Article

Spatial and temporal variability in spat settlement of intertidal oyster reefs support site-specific assessments for restoration practices

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| **Citation:** To be added by editorial staff during production.  Academic Editor: Firstname Lastname  Received: date  Revised: date  Accepted: date  Published: date    **Copyright:** © 2024 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/). |

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**Abstract:** Being some of the most productive ecosystems in the world, the declining condition and coverage of coastal habitats consequently results in the loss of the myriad economic and ecosystem services in which they provide. Mitigation efforts to address these deficits have been initiated in many places worldwide, however some of the tools for restoration and enhancement projects rely on place-based application as varying physical and biological characteristics of these sites drive the material and design of these projects. As such, increased place-based information to inform local management projects with the goals of reestablishing economic and ecological function of coastal habitats are imperative. As oysters are often used in many of these projects, particularly living shorelines, a form of natural stabilization that also provides habitat, this study quantified spatial and temporal patterns in eastern oyster spat settlement in a bar-built estuary in northeast Florida, USA that is host to a robust population of intertidal oyster reefs. Understanding how spat settlement varies in a dynamic region with two inlets and a short residence time is valuable for projects seeking to use this species behavior of gregarious settlement in establishing functional oyster reefs as part of restoration projects. There is variability in spat settlement in different regions of the area in which higher counts often coincide to watersheds with higher residence times and years with \_\_\_\_\_ conditions.

**Keywords:** restoration, spat, oysters, water quality

1. Introduction

Coastal ecosystems are some of the most productive ecosystems in the world providing numerous ecosystem services such as carbon sequestration, improvement of water quality, erosion control, and recreation (Brown et al., 2006; Barbier, 2007). Recognizing their value and aesthetic, these ecosystems are subject to constant human activity which has ultimately led to the steady deterioration in many habitats of these systems (Barbier, 2007). Worldwide, there has been an estimated loss of 35% of mangroves, 30% of coral reefs, 29% of seagrasses, 50% of salt marshes, and 85% of oyster reefs (Brown et al., 2006; Valiela et al., 2001; Orth et al., 2006; MEA 2006; FAO 2007; Beck et al., 2011). With the loss of these coastal ecosystems, the critical ecosystem services and economic value of those services decline as well such has been seen in the number of viable (non collapsed) fisheries (-33%), provision of nursery habitats (-69%), and filtering and detoxification services (-63%) (Worm et al. 2006). The mitigation of anthropogenic effects is of significant need in these systems.

As such, efforts in the restoration and enhancement of habitats in coastal ecosystems have increased with their decline in ecosystem and economic function. The use of organic materials in these projects has additional benefits beyond mere construction material. Living shorelines, a form of natural stabilization using organic materials, have been found to improve water quality, cease or reverse coastal erosion, and serve as critical habitats for plants, fishes, and invertebrates (Scyphers et al., 2011; Whalen et al., 2011; Kreeger and Padeletti, 2013). One common type of living shoreline is a shellfish-based living shoreline (Rupasinghe et al., 2024) which has the advantages of adapting with a changing climate; such as oyster reefs being able to grow at the pace of sea level rise (Rodriguez et al., 2014) and being able to self-repair after a destructive event (Gittman et al., 2014). Dame (1996) established oysters as “ecosystem engineers'' due to the fact that they can maintain, modify, and form habitats. As such, oysters are an ideal target material in mitigation projects in coastal ecosystems with oyster reef habitats as often a primary or secondary goal of these installations is utilizing their gregarious settlement behavior to continue to form and strengthen these installations over time.

Abundant and common to the southeastern United States, the eastern oyster Crassostrea virginica (Gmelin, 1791) forms three-dimensional reefs which enhance secondary and tertiary productivity within estuaries as juvenile fish and crustaceans recruit to and utilize these reefs as foraging grounds and refuge (Breitburg 1999; Coen and Luckenbach 2000; Harding and Mann 2003; Grabowski et al., 2005; Tolley and Volety 2005; Boudreaux et al. 2006; Rodney and Paynter 2006). Additionally, oyster reefs provide other types of ecosystem services such as water filtration, prevention of coastal erosion, boat wake mitigation, and carbon sequestration (Volety et al., 2014).

