8	87.8	NA	1.5
C3	28.8	28.8	20.3	21.4	6.70	6.33	94.1	94.6	3.0	2.25
RS1	29.2	29.3	20.9	21.4	6.31	5.97	92.2	88.9	4.5	2.0
RS2	30.4	30.4	21.2	21.2	6.47	6.40	96.3	93.5	5.0	2.0

Table A.2.c

Physical data recorded at first deployment (July), second deployment/first retrieval (August), third deployment/second retrieval (September), and final retrieval of settlement samples (October) at seven sites located throughout western Mississippi Sound in 2019. Temperature in °C, DO in mg/l, depth and Secchi depth in m, and DO saturation as percent.

July 2019										
Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
BB	30.7	29.3	1.42	1.91	8.17	6.10	NA	NA	3.5	0.3
BSL	30.6	29.1	1.82	2.16	8.40	6.13	NA	NA	1.3	0.5
HP	29.5	29.4	3.48	3.56	6.46	6.17	NA	NA	3.3	NA
PC	NA	NA	NA	NA	NA	NA	NA	NA	2.0	NA
PM	29.2	29.2	5.04	5.08	6.49	6.48	NA	NA	2.0	0.5
TR	28.9	28.9	3.90	3.90	5.68	5.68	NA	NA	2.8	0.5
SJ	29.5	29.3	1.29	1.29	6.57	6.11	NA	NA	4.0	0.3
August 2019										
Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
BB	30.6	29.6	2.70	6.10	7.75	2.95	104.9	40.9	3.5	0.5
BSL	31.6	29.6	3.50	6.00	8.26	3.67	113.5	44.5	3.5	0.5
HP	30.5	29.6	3.70	5.80	8.10	5.75	108.8	79.2	3.5	0.5
PC	30.6	30.0	11.10	12.10	7.96	4.97	116.5	68.3	4.0	0.5
PM	29.8	29.7	7.80	8.70	7.47	6.31	NA	NA	2.9	0.8
TR	29.5	29.5	6.30	6.40	7.51	7.02	102.7	99.6	2.9	0.8
SJ	29.3	29.2	1.50	2.00	6.20	5.65	80.6	74.3	4.0	0.5
September 2019										
Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
BB	31.0	30.5	13.8	15.0	6.89	4.71	100.3	68.3	6.6	0.5
BSL	31.5	30.6	13.8	15.3	8.78	4.43	127.9	65.1	5.8	NA
HP	31.0	30.6	16.7	17.4	6.85	5.01	102.6	72.8	7.0	0.8
PC	30.9	30.8	18.8	18.8	4.86	4.91	73.9	74.0	6.2	0.5
PM	30.6	30.6	20.2	19.9	5.37	5.37	79.4	80.1	5.2	1.0
TR	30.4	30.4	19.1	19.4	4.98	5.00	73.3	74.0	5.0	0.8
SJ	30.4	30.3	11.8	12.2	6.18	6.36	89.6	90.7	6.6	0.5
October 2019										
Site	Temperature		Salinity		DO		DO Saturation		Depth	Secchi
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom		
BB	24.8	24.8	13.9	14.3	7.92	7.75	101.9	94.8	3.5	0.5
BSL	25.3	25.0	14.6	14.8	7.32	7.06	95.9	93.8	3.0	NA
HP	24.9	24.9	14.9	14.9	8.8	10.21	114.3	127.12	3.5	0.5
PC	25.5	25.4	16.6	16.9	7.07	6.31	95.2	86.5	3.5	0.7
PM	25.4	25.4	17.0	17.3	7.18	6.63	95.5	88.7	3.5	0.7
TR	25.5	25.6	16.4	17.1	6.82	6.90	90.0	91.5	4.5	0.5
SJ	25.5	25.1	12.0	12.4	8.52	8.19	110.4	110.8	4.5	0.5

BB = Between Bridges; BSL = Bay Saint Louis; HP = Henderson Point; PC = Pass Christian; PM = Pass Marianne; TR = Telegraph Reef; SJ = Saint Joe.

