

Spatial and temporal variation of larval and spatfall index of the Eastern oyster (*Crassostrea virginica*) in the Mullica River Estuary



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Introduction

Oysters are an important part of ecosystem sustainability, such as being food for other organisms or filtering out toxins in the water. The life cycle of an oyster consists of three stages. The first stage is the larvae, which are microscopic plankton usually living for about 2-3 weeks. Then, larvae turn into juvenile spat, which settle onto hard substrate. Finally, the juveniles become adult oysters, which grow on clusters on oyster reefs. Understanding the relationship between the stages is important when assessing the health of an oyster population. For this project, we collected and analyzed larvae and spat samples from 2017-2019 at four sites in the Mullica River Estuary. We compared spatial and temporal variability in spat settlement and correlated spat and larval abundance at one of the sampling sites.



Figure 1: Map of the 4 sites across the Mullica River Estuary for spatfall (orange dots) and larval (green box) monitoring site location used in this study.

Methods

Spat Collection: Four sites were sampled for oyster spat across the Mullica River Estuary (Figure 1) from July to August in 2017, 2018 and 2019. Plastic mesh bags were filled with 20 shells each and distributed across each site. Bags were sampled and replaced biweekly. When collected, spat were counted on the smooth side of shells under a dissecting microscope and averaged for each shell. The data was analyzed with a Two-Way ANOVA separately for each year, with site and date as factors. Significant groups were determined by the Tukey pairwise comparisons with 95% confidence.

Larval collection: To sample oyster larvae, 100 liters were sampled at approximately the same time periods at the Fitney Bit site (Figure 1). The samples were stored in formalin and lab processed. Samples were then imaged on two 1 mL Sedgewick Rafter slides using a polarized light microscope. Images were then analyzed, using MATLAB to extract the bivalves. The bivalve images were identified as oysters using shell birefringence patterns (Goodwin et al. 2016) and were then sorted into three categories (Figure 3): small oyster larvae (D-stage), large oysters (veliger). The concentrations of larvae throughout the period were then compared to the average spatfall at Fitney Bit for each year.



Figure 2: Oyster spat.