Eastern oysters are non-incubatory oysters, meaning they release gametes into the water column and fertilization occurs outside of the organism (Galstoff, 1964). Eastern oyster larvae remain in the planktonic stage for about 2-3 weeks before settling on a suitable substrate, from which they are known as “spat”. Many abiotic and biotic factors influence the timing and extent of spawning to the recruitment and survival of juvenile oysters. Understanding the effect of these factors across this spectrum has great importance in the timing and success of many restoration projects with the goals of forming a functional oyster habitat as part of their design. Spawning response has been shown to be variable by the sex of the oyster (Galstoff, 1964), temperatures and salinity of the water (Ingle, 1952; Butler, 1949), and availability of food for adult oysters (Hofmann et al., 1992; Dekshenieks, 1993). Temperature, salinity, and circulation patterns are amongst the most notable factors to affect oyster larvae (Nelson and Perkins, 1931). Temperature and food supply have been found to affect the length of larval periods (Underwood and Fairweather, 1989). To a lesser degree, turbidity of the water also can affect larval growth as well as settlement. Larvae tend to settle on the underside of objects or in crevices, presumably to avoid light and silt, but have been found on the surfaces of objects in higher turbidity conditions (Kennedy, 1980; Nelson, 1953). Lastly, field studies have shown that settlement and recruitment of oyster larvae often has high inter-regional and interannual variability (Michener and Kenny, 1991; Horse et al., 1972; Lee, 1979; Sauod et al., 2000; Kim et al., 2010). Understanding spat settlement patterns within regions with restoration goals is valuable to planning for this installation and success.

The goal of this study was to quantify spatial and temporal variability in a dynamic bar-built estuary in northeast Florida, USA, that is host to a robust population of intertidal oyster reefs and to establish patterns of spat settlement to inform oyster-based restoration and enhancement projects. Understanding the variability of spat settlement in previous field studies in the southeast, as well as differences in oyster densities in the region (Dix 2009; Marcum et al., 2018), it was expected that there would be spatial variability amongst study sites. Therefore, potential drivers of this variability were also examined to provide further insight into differing characteristics amongst regions and years.

2. Materials and Methods

2.1. Study Sites

The Guana Tolomato Matanzas (GTM) estuary is a bar-built estuary with enclosed lagoons “rivers” (the Guana, Tolomato, and Matanzas) that trifurcate at the St. Augustine Inlet (Figure 1). This inlet is one of two in the system, and it is maintained and stabilized with a jetty by the United States Army Corps of Engineers to a depth of 5-m. The other, the Matanzas Inlet, is an unstructured inlet just north of Marineland, Florida, USA. The estuary is well-flushed with a short residence time of approximately 12.6 days (Phlips et al. 2004; Sheng et al. 2008; Gray et al. 2021) and is well-mixed, meaning vertical stratification in salinity is homogenous. The GTM estuary hosts exceptionally intact and robust populations of eastern oysters that filter approximately 60% of the estuary’s volume within a single residence time (Gray et al. 2021). There is also a functional oyster fishery (commercial and recreational) in several regions.

**Map

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**Figure 1.** Map of the Guana Tolomato Matanzas National Estuarine Research Reserve boundary (black line, bottom inset: red), spat collector locations (black dots), water quality monitoring stations (black triangles, not included in insets), and regions: Tolomato River (turquoise), Guana River (pink), Salt Run (yellow), St. Augustine (red), and Fort Matanzas (blue). Water quality stations are from the System-Wide Monitoring Program and are Pine Island (“PIWQ”), San Sebastian (“SSWQ”), and Fort Matanzas (“FMWQ”).

The GTM National Estuarine Research Reserve (GTMNERR) initiated a monitoring program for local oysters in 2014 in which a regional approach was adopted based on perceived differences in water quality, food availability, hydrodynamics, harvesting, and management (Marcum et al. 2018). Regions were created based on the major waterways along the Intracoastal Waterway (ICW): Tolomato River, Guana River, Salt Run and Matanzas River. A similar approach was used in this study; however, the Matanzas River region was further subdivided into St. Augustine (the northern portion of the river) and Fort Matanzas (the remaining portion of the river to the south) due to hydrological differences associated with the two inlets (Figure 1).

The GTMNERR maintains continuous long-term water quality monitoring stations within the GTM estuary as part of the System-Wide Monitoring Program (SWMP) of the National Estuarine Research Reserves (NERRS). All SWMP data are publicly available through the NERRS Centralized Data Management Office (CDMO) at nerrsdata.org (NERRS 2022).

