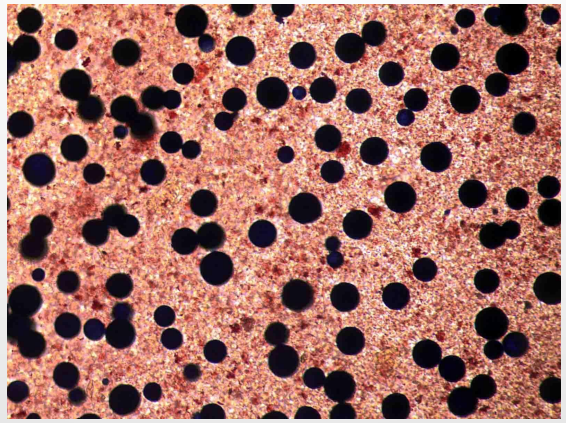
Status of Dermo Disease *(Perkinsus marinus)* in Patuxent River Oysters *(Crassostrea virginica)*



Victoria Roberts

PEARL Internship Program

Summer 2017

Background:
The Oyster Decline

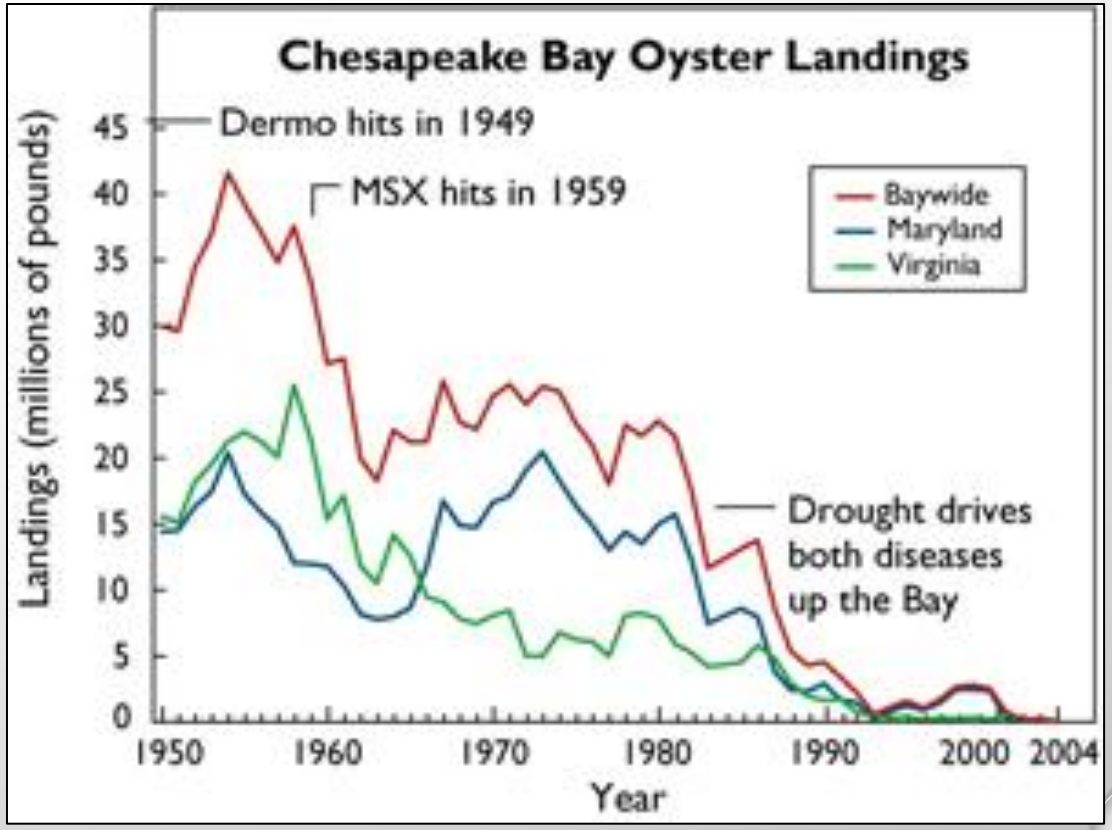
Chesapeake Bay oyster populations

- Valuable ecological and economic resource
- 2.5 million bu (1920-1969)
- 80,000 bu (1993-1994)
- 100,000 bu (2000-present)

Historic population decline

- Over-harvesting
- Habitat loss
- Disease

(Abbe et al, 2010; Greer and Fincham, 2006; Maryland Department of Natural Resources, 2016)



https://http://www.chesapeakequarterly.net/V05N2/side1/

Background:
Dermo Disease

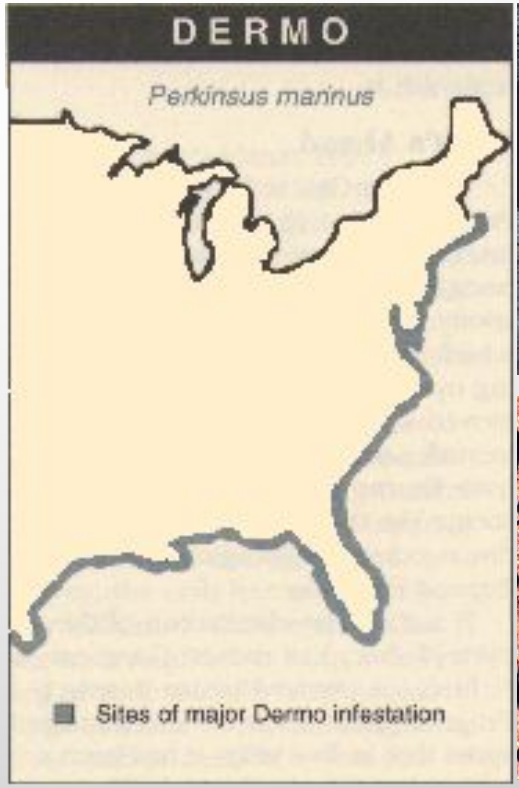
Perkinsus marinus

- Common oyster parasite on the east coast
- Infection spread by waterborne spores
- Results in poor condition and mortality
- Not harmful to humans