Table A.3
Linear Mixed Model tests of fixed effects within the full model for the eight settlement plate metrics in 2018.

Metric	Source	Numerator df	Denominator df	F	Sig.
Large Spat Density	Intercept	1	42	697.38	< **0.001**
	Month	1	42	40.29	< **0.001**
	Site	7	42	5.57	< **0.001**
	Surface	1	126	224.70	< **0.001**
	Exclusion	1	126	2.08	0.152
	Month × Site	7	42	2.50	**0.031**
	Month × Surface	1	126	59.27	< **0.001**
	Month × Exclusion	1	126	0.35	0.558
	Site × Surface	7	126	5.08	< **0.001**
	Site × Exclusion	7	126	2.33	**0.028**
	Surface × Exclusion	1	126	13.40	< **0.001**
	Month × Site × Surface	7	126	2.04	0.055
	Month × Site × Exclusion	7	126	1.87	0.080
	Month × Surface × Exclusion	1	126	0.54	0.465
	Site × Surface × Exclusion	7	126	1.15	0.339
	Month × Site × Surface × Exclusion	7	126	0.46	0.861

Metric	**Source**	**Numerator df**	**Denominator df**	**F**	**Sig.**
Early Spat Density	Intercept	1	42	176.46	< **0.001**
	Month	1	42	65.27	< **0.001**
	Site	7	42	0.61	0.745
	Surface	1	126	17.95	< **0.001**
	Exclusion	1	126	0.06	0.803
	Month × Site	7	42	3.12	**0.010**
	Month × Surface	1	126	8.92	**0.003**
	Month × Exclusion	1	126	1.73	0.191
	Site × Surface	7	126	4.85	< **0.001**
	Site × Exclusion	7	126	1.94	0.068
	Surface × Exclusion	1	126	12.91	< **0.001**
	Month × Site × Surface	7	126	1.92	0.072
	Month × Site × Exclusion	7	126	2.20	**0.038**
	Month × Surface × Exclusion	1	126	0.19	0.665
	Site × Surface × Exclusion	7	126	3.29	**0.003**
	Month × Site × Surface × Exclusion	7	126	0.96	0.467

Metric	**Source**	**Numerator df**	**Denominator df**	**F**	**Sig.**
Mean Spat Size	Intercept	1	42	1738.19	< **0.001**
	Month	1	42	48.02	< **0.001**
	Site	7	42	1.48	0.199
	Surface	1	126	67.97	< **0.001**
	Exclusion	1	126	1.37	0.244
	Month × Site	7	42	7.61	< **0.001**
	Month × Surface	1	126	3.44	0.066
	Month × Exclusion	1	126	5.59	**0.020**
	Site × Surface	7	126	2.45	**0.022**
	Site × Exclusion	7	126	4.38	< **0.001**
	Surface × Exclusion	1	126	2.06	0.153
	Month × Site × Surface	7	126	2.86	**0.008**
	Month × Site × Exclusion	7	126	4.20	< **0.001**
	Month × Surface × Exclusion	1	126	3.94	**0.049**
	Site × Surface × Exclusion	7	126	6.90	< **0.001**
	Month × Site × Surface × Exclusion	7	126	1.76	0.101
	Site × Surface × Exclusion	7	126	1.69	0.116
	Month × Site × Surface × Exclusion	7	126	0.79	0.598

Metric	**Source**	**Numerator df**	**Denominator df**	**F**	**Sig.**
Maximum Spat Size	Intercept	1	42	2498.45	< **0.001**
	Month	1	42	38.49	< **0.001**
	Site	7	42	1.88	0.098
	Surface	1	126	190.79	< **0.001**
	Exclusion	1	126	3.49	0.064
	Month × Site	7	42	7.16	< **0.001**
	Month × Surface	1	126	4.67	**0.033**
	Month × Exclusion	1	126	1.87	0.174
	Site × Surface	7	126	1.11	0.361
	Site × Exclusion	7	126	2.06	0.053
	Surface × Exclusion	1	126	1.25	0.266
	Month × Site × Surface	7	126	1.46	0.188
	Month × Site × Exclusion	7	126	0.91	0.500
	Month × Surface × Exclusion	1	126	0.89	0.348
	Site × Surface × Exclusion	7	126	1.72	0.109
	Month × Site × Surface × Exclusion	7	126	1.18	0.322