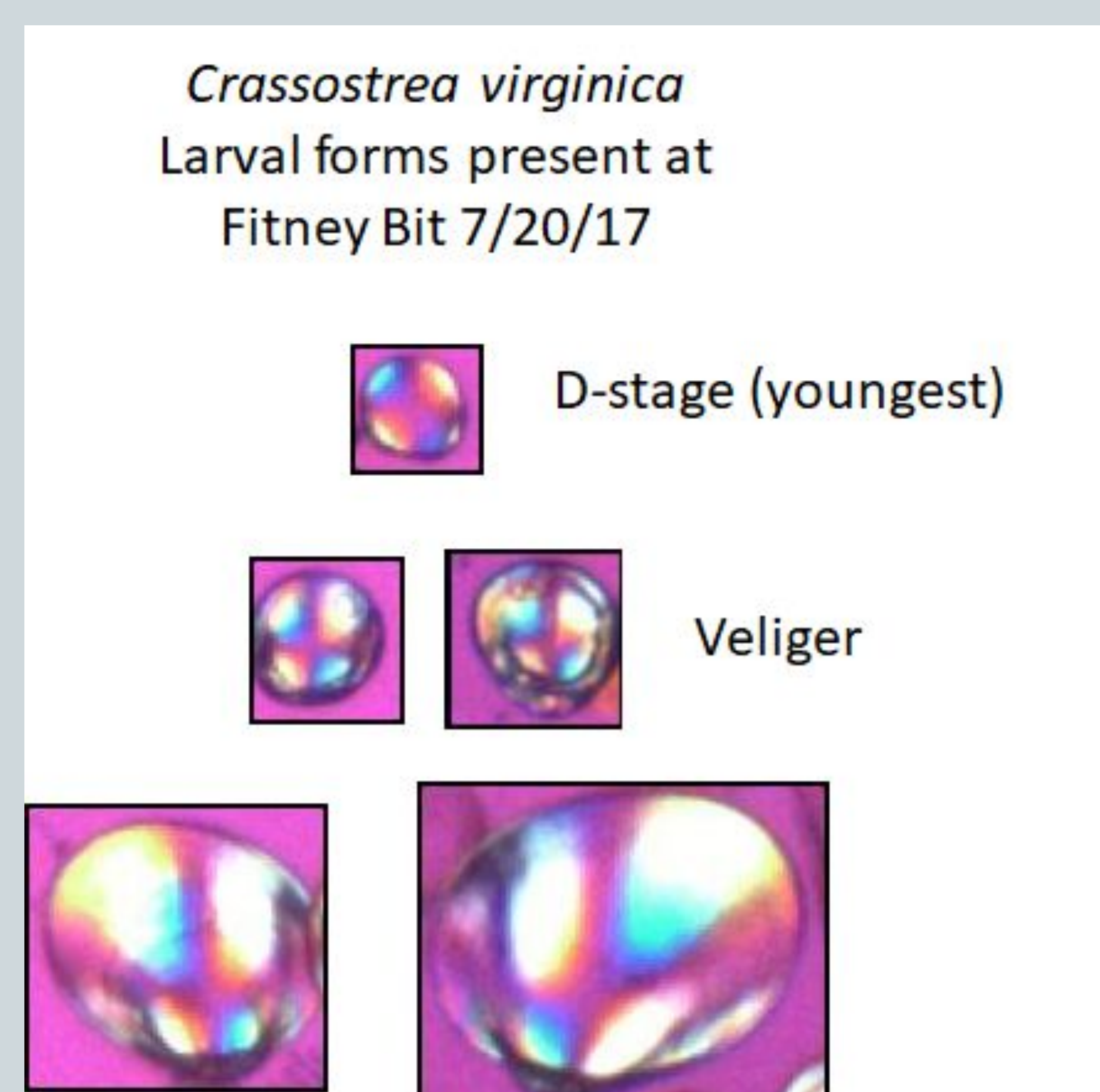


Figure 3: Examples of D-stage and veliger oysters

References and Acknowledgements

- Goodwin, J. D., North, E. W., & Kennedy, V. S. (2016). Identification of Eastern Oyster *Crassostrea virginica* larvae using polarized light microscopy in a Mesohaline region of Chesapeake Bay. *Journal of Shellfish Research*, 35(1), 157-168. doi:10.2983/035.035.0117
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- Thanks to Sadie Gramuglia and other past Stockton student researchers for additional contributions to the project.
- Thanks to Steve Evert and David Ambrose and the Stockton Marine Field Station for spat data contribution.