Researching disease

- Create prediction models
- Benefit ecological restoration
- Benefit commercial oyster industry

(Abbe et al, 2010; Virginia Institute of Marine Science)



http://ww2.mdsg.umd.edu/marinenotes/Summer95/index.html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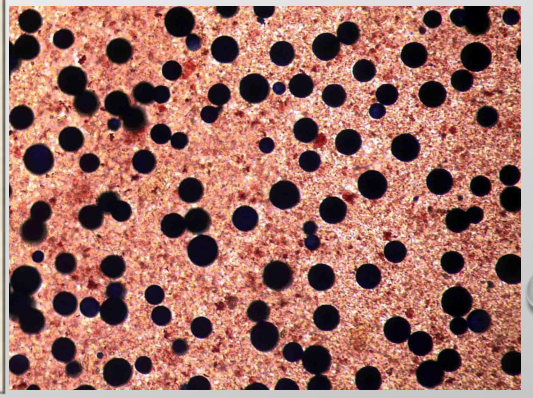
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Background:
Measuring Infection

Prevalence

- Percentage (0-100%)
- How many are infected?

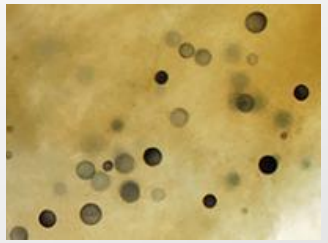
Intensity

- Numerical rank (0-7)
- How badly are they infected?

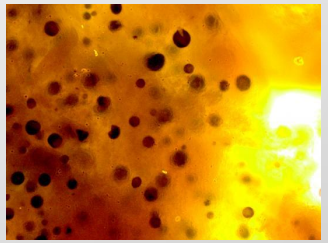
(Abbe et al, 2010; Dungan, 2016)

Table 3. *Perkinsus* sp. infection intensity ranking criteria for RFTM assays of oyster rectum tissues by RFTM assays conducted by Maryland DNR.

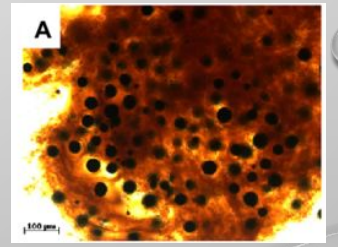
Infection intensity rank	Abundance of *Perkinsus* sp. cells and related evaluation criteria
0	No *Perkinsus* sp. cells (hypnospores) in entire sample.
1	1 - 4 *Perkinsus* sp. cells in entire sample.
2	5 - 25 *Perkinsus* sp. cells in entire sample.
3	26 - 200 *Perkinsus* sp. cells in entire sample. Parasites may occur in isolated clusters of 10-20 cells, and/or be disbursed throughout to show 1-2 cells in each 100x field.
4	About 50% of the tissue sample is occupied by *Perkinsus* sp. cells. Each 100x field shows several cells. Dense masses of hypnospores may occur locally with uninfected tissues surrounding, but blue-black staining is not macroscopically evident.
5	*Perkinsus* sp. hypnospores are present in large numbers in all areas of the tissue sample. Uninfected tissues commonly occur between parasite cells. Less than half of the tissue sample area appears blue-black macroscopically.
6	*Perkinsus* sp. hypnospores are abundant in most areas of the tissue sample. Narrow areas of uninfected tissues occur between parasite cells. The majority of the sample appears blue-black or pigmented, macroscopically.
7	*Perkinsus* sp. hypnospores occur in enormous numbers throughout the tissue sample. Areas of uninfected tissues are rare or absent between parasite cells. The entire tissue appears blue-black macroscopically.



Light (1-2)



Moderate (3-4)



Heavy (5-7)

Background:
Environmental Influences

George Abbe *et al* (2010)