2.2. Data Collection

2.2.1. Spat Tree Deployment

A stratified random sample of three reefs in each region of interest (except for the Tolomato River) were selected to deploy spat collectors. The Tolomato River region had two spat collectors deployed at either end of an oyster enhancement area known as Wright’s Landing, where 275 m2 of oyster reefs (28 individual reefs) were created from bagged shell in 2012 and 2013, and one across from the site on a natural reef. In June 2016 and June 2020, one additional spat collector was deployed in the St. Augustine and Tolomato regions, respectively.

Patterns in spat settlement were monitored using the hanging shell method. Samples were collected using T-shaped structures (trees) made from PVC, with shell “stringers” suspended from each side of the crossbar (Figure; Arnold et al. 2008, Haven and Fritz 1985, Parker 2015, Volety and Savarese 2001, Wilson et al. 2005). Each stringer was composed of six cleaned eastern oyster shells that were between 5 to 10 cm in shell height. Holes were drilled through the shells, and they were strung onto galvanized wire oriented with the inner concave surface facing down. Prior to deployment, shells were cleaned by soaking in bleach water for 48 hours followed by removal of all fouling organisms by scrubbing with a wire brush. After bleaching, the shells were then soaked for at least 24 hours in freshwater.

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**Figure 2.** This is a figure. Schemes follow the same formatting.

Trees were inserted into the reef at the apparent densest portion of live oyster on the reef and situated so that the shells were at the approximate height of the surrounding live oysters. All regions had trees deployed starting in February 2015 except for the Tolomato River, which was initiated in September 2015. These trees were left to soak for approximately one month upon which they were collected. During collection, any fouling organisms were removed from the tree, and new stringers were deployed. The retrieved stringers were labeled and stored in a -4°C freezer until processed. Efforts were made for the stringers to remain deployed for one month, however due to logistics, there was some variation in how long they were left in the field before collection. Trees were deployed for approximately 30 days on average with a range in the project of 21-43 days. Hurricane Matthew affected the study area in October 2016 and spat trees were unable to be collected, resulting in missing data from September and October of that year.

2.2.2. Shell Processing and Counting

Shells were assigned numerical IDs based on their position on each stringer, with the topmost shell designated number one and the bottommost number six. The top and bottom shells (one and six, respectively) were discarded and shells two through five were evaluated for spat abundance.

In early years of the monitoring, spat were counted using the naked eye or a magnifying glass on both sides of the shells (interior and exterior). A small-scale comparison study determined that spat abundance was significantly higher using a dissection microscope and on the inner surface (bottom of the shell) only. Beginning in December 2017, all processing was done under microscope on the inner surface of the shells. A linear regression equation based on the comparison was developed to correct the non-microscope data for analysis:

|  |  |
| --- | --- |
| S = 1.4658*b* + 1.0378 | (1) |

where S was the adjusted number of spat counted and *b* was the observed number of spat counted on the bottom of the shell by naked eye. The average number of spat per shell was calculated for each tree deployed within each region each month. These values were then rounded to convert the number of spat to integers.

2.3 Water Quality

Water quality data used in this study were downloaded from the CDMO for the Pine Island (gtmpiwq), San Sebastian (gtmsswq), and Fort Matanzas (gtmfmwq) stations for the continuous water quality information (Figure)(DATA CITATION). These stations were equipped with YSI EXO2 data sondes deployed approximately one meter from the bottom. The sondes measure a variety of parameters every 15-minutes including water temperature (°C), salinity (psu), and turbidity (NTU). Discrete water samples were collected in duplicate at these same stations once a month for chlorophyll *a* (chl-*a)* on a morning ebb tide from as close to the sonde depth as possible. Samples were filtered in the field, placed on ice in the dark and shipped overnight to the Florida Department of Environmental Protection’s Central Laboratory in Tallahassee, FL. Chl-*a* was extracted from frozen filters within 28 days and analyzed using Standard Methods (SM10200H; citation).

The data had undergone the quality assurance and quality checks of the CDMO and data flagged as “rejected” and “suspect” were removed from the dataset. The duplicate chl-*a* samples from each station were averaged by month. All water quality data was then aggregated inside and outside the defined spat settlement periods for descriptive statistics.

