Article

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

Shannon D. Kimmel1, Hans Prevost1, Alexandria Knoell1, Pamela Marcum2 and Nicole Dix 1,\*

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1 Guana Tolomato Matanzas National Estuarine Research Reserve, Ponte Vedra Beach, FL 32082, USA;   
[Shannon.Dunnigan@dep.state.fl.us](mailto:Shannon.Dunnigan@dep.state.fl.us) (S.D.K); [Hans.Prevost@dep.state.fl.us](mailto:Hans.Prevost@dep.state.fl.us) (H.P.); [AKnoell@hotmail.com](mailto:AKnoell@hotmail.com) (A.K.)

2 South Carolina Department of Natural Resources, Charleston, SC 29412, USA; [marcump@dnr.sc.gov](mailto:marcump@dnr.sc.gov)

**\*** Correspondence: [Nikki.Dix@dep.state.us](mailto:Nikki.Dix@dep.state.us)

**Abstract:** A single paragraph of about 200 words maximum. For research articles, abstracts should give a pertinent overview of the work. We strongly encourage authors to use the following style of structured abstracts, but without headings: (1) Background: Place the question addressed in a broad context and highlight the purpose of the study; (2) Methods: briefly describe the main methods or treatments applied; (3) Results: summarize the article’s main findings; (4) Conclusions: indicate the main conclusions or interpretations. The abstract should be an objective representation of the article and it must not contain results that are not presented and substantiated in the main text and should not exaggerate the main conclusions.

**Keywords:** keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article yet reasonably common within the subject discipline.)

1. Introduction

The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance. The current state of the research field should be carefully reviewed and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your particular field of research. References should be numbered in order of appearance and indicated by a numeral or numerals in square brackets—e.g., [1] or [2,3], or [4–6]. See the end of the document for further details on references.

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. 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.

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). 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.

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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), and regions: Tolomato River (turquoise), Guana River (pink), Salt Run (yellow), St. Augustine (red), and Fort Matanzas (blue).

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). These stations are equipped with YSI EXO2 data sondes deployed approximately one meter from the bottom which measure a variety of parameters every 15-minutes: 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* 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. Chlorophyll a was extracted from frozen filters within 28 days and analyzed using Standard Methods (SM10200H; citation). All SWMP data are publicly available through the NERRS Centralized Data Management Office (CDMO) at nerrsdata.org (NERRS 2024). Data used in this study was downloaded from the CDMO for the Pine Island (gtmpiwq), San Sebastian (gtmsswq), and Fort Matanzas (gtmfmwq) stations for the continuous water quality information (Figure).

2.2. Data Collection

2.2.1. Spat Tree Deployment

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). Each stringer was composed of six cleaned eastern oyster shells, 5 to 10 cm shell height, with holes drilled through, 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. Cleaned 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, 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. The inner surface of the shells was observed under a dissecting microscope and total number of spat was recorded for each shell.

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.

2.3. Data Preparation

2.3.1. Spat Counts

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. To account for the variation in deployment of the trees each month for analysis, this count value was standardized by the number of days the stringers were left out to soak, thus creating a rate: mean number of spat per shell per deployment of tree (days). This standardized monthly spat value was also rounded to convert the number to an integer.

2.4. Data Analysis

All data analysis and visualizations were created using R programming language (R Core Team, 2023). 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). The dependent variable was the standardized average spat count for each collector by deployment. The independent variables (main effects) were both categorical the region and year. Multiple models were fitted to the data: Model 0 included region, Model 1 included region and the year, Model 2 included the interaction of region and year, and Model 3 included just year.

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 (Burnham and Anderson 2002). Models were fit to the data using the glmmTMB package (Brooks et al. 2017) 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.

2.5 Water Quality

To understand environmental conditions during each month the spat collectors were deployed, water quality data from the GTMNERR SWMP stations (Pine Island, gtmpiwq; San Sebastian, gtmsswq; Fort Matanzas, gtmfmwq) were downloaded from the NERRS CDMO. This 15-minute data (temperature, salinity, and turbidity) had undergone the quality assurance and quality checks of the CDMO and data flagged as “rejected” and “suspect” were removed from the dataset. Chlorophyll *a* data from each station was averaged by month and, like the continuous data, values flagged as “suspect” or “rejected” were removed. All water quality data was then aggregated by inside and outside the defined spat settlement periods for descriptive statistics. Several helpful import, filtering, and aggregating functions from the SWMPr package in R were used for this compilation (Beck et al. 2024).

3. Results

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.

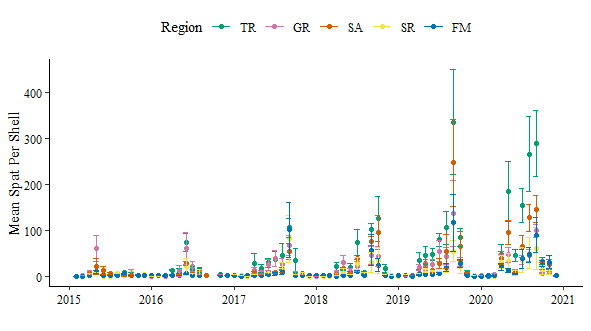
Numbered lists can be added as follows:

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

The text continues here.

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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.

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

**Table 1.** 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) |

**Table 2.** 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 standardized 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 1: spat\_std ~ region + year |  |  | 11 | 2692.97 | 0.00 | 1 |
| Model 2: spat\_std ~ region + year + (region\*year) |  |  | 31 | 2714.69 | 21.72 | 0 |
| Model 3: spat\_std ~ year |  |  | 7 | 2746.48 | 53.51 | 0 |
| Model 0: spat ~ region |  |  | 6 | 2749.00 | 56.03 | 0 |

**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** |
| entry 1 | data | data |
| entry 2 | data | data 1 |

1 Tables may have a footer.

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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.

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

\* Tables may have a footer.

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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.

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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.

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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.

**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

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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.
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