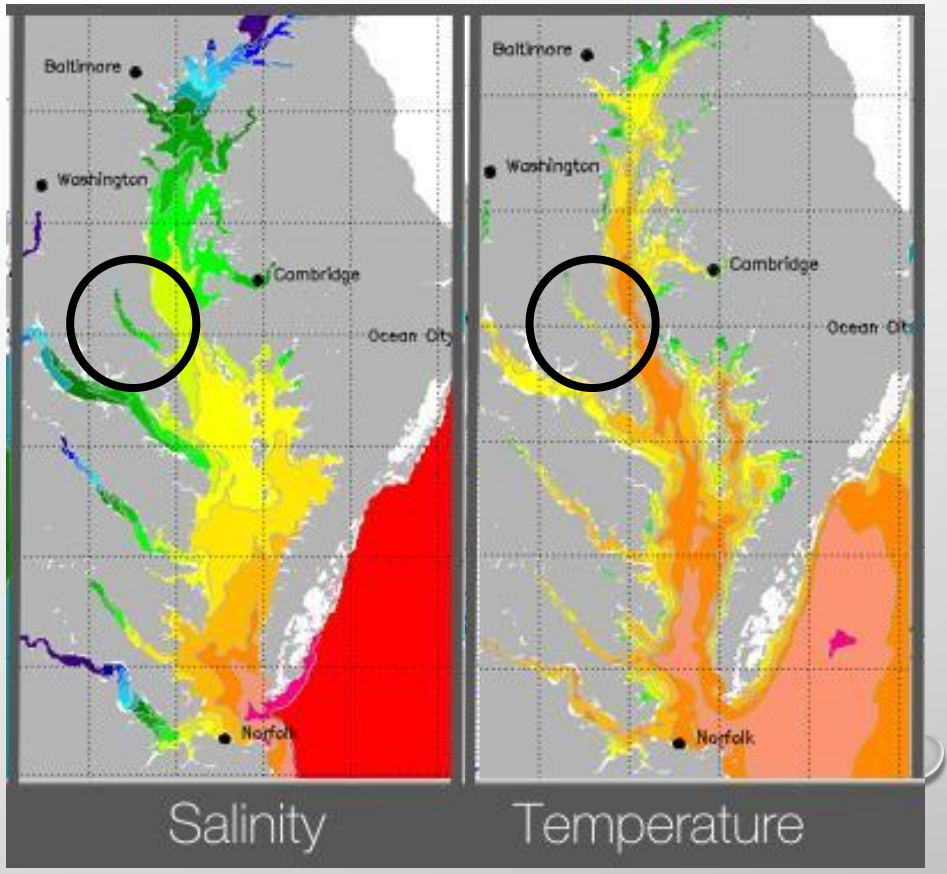
Environmental Influences

- Temperature increases, infection rate increases
- Salinity increases, infection rate increases
- Temperature and salinity gradient

Effect of climate change

- Increasing temperatures
- Increasing salinity

(Abbe et al, 2010; Environmental Protection Agency, 2017; Virginia Institute of Marine Science)



https://aamboceanservice.blob.core.windows.net/oceanservice-prod/facts/ofs.jpg

Background:
Environmental Influences

Question 1: Is there a difference in July 2017 disease status between sites?

- $H1_o$: There is no difference in total prevalence, lethal prevalence, or intensity spread between sites
- H1: There is a difference in total prevalence, lethal prevalence, or intensity spread between sites

Question 2: How does July 2017 disease status compare to past years?

- $H2_o$: 2017 does not have higher disease presence than historical data in terms of total prevalence, lethal prevalence, or mean intensity
- H2: 2017 has a higher disease presence than historical data in terms of total prevalence, prevalence lethal, or mean intensity

Question 3: Can environmental data predict July 2017 infection intensity?

- $H3_o$: We cannot predict intensity based on temperature or salinity data
- H3: We can predict intensity based on temperature or salinity data

Methods:
Procedures

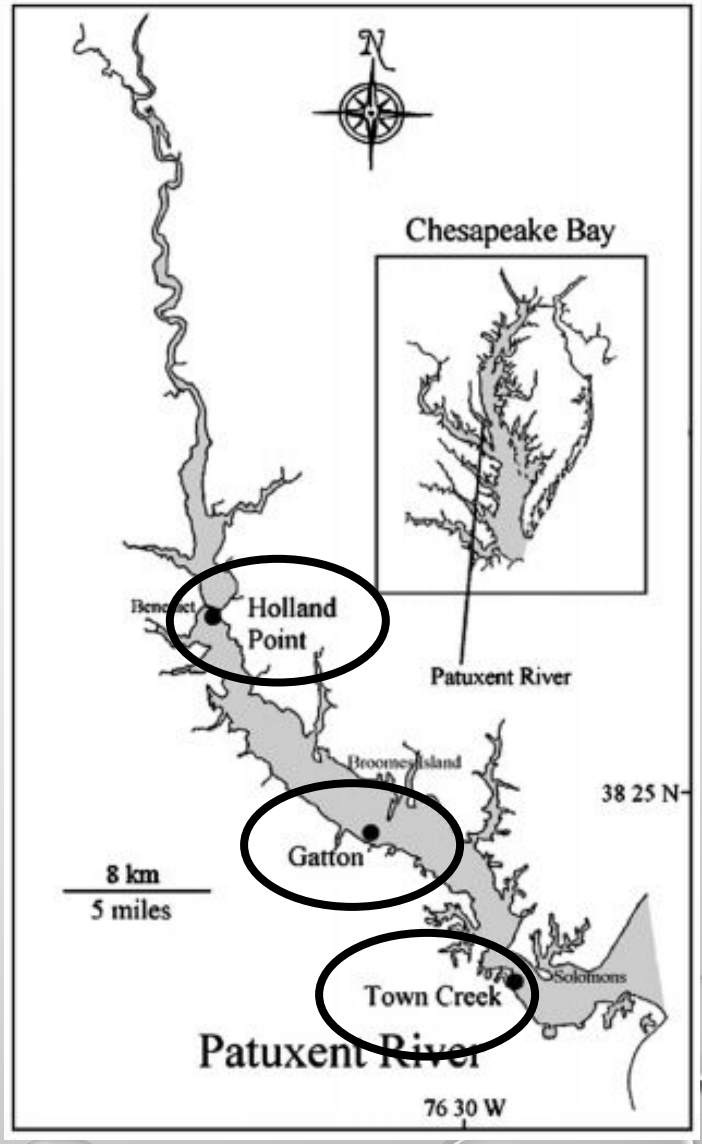
Site selection

- 30 feral oysters obtained from 3 sites in Patuxent River
- Holland Point (upper river, lowest temperature, lowest salinity)
- Gatton (middle river, higher temperature, higher salinity)
- Town Creek (lower river, highest temperature, highest salinity)