2.4. Data Analysis

All data analysis and visualizations were created using R programming language (R Core Team, 2023). Several helpful import, filtering, and aggregating functions from the SWMPr package in R were used for the compilation of the water quality data (Beck 2016).

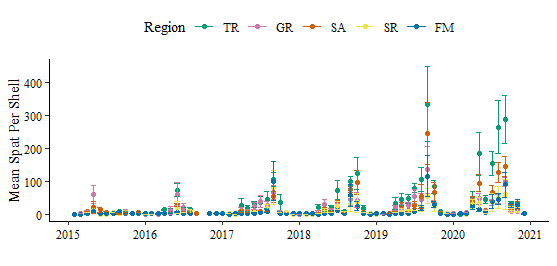
Since oyster count data has been shown to fit a negative binomial distribution, a generalized linear regression model with a negative binomial distribution was used to quantify spatial and temporal variability (Moore et al. 2020). Spat counts per shell were assumed to be related to the amount of time they were left “soaking” during deployment; therefore, to control for this the number of soak days was included as an effort offset (log link function; Zuur et al. 2013). This causes the models to predict the rate measured as count per deployment, while maintaining the dependent variable as an integer of counts. The dependent variable was the average spat count for each collector rounded as an integer. The independent variables (main effects) were both categorical and these were region and year. Multiple models were fitted to the data: Model 0 was the intercept only (set to 1), Model 1 included region, Model 2 included region and the year, Model 3 included the interaction of region and year, and Model 4 included just year (Table 1). The default glmmTMB optimizer (nlminb) was used.

Comparisons were made between models with different combinations of independent variables using Akaike’s Information Criterion (AICc). The lowest AICc value represents the best fit of the models tested (Table 1; Burnham and Anderson 2002). Models were fit to the data using the glmmTMB package (Brooks et al. 2017), assessed with the performance package (Lüdecke) and predicted values (marginal means) and pairwise comparisons with tukey adjustments were made from the best fit model using the emmeans (Lenth 2024) packages in R.

3. Results

3.1. Spatial and Temporal Variability in Spat Settlement

Discuss overall patterns in spat across the entire study period

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**Figure 2.** Monthly mean spat per shell (with standard error bars) for each region from 2015-2020: Tolomato River (TR, green); Guana River (GR, pink); Saint Augustine (SA; orange); Salt Run (SR, yellow); and Fort Matanzas (FM, blue).

Then go into the analysis between regions and years. Present model results, then plots comparing the regions and the years.

Five different models were fit to the spat per shell data (Table 2; Appendix). The best fitting model included terms for region and year. The dispersion ratio from the negative binomial distribution was 1.527 (*p* < 0.001), suggesting overdispersion. No significant interaction was found between year and regions; however, autocorrelation in the residuals was detected in the model (Kolmogorov-Smirnov test: *p* = 0.034, Durbin-Watson test: *p* < 0.001).

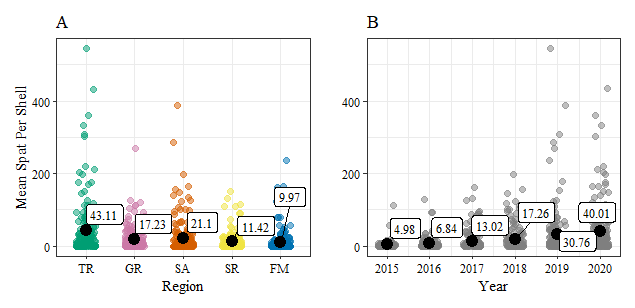
**Table 1.** Model selection table for generalized linear model of eastern oyster spat count data standardized by days left out in field for settlement on deployed collectors (“trees”) in different regions within the Guana Tolomato Matanzas estuary, Florida, USA. The predicted response is the average spat per shell per collector (spat\_std). Akaike’s Information Criterion (AICc) and the AICc difference (ΔAICc) are provided to inform comparisons of the model statistical fit to the data (*k* is the number of parameters) and are ranked from lowest AICc to highest in the table. Region and year are both categorical variables in which region describes the location of the collection and year is the year of study.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model** |  |  | ***k*** | **AICc** | Δ**AICc** | **AICc weight** |
| Model 2: spat\_count ~ region + year + offset[log(soak\_days)] |  |  | 11 | 6484.63 | 0.00 | 1 |
| Model 3: spat\_count ~ region + year + region:year +  offset[log(soak\_days)] |  |  | 31 | 6504.43 | 19.81 | 0 |
| Model 4: spat\_count ~ year + offset[log(soak\_days)] |  |  | 7 | 6559.31 | 74.69 | 0 |
| Model 1: spat\_count ~ region + offset[log(soak\_days)] |  |  | 6 | 6613.63 | 129.00 | 0 |
| Model 0: spat\_count ~ offset[log(soak\_days)] |  |  | 2 | 6688.57 | 203.94 | 0 |