Figures

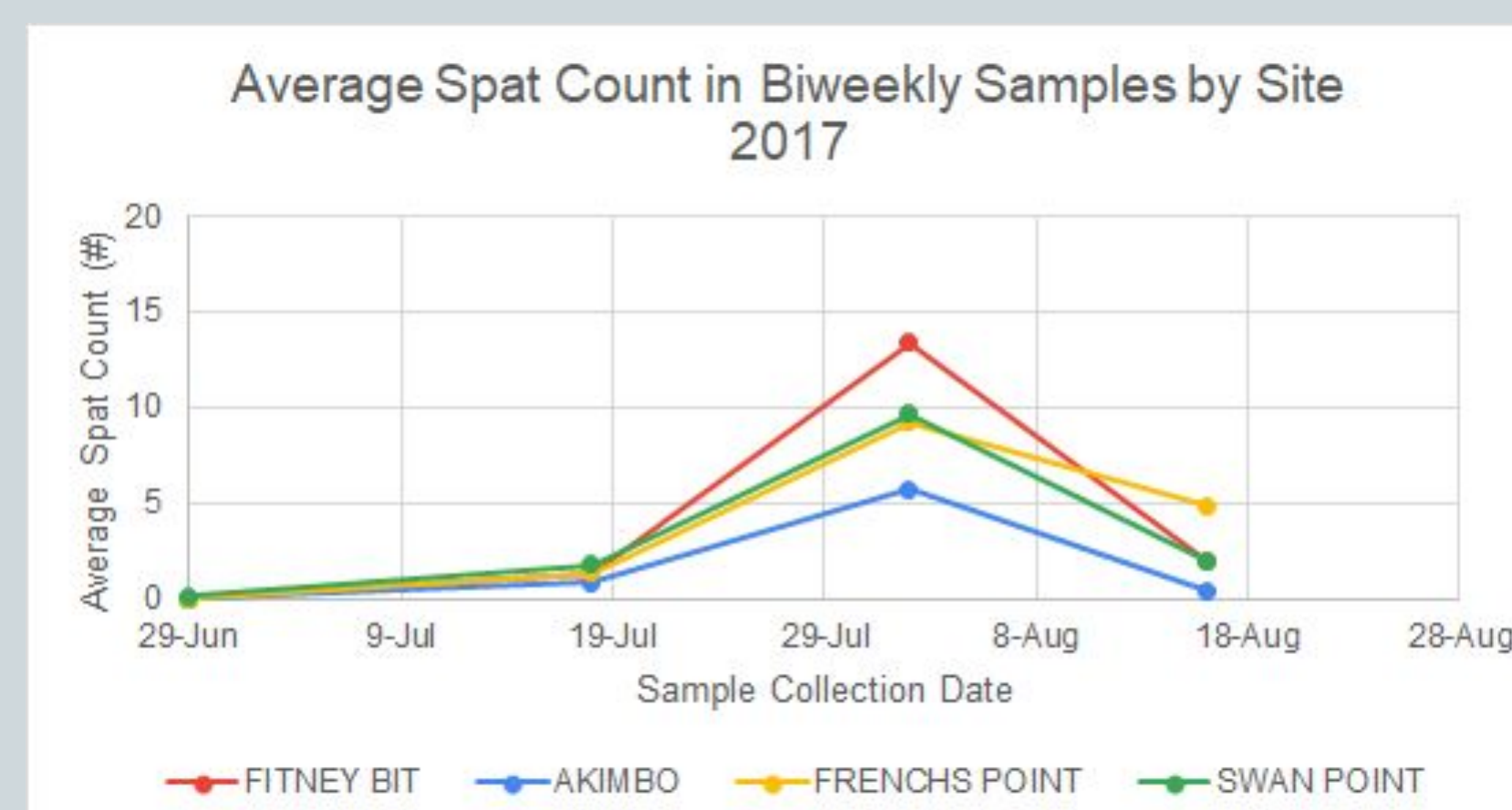


Figure 4a: Graph of average spat count per shell from 20 shell bags in biweekly 2017 samples for site. Date is retrieval after two week deployment.