Slide preparation

- DNR procedures (ARFTM assay)
- Tissues stained and examined on slides
- Ranked on intensity scale (0-7)

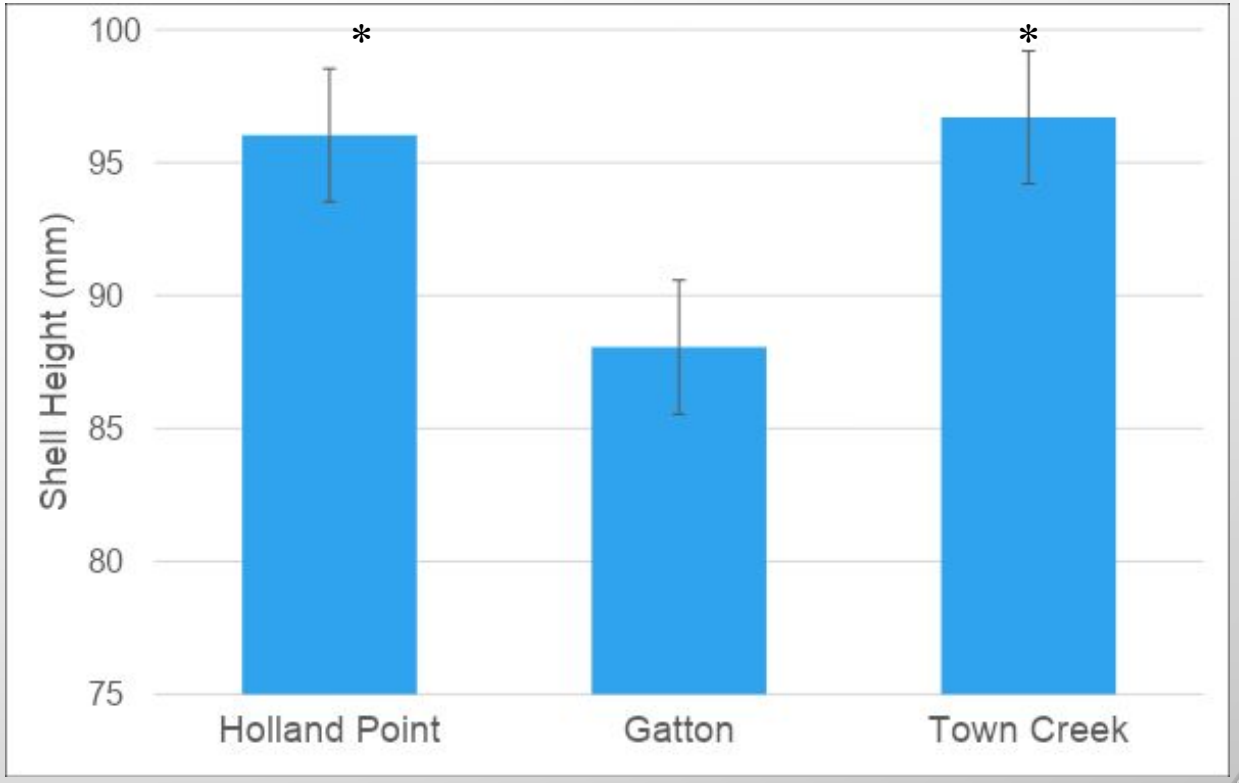
(Abbe et al, 2010; Chesapeake Bay Program, 2017, Eyes on the Bay, 2017)



Methods:
Oyster Characteristics

Shell height

- Oysters selected > 50 mm, 2-3 year
- Significant difference between sites (ANOVA, P-value < 0.05)
- No significant difference between HP and GA (ANOVA, P-value = 0.453)
- No presumed difference in age