**Table 2.** Estimated marginal means for mean spat per shell in the five regions in the Guana Tolomato Matanzas estuary. Results are averaged over the levels of year (2015-2020) and are given on the log (not the response) scale. SE = standard error, CI = confidence level of 0.95.

|  |  |  |  |
| --- | --- | --- | --- |
| **Region** | **Estimated Marginal Mean** | **SE** | **CI** |
| Tolomato River (TR) | 3.33 | 0.13 | 3.09 – 3.58 |
| Guana River (GR) | 2.77 | 0.12 | 2.54 – 3.00 |
| Saint Augustine (SA) | 2.63 | 0.12 | 2.39 – 2.86 |
| Salt Run (SR) | 2.08 | 0.13 | 1.83 – 2.34 |
| Fort Matanzas (FM) | 1.91 | 0.12 | 1.68 – 2.14 |

Tolomato River (TR) had the greatest mean spat per shell (estimated mean (log): 3.33, CI = 3.09 – 3.58; untransformed mean: 43.11) and Fort Matanzas (FM) the least (emm: 1.91, CI = 1.68 – 2.14; 9.97) (Figure 2; Table 2).

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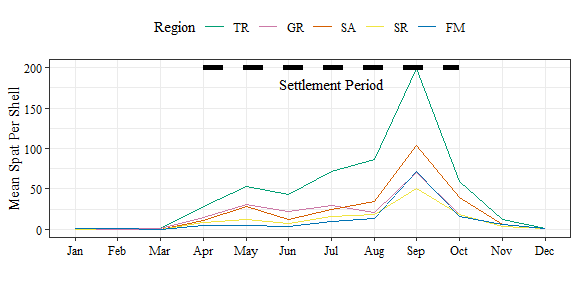
**Figure 3.** Mean spat per shell in the (A) five regions of the Guana Tolomato Matanzas estuary: Tolomato River (TR, green); Guana River (GR, pink); Saint Augustine (SA; orange); Salt Run (SR, yellow); and Fort Matanzas (FM, blue). Mean spat per shell in all regions for each year of the study (B). Group means (raw and untransformed) are represented by the large black dots with the mean presented in a call-out box next to the dot in each plot. Each point represents a sampled collector per month.

**Table 3.** Estimated marginal means for mean spat per shell between 2015-2020 in the Guana Tolomato Matanzas estuary. Results are averaged over the levels of region and are given on the log (not the response) scale. SE = standard error, CI = confidence level of 0.95.

|  |  |  |  |
| --- | --- | --- | --- |
| **Year** | **Estimated Marginal Mean** | **SE** | **CI** |
| 2015 | 1.57 | 0.16 | 1.25 – 1.88 |
| 2016 | 1.73 | 0.14 | 1.44 – 2.01 |
| 2017 | 2.41 | 0.13 | 2.15 – 2.66 |
| 2018 | 2.79 | 0.13 | 2.55 – 3.04 |
| 2019 | 3.22 | 0.12 | 2.98 – 3.46 |
| 2020 | 3.56 | 0.12 | 3.31 – 3.80 |

3.2. Patterns in Annual Spat Settlement

SPAT

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**Figure 1.** Monthly mean spat per shell with settlement period indicated as the thick dashed line between April and October for the five regions in the Guana Tolomato Matanzas estuary: Tolomato River (TR, green); Guana River (GR, pink); Saint Augustine (SA; orange); Salt Run (SR, yellow); and Fort Matanzas (FM, blue) based on data collected from 2015-2020.