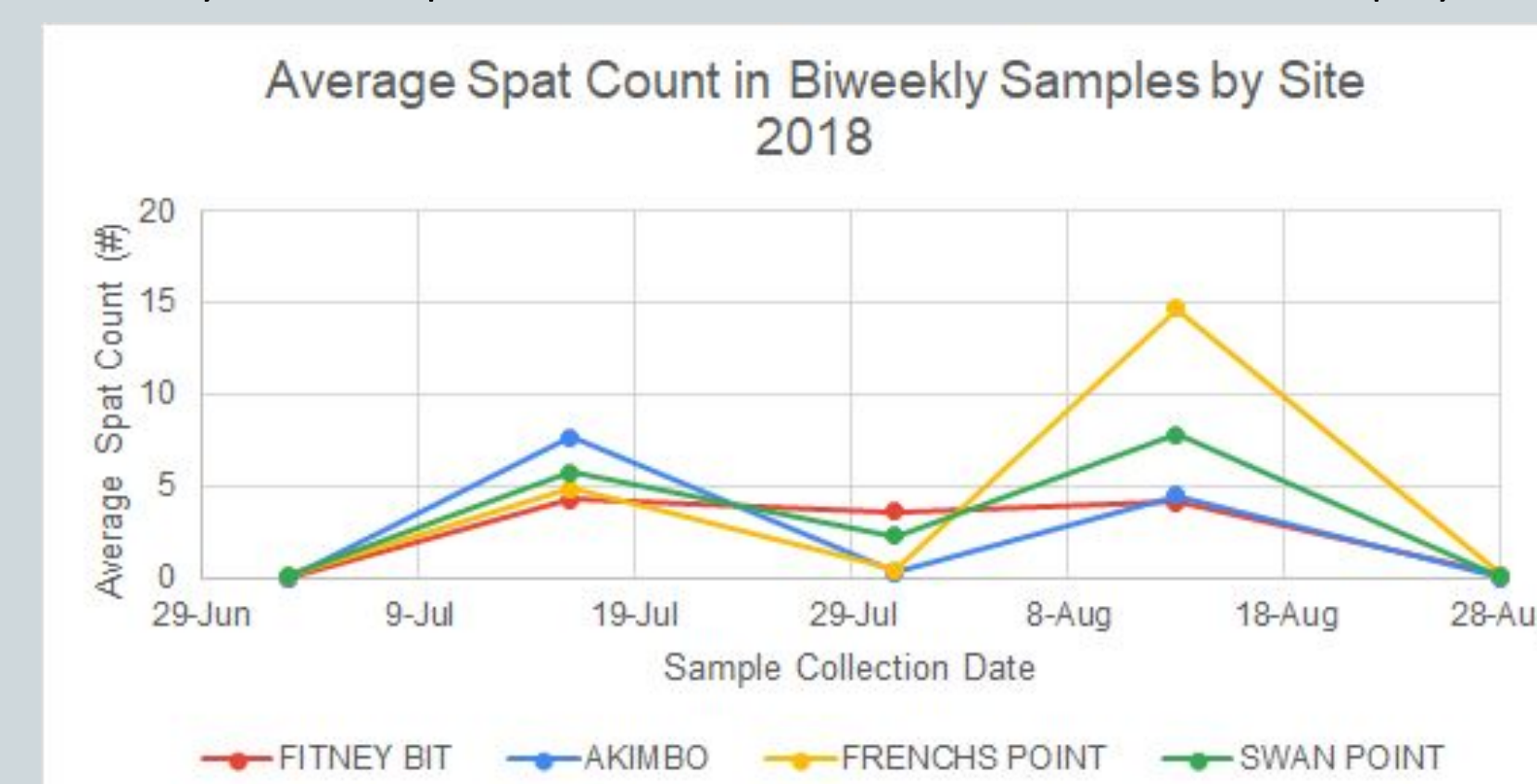


Figure 4b: Graph of average spat count per shell from 20 shell bags in biweekly 2018 samples for site. Date is retrieval after two week deployment.