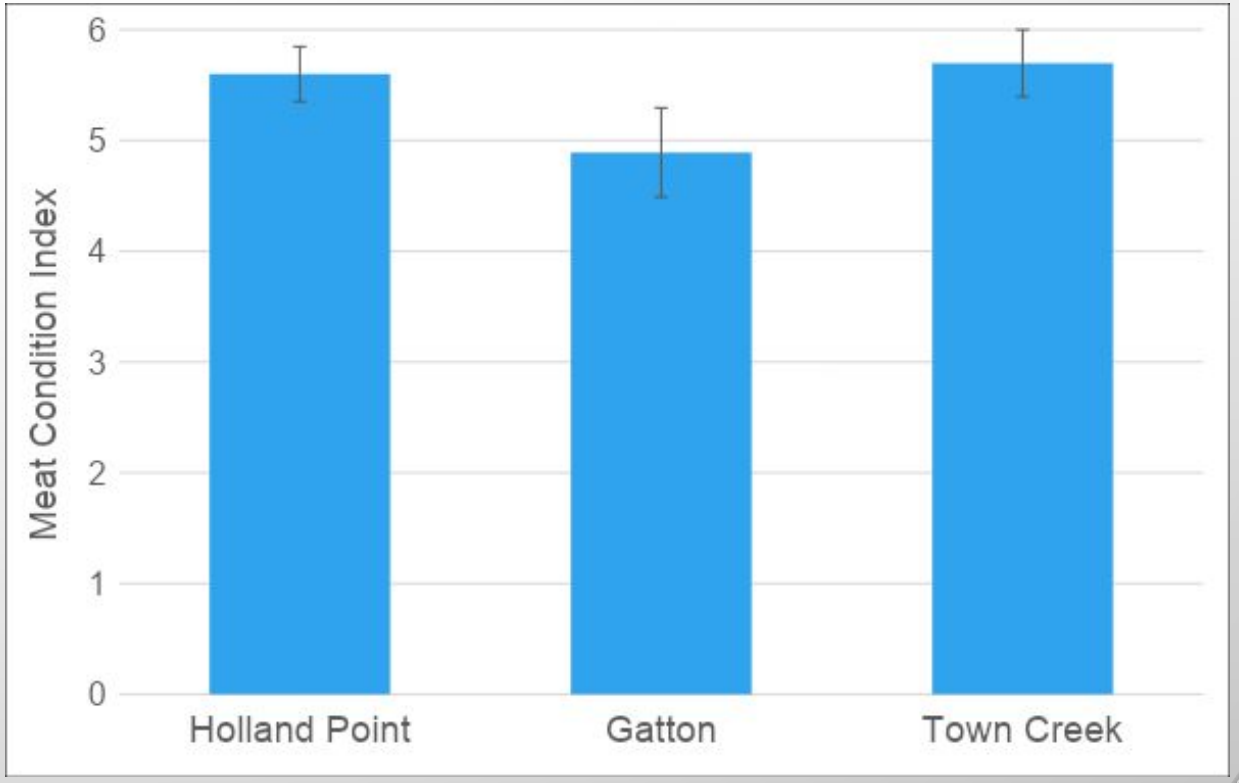


(Abbe et al, 2010; Virginia Institute of Marine Science)

Methods:
Oyster Characteristics

Meat condition index

- Ratio of internal volume to shell size
- No significant difference between sites (ANOVA, P-value = 0.36)



(Albright, 2007)

Methods:
Statistical Analysis

Question 1: Is there a difference in July 2017 disease status between sites?

- Prevalence: Percentage (0-100%)
- Intensity spread: Pearson Chi-squared (X^2) and likelihood tests (P-value)

Question 2: How does July 2017 disease status compared to past years?

- Prevalence: Percentage (0-100%)
- Mean intensity: No statistical tests

Question 3: Can environmental data predict July 2017 infection rates?

- Goodness of fit: Correlation (R), GLM and effect tests (P-value), AIC score

(Abbe et al, 2010; Dungan, 2016)

Results:
Question 1

Is there a difference in July 2017 disease status between sites?

- $H1_o$: There is no difference in total prevalence, lethal prevalence, or intensity spread between sites
- H1: There is a difference in total prevalence, lethal prevalence, or intensity spread between sites

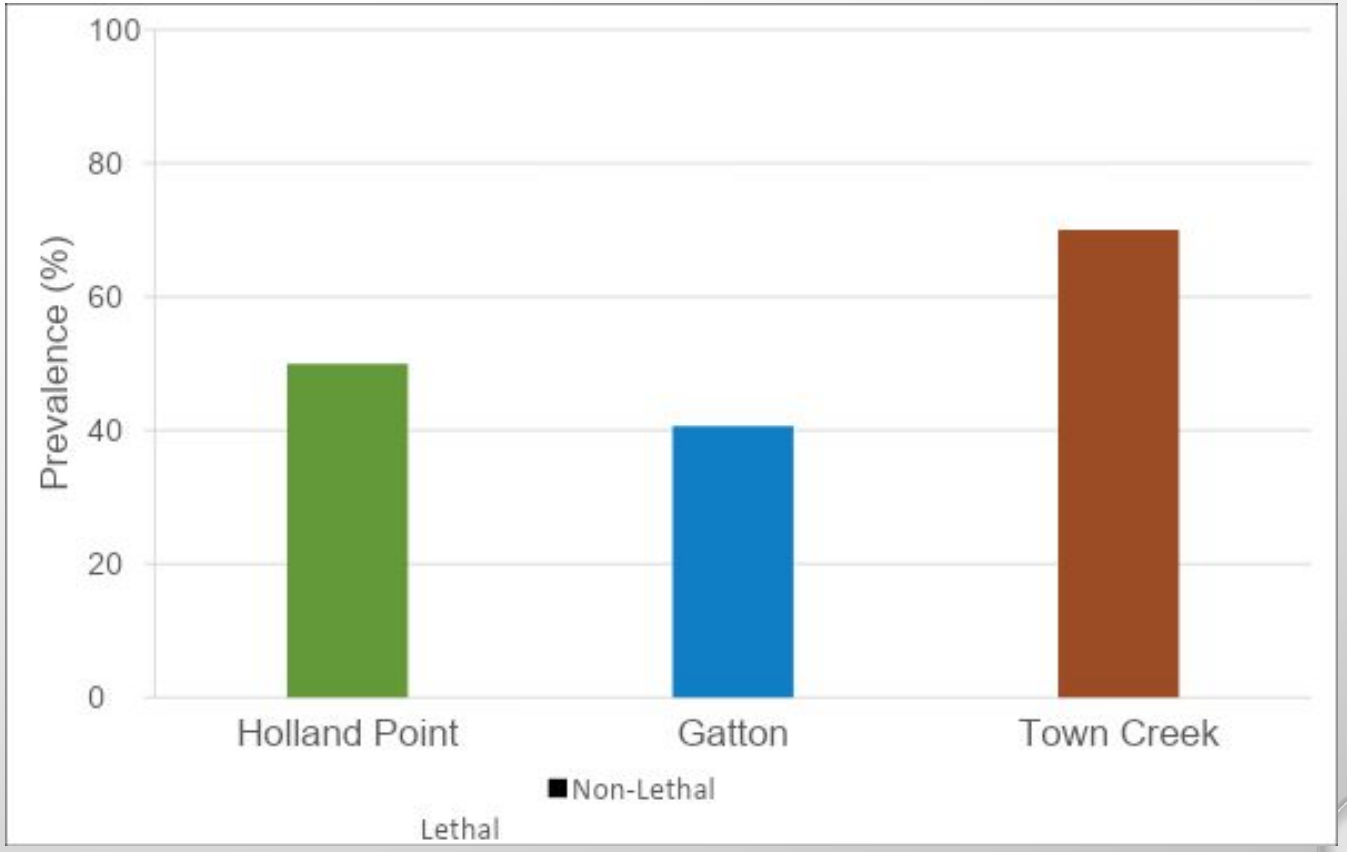
Results:
Prevalence

Total Prevalence (0-100%)