**Table X.** Summary information for spat settlement per shell in regions in the Guana Tolomato Matanzas estuary inside the annual settlement period (April – October) and outside of the settlement period (January – March and November – Dec). Metrics include average total settlement per shell per year (standard error) and average settlement per shell (standard error).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***Inside Settlement Period*** | | ***Outside Settlement Period*** | |
| **Region** | **Avg Total Settlement per Shell per Year** | **Avg Settlement per Shell** | **Avg Total Settlement per Shell per Year** | **Avg Settlement per Shell** |
| Tolomato River (TR) | 1234 (466) | 75 (10) | 38 (13) | 3 (1) |
| Guana River (GR) | 571 (18) | 29 (4) | 18 (5) | 2 **(**1) |
| St. Augustine (SA) | 677 (249) | 35 (6) | 27 (11) | 2 **(**1) |
| Salt Run (SR) | 305 (100) | 19 **(**4) | 16 (5) | 2 **(**1) |
| Fort Matanzas (FM) | 313 (110) | 17 **(**4) | 26 (14) | 2 **(**1) |

3.3. Drivers of Variability

WATER QUALITY

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Subsection

3.1.1. Subsubsection

Bulleted lists look like this:

* First bullet;
* Second bullet;
* Third bullet.

Numbered lists can be added as follows:

1. First item;
2. Second item;
3. Third item.

The text continues here.

3.2. Figures, Tables and Schemes

All figures and tables should be cited in the main text as Figure 1, Table 1, etc.

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**Figure 1.** This is a figure. Schemes follow the same formatting.

**Table 1.** This is a table. Tables should be placed in the main text near to the first time they are cited.

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| **Title 1** | **Title 2** | **Title 3** |
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| entry 2 | data | data 1 |

1 Tables may have a footer.

The text continues here (Figure 2 and Table 2).

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| (**a**) | (**b**) |

**Figure 2.** This is a figure. Schemes follow another format. If there are multiple panels, they should be listed as: (**a**) Description of what is contained in the first panel; (**b**) Description of what is contained in the second panel. Figures should be placed in the main text near to the first time they are cited.

**Table 2.** This is a table. Tables should be placed in the main text near to the first time they are cited.

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| **Title 1** | **Title 2** | **Title 3** | **Title 4** |
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| entry 4 | data | data | data |
| data | data | data |

\* Tables may have a footer.

3.3. Formatting of Mathematical Components

This is example 1 of an equation:

|  |  |
| --- | --- |
| a = 1, | (1) |

the text following an equation need not be a new paragraph. Please punctuate equations as regular text.

This is example 2 of an equation:

|  |  |
| --- | --- |
| a = b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z | (2) |

the text following an equation need not be a new paragraph. Please punctuate equations as regular text.

Theorem-type environments (including propositions, lemmas, corollaries etc.) can be formatted as follows:

**Theorem 1.** Example text of a theorem. Theorems, propositions, lemmas, etc. should be numbered sequentially (i.e., Proposition 2 follows Theorem 1). Examples or Remarks use the same formatting, but should be numbered separately, so a document may contain Theorem 1, Remark 1 and Example 1.

The text continues here. Proofs must be formatted as follows:

**Proof of Theorem 1.** Text of the proof. Note that the phrase “of Theorem 1” is optional if it is clear which theorem is being referred to. Always finish a proof with the following symbol. □

The text continues here.

4. Discussion

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

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**Appendix A**

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

**Table 2.** This is a table. Tables should be placed in the main text near to the first time they are cited.

|  |  |  |  |
| --- | --- | --- | --- |
| **Title 1** |  | **Title 2** | **Title 3** |
| entry 1 |  | data | data |
| entry 2 |  | data | data 1 |

1 Tables may have a footer.

**Table 2.** Model results for the best-fitting generalized linear model (Table # in text) of oyster spat counts per shell on collectors deployed in five regions in the Guana Tolomato Matanzas estuary from 2015-2020 where spat count per shell = region + year + offset[log(soak time)]. Parameter estimates are on a log scale. Dispersion parameter 0.374.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Estimate** | **SE** | ***z*-value** | **Pr(>*z*)** |
| Intercept | -1.06 | 0.21 | -5.06 | < 0.001 |
| Guana River (GR) | -0.56 | 0.17 | -3.24 | < 0.01 |
| Saint Augustine (SA) | -0.71 | 0.17 | -4.12 | < 0.001 |
| Salt Run (SR) | -1.25 | 0.18 | -6.96 | < 0.001 |
| Fort Matanzas (FM) | -1.42 | 0.17 | -8.25 | < 0.001 |
| 2016 | 0.16 | 0.22 | 0.74 | 0.46 |
| 2017 | 0.84 | 0.21 | 4.07 | < 0.001 |
| 2018 | 1.23 | 0.21 | 5.95 | < 0.001 |
| 2019 | 1.65 | 0.2 | 8.12 | < 0.001 |
| 2020 | 1.99 | 0.21 | 9.70 | < 0.001 |