Table A.3 (*continued*)

Metric	Source	Numerator df	Denominator df	F	Sig.
Spat Scar Index	Intercept	1	168	1316.78	< **0.001**
	Month	1	168	0.71	0.399
	Site	7	168	4.95	< **0.001**
	Surface	1	168	58.12	< **0.001**
	Exclusion	1	168	0.29	0.592
	Month × Site	7	168	1.78	0.094
	Month × Surface	1	168	0.95	0.332
	Month × Exclusion	1	168	0.11	0.737
	Site × Surface	7	168	0.94	0.479
	Site × Exclusion	7	168	0.90	0.509
	Surface × Exclusion	1	168	0.22	0.642
	Month × Site × Surface	7	168	1.52	0.163
	Month × Site × Exclusion	7	168	0.88	0.521
	Month × Surface × Exclusion	1	168	0.82	0.367
	Site × Surface × Exclusion	7	168	1.22	0.292
	Month × Site × Surface × Exclusion	7	168	1.00	0.431

Metric	Source	Numerator df	Denominator df	F	Sig.
Implicit Specific Growth	Intercept	1	168	10819.00	< **0.001**
	Month	1	168	203.75	< **0.001**
	Site	7	168	2.87	**0.007**
	Surface	1	168	164.17	< **0.001**
	Exclusion	1	168	0.94	0.333
	Month × Site	7	168	3.96	**0.001**
	Month × Surface	1	168	0.89	0.346
	Month × Exclusion	1	168	0.09	0.761
	Site × Surface	7	168	0.65	0.716
	Site × Exclusion	7	168	1.98	0.060
	Surface × Exclusion	1	168	0.63	0.429
	Month × Site × Surface	7	168	2.47	**0.020**
	Month × Site × Exclusion	7	168	0.67	0.697
	Month × Surface × Exclusion	1	168	0.09	0.761
	Site × Surface × Exclusion	7	168	1.68	0.117
	Month × Site × Surface × Exclusion	7	168	1.03	0.412

Metric	Source	Numerator df	Denominator df	F	Sig.
Spat Cover/ Soak time	Intercept	1	186.86	1838.97	< **0.001**
	Month	1	186.86	43.70	< **0.001**
	Site	7	186.86	6.04	< **0.001**
	Surface	1	137.03	279.64	< **0.001**
	Exclusion	1	137.03	2.39	0.124
	Month × Site	7	186.86	4.18	< **0.001**
	Month × Surface	1	137.03	54.02	< **0.001**
	Month × Exclusion	1	137.03	0.44	0.509
	Site × Surface	7	137.03	5.70	< **0.001**
	Site × Exclusion	7	137.03	2.48	**0.020**
	Surface × Exclusion	1	137.03	8.40	**0.004**
	Month × Site × Surface	7	137.03	1.99	0.061
	Month × Site × Exclusion	7	137.03	1.66	0.124
	Month × Surface × Exclusion	1	137.03	1.24	0.268
	Site × Surface × Exclusion	7	137.03	1.30	0.254
	Month × Site × Surface × Exclusion	7	137.03	0.49	0.844

Metric	Source	Numerator df	Denominator df	F	Sig.
Barnacle Cover/Soak Time	Intercept	1	61.891	836.91	< **0.001**
	Month	1	61.891	49.07	< **0.001**
	Site	7	61.891	24.42	< **0.001**
	Surface	1	231.354	342.93	< **0.001**
	Exclusion	1	231.354	41.42	< **0.001**
	Month × Site	7	61.891	2.91	**0.011**
	Month × Surface	1	231.354	2.71	0.101
	Month × Exclusion	1	231.354	2.28	0.132
	Site × Surface	7	231.354	9.32	< **0.001**
	Site × Exclusion	7	231.354	5.05	< **0.001**
	Surface × Exclusion	1	231.354	11.81	**0.001**
	Month × Site × Surface	7	231.354	0.63	0.731
	Month × Site × Exclusion	7	231.354	1.82	0.084
	Month × Surface × Exclusion	1	231.354	0.08	0.782
	Site × Surface × Exclusion	7	231.354	3.15	**0.003**
	Month × Site × Surface × Exclusion	7	231.354	1.99	0.057

Table A.4
Significant responses for eight custom contrasts. Near = nearshore; Off = offshore; Relic = relic shell; Lime = limestone; Ref = reference; Expos = exposed; Restrc = restricted.