- Proportion of infected oysters
- Holland Point 70% (low)
- Town Creek 100% (high)

Lethal Prevalence (0-100%)

- Proportion of lethally infected oysters (>5)
- Holland Point 20% (low)
- Gatton 40% (high)

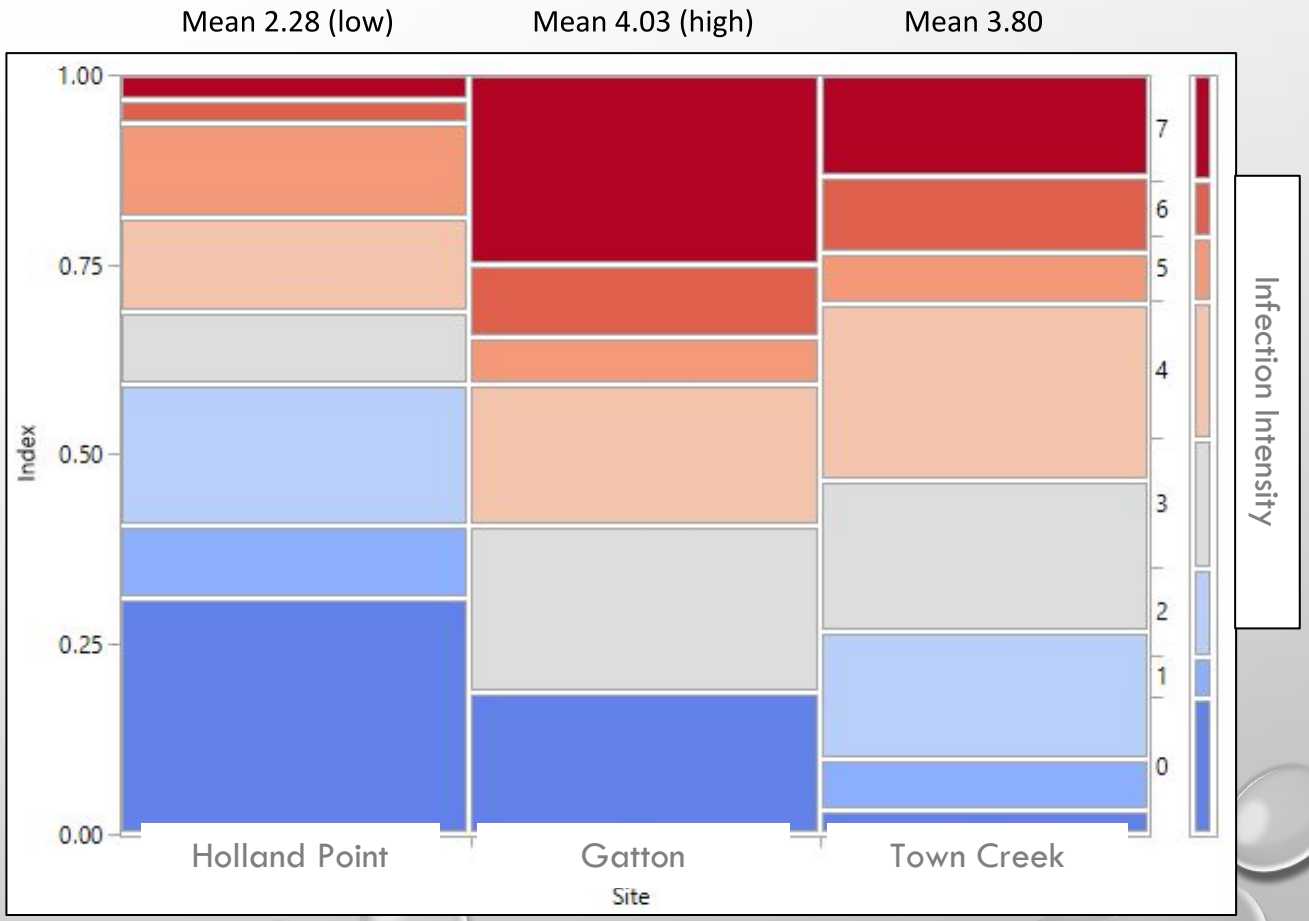


Results:
Intensity Spread

Chi-squared (X^2) test

- Comparison of intensity spread between sites
- Pearson's Chi-squared P-value < 0.05 (significant)
- Likelihood ratio P-value < 0.05 (significant)

Table 1. Pearson chi-squaured results				
Test	N	DF	ChiSquare	Prob>ChiSq
Likelihood Ratio	94	14	32.884	0.003
Pearson	94	14	25.608	0.029



Results:
Question 2

How does July 2017 disease status compare to past years?