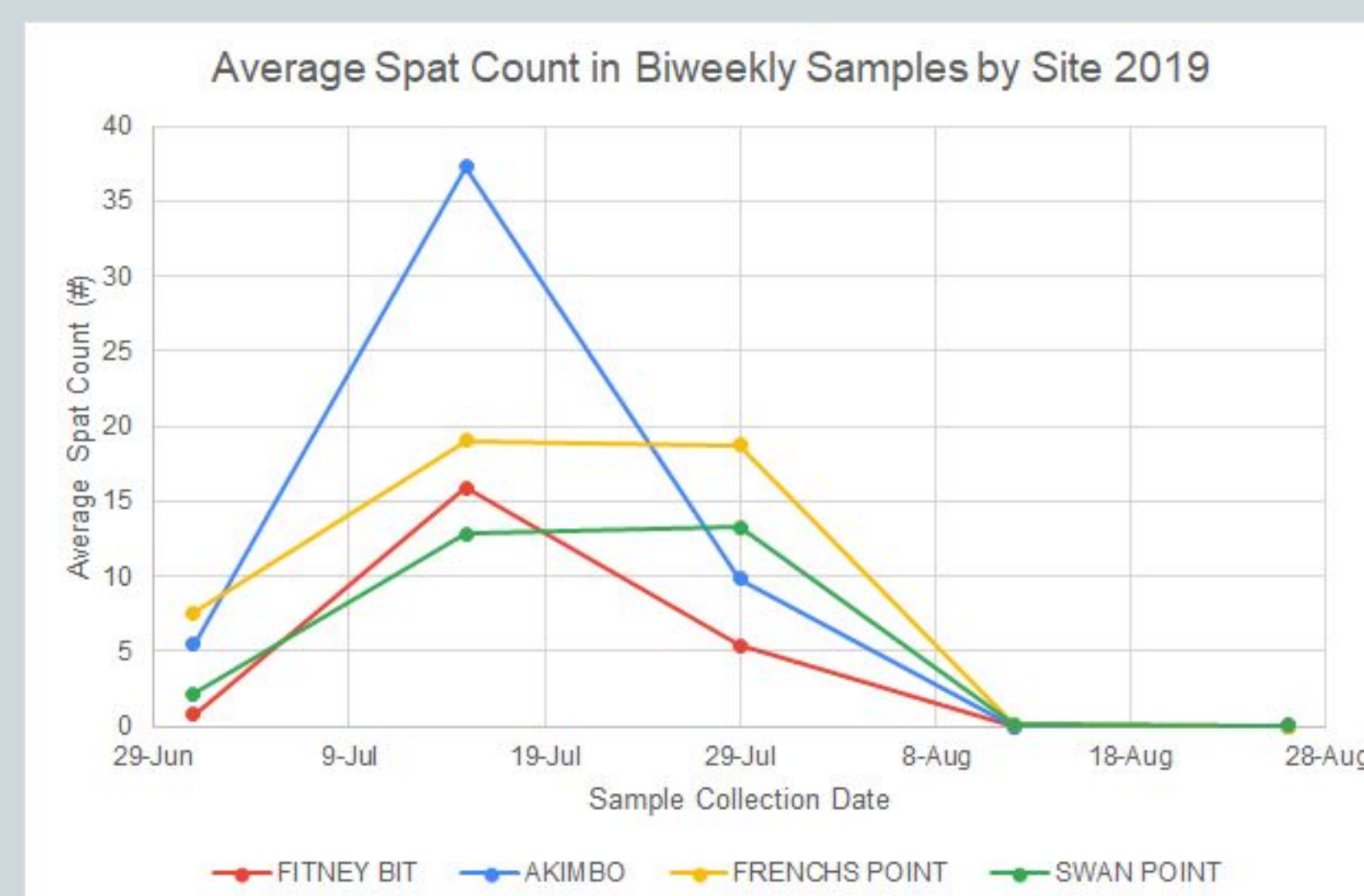


Figure 4c: Graph of average spat count per shell from 20 shell bags in biweekly 2019 samples for site. Date is retrieval after two week deployment.