**Table 3a.** Pairwise comparisons of mean spat per shell between sampled regions in the Guana Tolomato Matanzas estuary: Tolomato River (TR), Guana River (GR), Saint Augustine (SA), Salt Run (SR), and Fort Matanzas (FM). Results are averaged over the level of year and given on the log (not the response) scale. Tukey method for post-hoc tests. SE = standard error.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Contrast** | **Estimate** | **SE** | ***z*-ratio** | ***p*-value** |
| TR – GR | 0.56 | 0.17 | 3.24 | 0.0105 |
| TR – SA | 0.71 | 0.17 | 4.12 | 0.0004 |
| TR – SR | 1.25 | 0.18 | 6.96 | < 0.0001 |
| TR – FM | 1.43 | 0.17 | 8.25 | < 0.0001 |
| GR – SA | 0.14 | 0.17 | 0.85 | 0.916 |
| GR – SR | 0.69 | 0.18 | 3.90 | 0.0009 |
| GR – FM | 0.86 | 0.17 | 5.12 | < 0.0001 |
| SA – SR | 0.54 | 0.18 | 3.12 | 0.0162 |
| SA – FM | 0.72 | 0.17 | 4.30 | 0.0002 |
| SR – FM | 0.18 | 0.18 | 0.998 | 0.8565 |

**Table 3b.** Pairwise comparisons of mean spat per shell among sampling years in the Guana Tolomato Matanzas estuary. Results are averaged over the levels of region and given on the log (not the response) scale. Tukey method for post-hoc tests. SE = standard error.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Estimate** | **SE** | ***z*-value** | **Pr(>*z*)** |
| 2015 – 2016 | -0.16 | 0.22 | -0.74 | 0.9775 |
| 2015 – 2017 | -0.84 | 0.21 | -4.07 | 0.0007 |
| 2015 – 2018 | -1.23 | 0.21 | -5.95 | < 0.0001 |
| 2015 – 2019 | -1.65 | 0.20 | -8.12 | < 0.0001 |
| 2015 – 2020 | -1.99 | 0.21 | -9.70 | < 0.0001 |
| 2016 – 2017 | -0.68 | 0.19 | -3.52 | 0.0057 |
| 2016 – 2018 | -1.07 | 0.19 | -5.55 | < 0.0001 |
| 2016 – 2019 | -1.49 | 0.19 | -7.88 | < 0.0001 |
| 2016 - 2020 | -1.83 | 0.19 | -9.59 | < 0.0001 |
| 2017 – 2018 | -0.38 | 0.18 | -2.12 | 0.2745 |
| 2017 - 2019 | -0.81 | 0.18 | -4.53 | 0.0001 |
| 2017 – 2020 | -1.15 | 0.18 | -6.39 | < 0.0001 |
| 2018 - 2019 | -0.42 | 0.18 | -2.40 | 0.1572 |
| 2018 - 2020 | -0.76 | 0.18 | -4.31 | 0.0002 |
| 2019 - 2020 | -0.34 | 0.17 | -1.96 | 0.3635 |

**Appendix B**

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled starting with “A”—e.g., Figure A1, Figure A2, etc.

References

References must be numbered in order of appearance in the text (including citations in tables and legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. Include the digital object identifier (DOI) for all references where available.

Citations and references in the Supplementary Materials are permitted provided that they also appear in the reference list here.

In the text, reference numbers should be placed in square brackets [ ] and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10), or [6] (pp. 101–105).

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EXAMPLES

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.
2. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
3. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, 2008; pp. 154–196.
4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* year, *phrase indicating stage of publication (submitted; accepted; in press)*.
5. Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, 2012.
6. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference (Day Month Year).
7. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.
8. Title of Site. Available online: URL (accessed on Day Month Year).

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