- $H2_o$: 2017 does not have higher disease presence than historical data in terms of prevalence, lethal infection or mean intensity
- H2: 2017 has a higher disease presence than historical data in terms of prevalence, lethal infection or mean intensity

Results: Prevalence

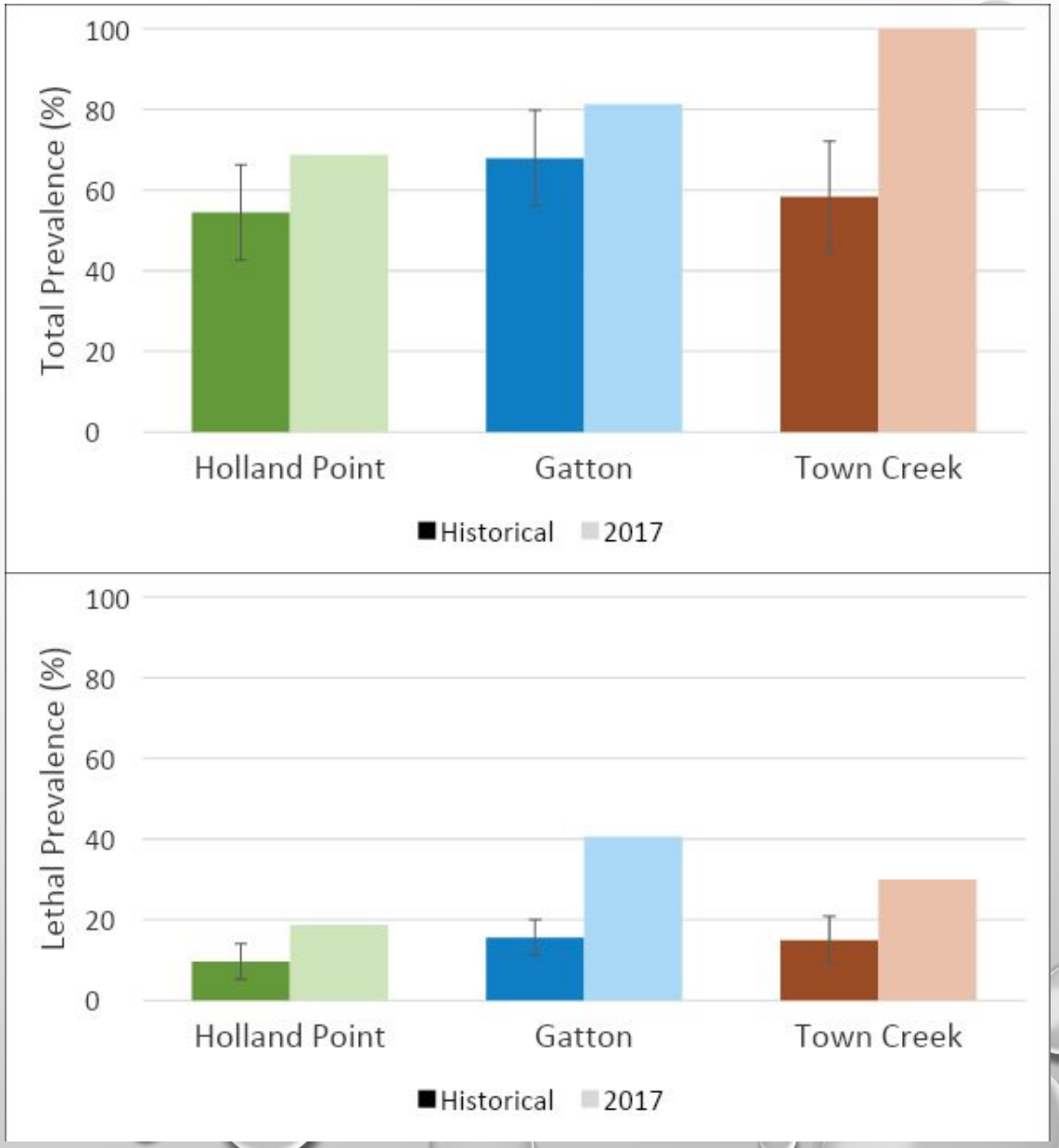
Total Prevalence (0-100%)

- Proportion of infected oysters
- Historical data taken July 1997-2007
- Above average

Lethal Prevalence (0-100%)

- Proportion of lethally infected oysters (>5)
- Historical data taken July 1997-2007
- Above average