Contrast 1	Late vs. Early sample period	Mean Early	Mean Late	t-value	Df	P
Response	Large spat density per day	134.35	44.78	−6.35	42	< **0.001**
	Early spat density per day	11.30	70.87	8.08	42	< **0.001**
	Maximum spat size	11.18	14.02	6.20	42	< **0.001**
	Implicit specific growth	0.44	0.33	−14.27	168	< **0.001**
	Mean spat size	5.19	3.38	−6.93	42	< **0.001**
	Spat cover/soak time (d)	0.78	0.47	−6.61	186.86	< **0.001**
	Barnacle cover/soak time (d)	1.28	0.77	−2.21	42	**0.033**
Contrast 2	**Offshore sites vs. Nearest inshore sites**	Mean Near	Mean Off	t-value	Df	P
Response	Large spat density per day	62.17	90.43	2.78	42	**0.008**
	Spat scar index	0.16	0.31	3.76	168	< **0.001**
	Spat cover/soak time (d)	0.52	0.59	1.81	186.86	0.073
	Barnacle cover/soak time (d)	1.45	0.04	−10.92	61.89	< **0.001**
Contrast 3	**Relic Shell vs. Nearshore reference sites**	Mean Relic	Mean Ref	t-value	Df	P
Response	Barnacle cover/soak time (% d^{-1})	1.76	0.99	3.99	61.89	< **0.001**
Contrast 4	**Nearshore reference vs. Limestone sites**	Mean Lime	Mean Ref	t-value	Df	P
Response	Barnacle cover/soak time (% d^{-1})	1.30	0.99	−1.99	61.89	0.051
Contrast 5	**Relic shell vs. Limestone sites**	Mean Relic	Mean Lime	t-value	Df	P
	Barnacle cover/soak time (% d^{-1})	1.76	1.30	2.32	61.89	**0.024**
Contrast 6	**Nearshore reference vs. Both restored types**	Mean Both	Mean Ref	t-value	Df	P
Response	Barnacle cover/soak time (% d^{-1})	1.51	0.99	−3.60	61.89	**0.001**
Contrast 7	**Upper excluded surface vs. Upper exposed surface**	Mean Caged	Mean Expos	t-value	Df	P
Response	Large spat density per day	52.61	30.00	3.61	126	< **0.001**
	Early spat density per day	42.17	23.91	2.72	126	**0.008**
	Maximum spat size (mm)	9.60	8.64	2.11	126	**0.037**
	Spat cover/soak time (% d^{-1})	9.53	5.26	3.14	137.03	**0.002**
	Barnacle cover/soak time (% d^{-1})	34.64	57.45	−6.98	231.35	< **0.001**
Contrast 8	**Lower restricted vs. Upper exposed surface**	Mean Restrc	Mean Expos	t-value	Df	P
Response	Large spat density per day	150.43	30.00	13.19	126	< **0.001**
	Early spat density per day	48.70	24.35	5.54	126	< **0.001**
	Spat scar index	0.10	0.26	−5.06	168	< **0.001**
	Maximum spat size (mm)	15.71	8.64	10.56	126	< **0.001**
	Implicit specific growth (mm d^{-1})	0.044	0.033	9.26	168	< **0.001**
	Mean spat size (mm)	4.75	3.74	4.81	126	< **0.001**
	Spat cover/soak time (% d^{-1})	27.41	5.26	13.87	137.03	< **0.001**
	Barnacle cover/soak time (% d^{-1})	10.09	57.45	−15.52	231.35	< **0.001**

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