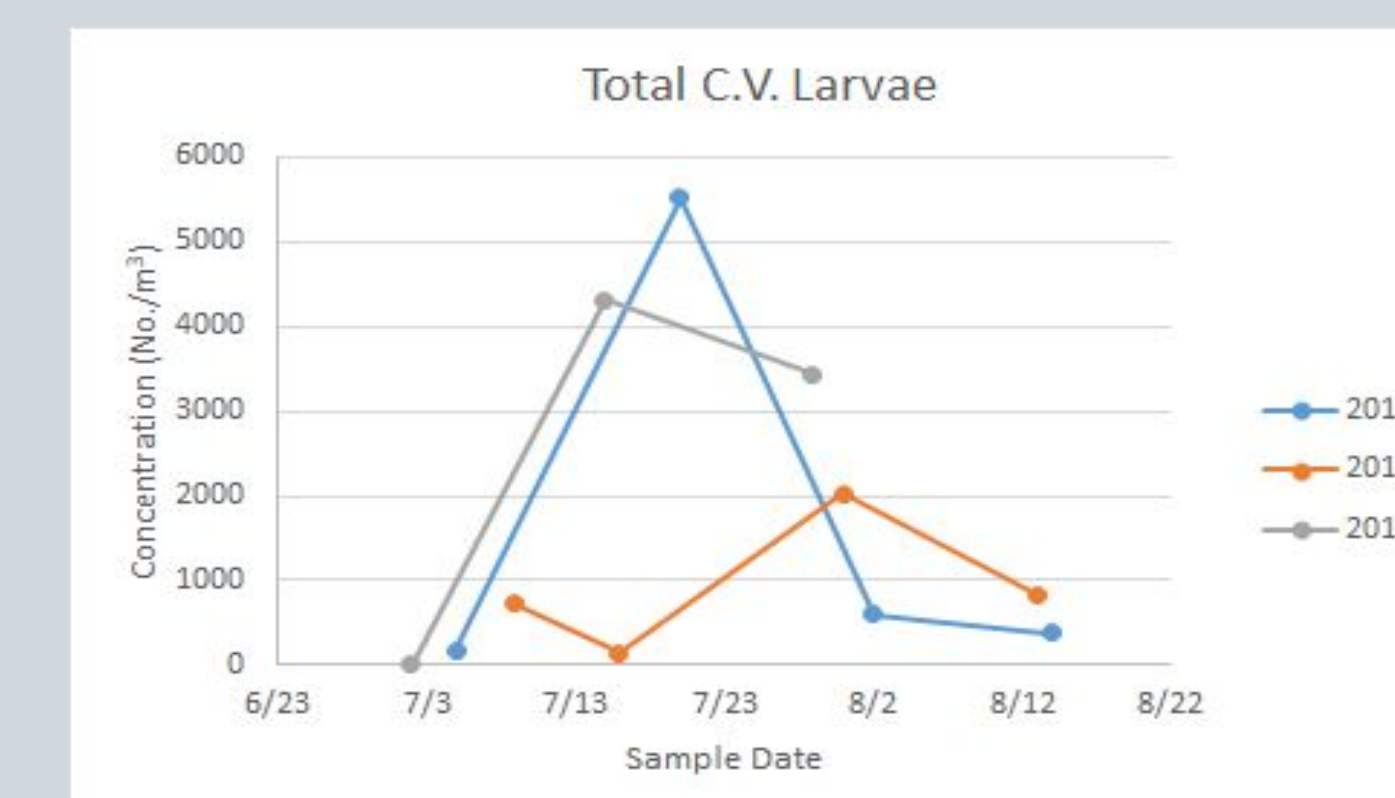


Figure 5a: Scatterplot graph showing the concentration data of the total oyster larvae from July through August each year.

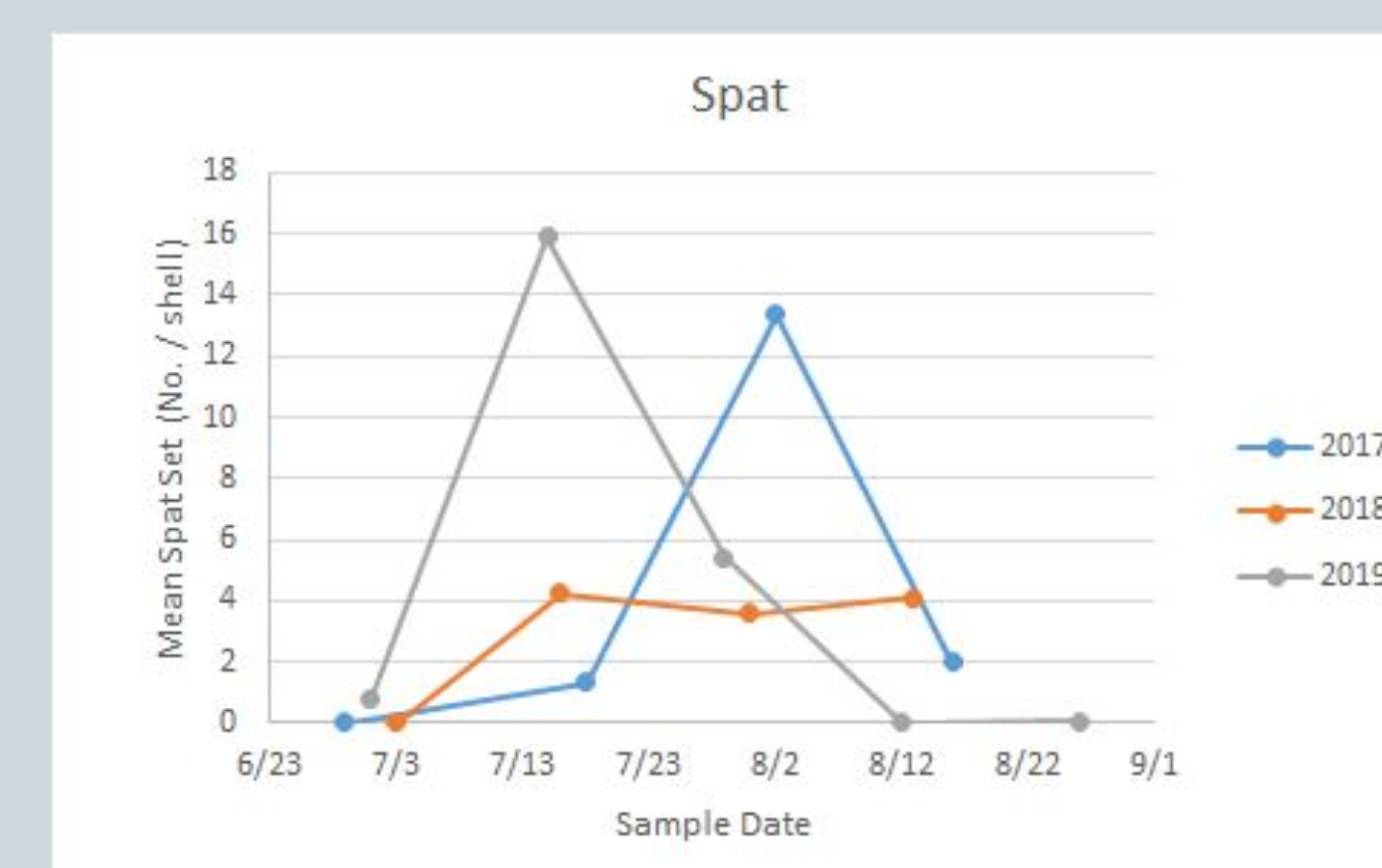


Figure 5b : Scatterplot graph showing the average spat per shell data from July through August for each year.

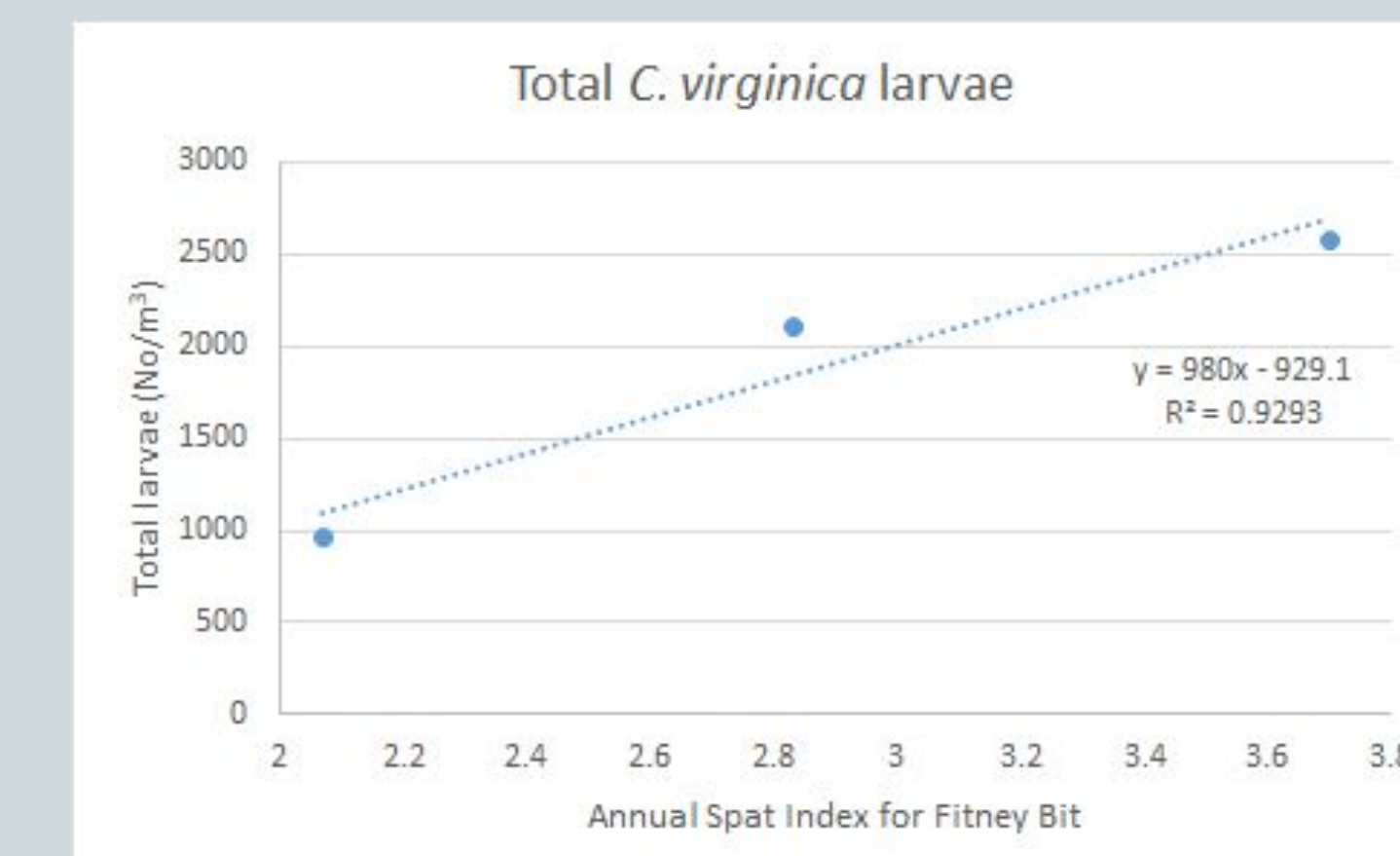


Figure 5c: Linear regression showing the relationship between annual spat index (average spat per shell across all dates) and average larval concentration for the first three dates across 2017-2019 years.

Results

Figure 4 Results: Spat Count in 2017 peaked in the August 2nd sample with a decline for all sites in the following sample period (Fig 4a). Spat Count in 2018 had two peaks. A smaller peak in July 16th with a small decline to July 31st. The largest peak was reached in the August 13th sample with a drop to near zero from all sites in the next sampling period. Fitney Bit did not fluctuate between the two peaks for the the other sites (Fig 4b). Spat Count in 2019 was far greater than previous years at all sites. Spat count peaked in the July 15th sample with Akimbo having the greatest spat count. The Akimbo and Fitney Bit counts declined to the July 29th sample while Swan Point and French's Point showed similar spat counts. All sites then decreased to almost zero in the August 12th sample and showed no further increase.

Figure 5 Results: The total larval concentration had similar early peaks in 2017 and 2019, where 2017 experienced the biggest increase in larval concentration in mid-July. The 2018 peak was later and overall lower. Timing of peaks of spat and larvae vary with year. In 2017, peaks of total larval concentration (Fig 5a) each year precede peaks of average spat count (Fig 5b) showing a spike in larval abundance prior to elevated spat settlement. 2018 and 2019 spat peaks are not preceded by a larval spike. Highest spatfall was observed in 2019 with all sites showing higher average spat counts than the other years (Fig 4c and 5b). When comparing yearly variation of spatfall with average larval concentration at Fitney Bit, the data showed that as spat index increased, the larval concentration increased, as well.

Discussion

Peaks of spatfall did not show consistent timing each year. Using the ANOVA test, temporal variation between dates of July-August each year was analyzed. In 2017, mid-July showed a significant difference from the other dates with a higher spatfall abundance. In addition, mid-August showed a significantly lower spatfall abundance than the rest of the dates. In 2018, all dates were significantly different from each other, with mid-July and mid-August showing the highest spatfall abundance, while the other dates showed the lowest spatfall abundance. In 2019, the July dates were significantly higher than the August dates.

The spatial variation of spatfall also followed different trends each year. In 2017 the Akimbo site was significantly lower than other sites. In 2018, Akimbo showed significant difference from Swan Point and French's Point which were not different from each other. The Fitney Bit site showed no significant difference from any of the other sites using the Tukey Method. In 2019, all sites were significantly different from each other besides Akimbo and French's Point which were significantly higher.

Looking at the larval and spat abundance each year for Fitney Bit shows the two are correlated, but the timing of peaks between spat and larvae differ each year. In 2017 and 2019, the larval abundance peaked in mid-July. Spatfall peaked in early August for 2017 but in mid-July alongside the larval in 2019. In 2018 larval abundance was low in mid-July and the year saw a low spatfall count compared to 2017 and 2019. The linear regression model (Fig 5c) shows that the abundance of larval positively correlates with the abundance of spatfall.

Spatial variation between sites seemed to be slightly significant within each year as spat settlement varied significantly between sites, but it did not seem significant between years since the amount of spatfall was not consistently higher or lower at any given site. Temporal variation between dates was significant each year, where the time period of mid-July through early August typically experienced the highest abundance of spatfall.

Following this study we hope to sort larval samples from the other three sites from 2017-2019 to examine how abundance of Eastern Oyster larvae relate to the temporal and spatial variation at all four sites.