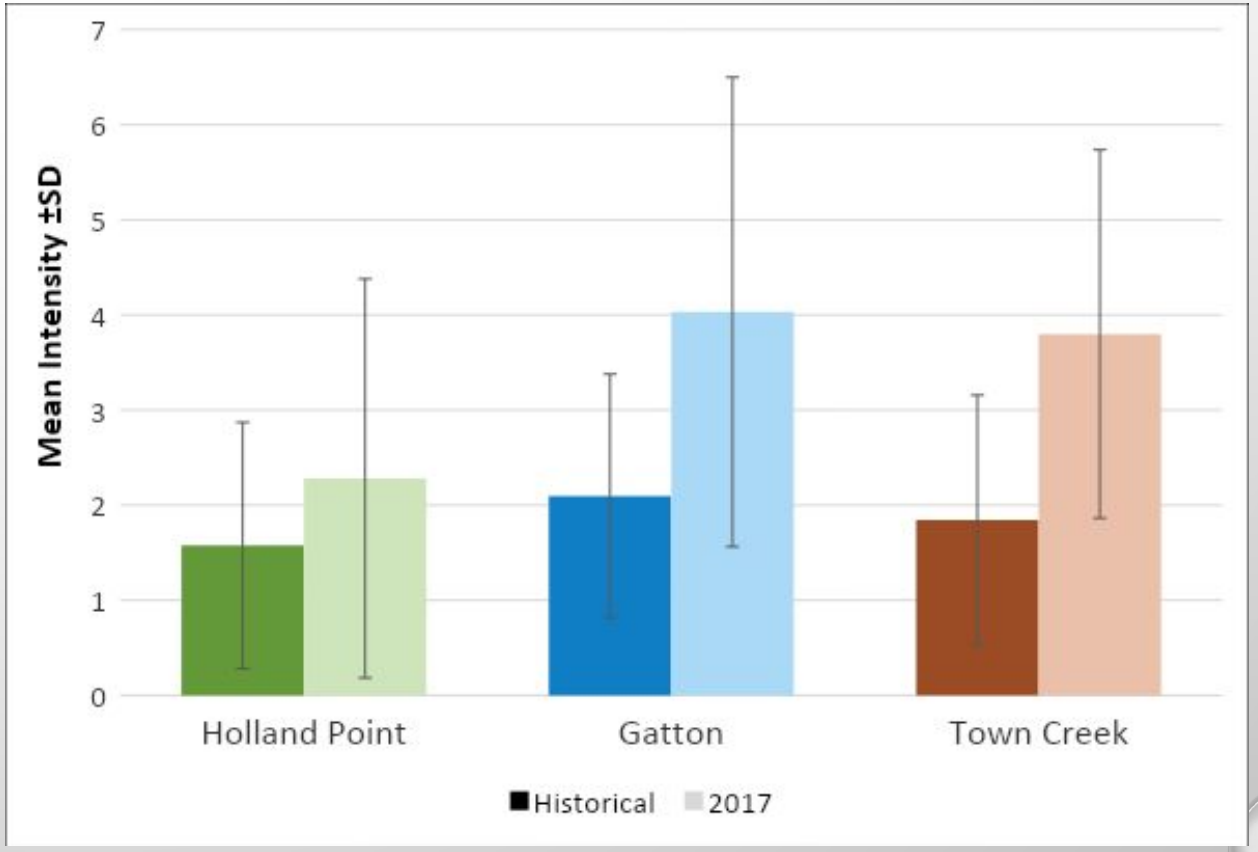
(Abbe et al, 2010)



Results:
Mean Intensity

Mean intensity

- Average intensity of infected oysters
- Historical data taken July 1997-2007
- Not different



(Abbe et al, 2010)

Results:
Question 3

Can environmental data predict July 2017 infection intensity?

- $H3_o$: We cannot predict intensity based on temperature and salinity data
- H3: We can predict intensity based on temperature and salinity data

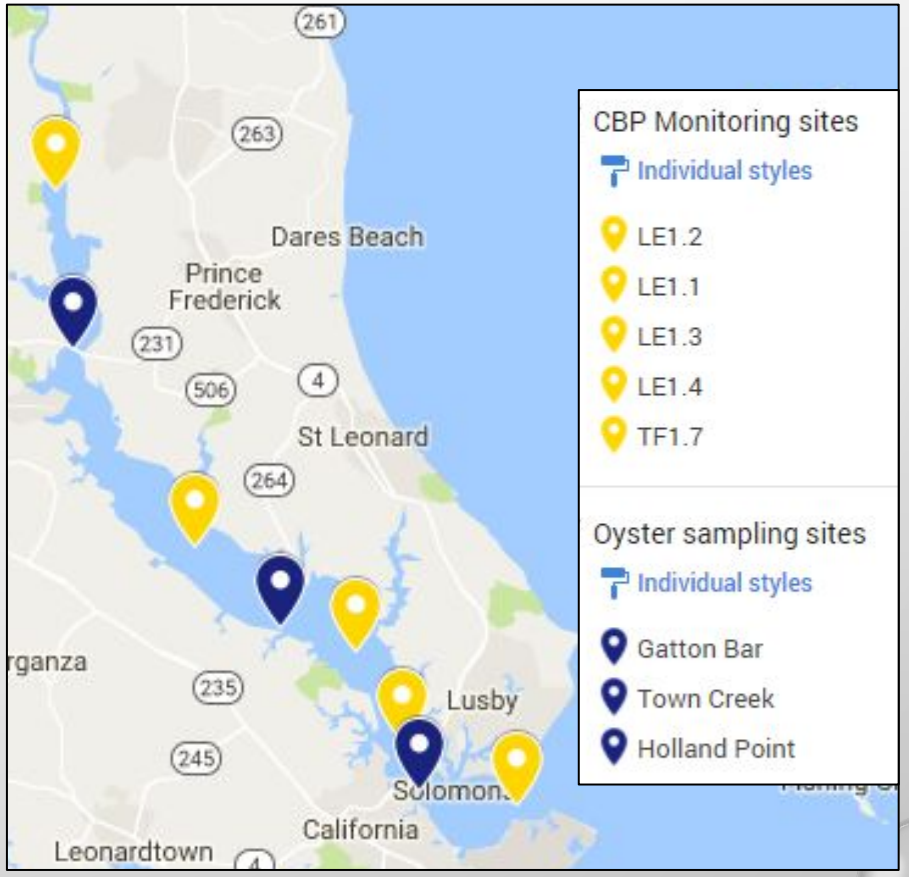
Results:
Water Quality Data

Predictive modeling

- Intensity as a function of salinity and temperature
- Requires extensive historic database

Water quality data

- 5 sites from Chesapeake Bay Program and Eyes on the Bay
- Temperature and salinity data retrieved from 1989-2017
- Interpolated salinity and temperature at oyster sites



(Chesapeake Bay Program, 2017, Eyes on the Bay, 2017)

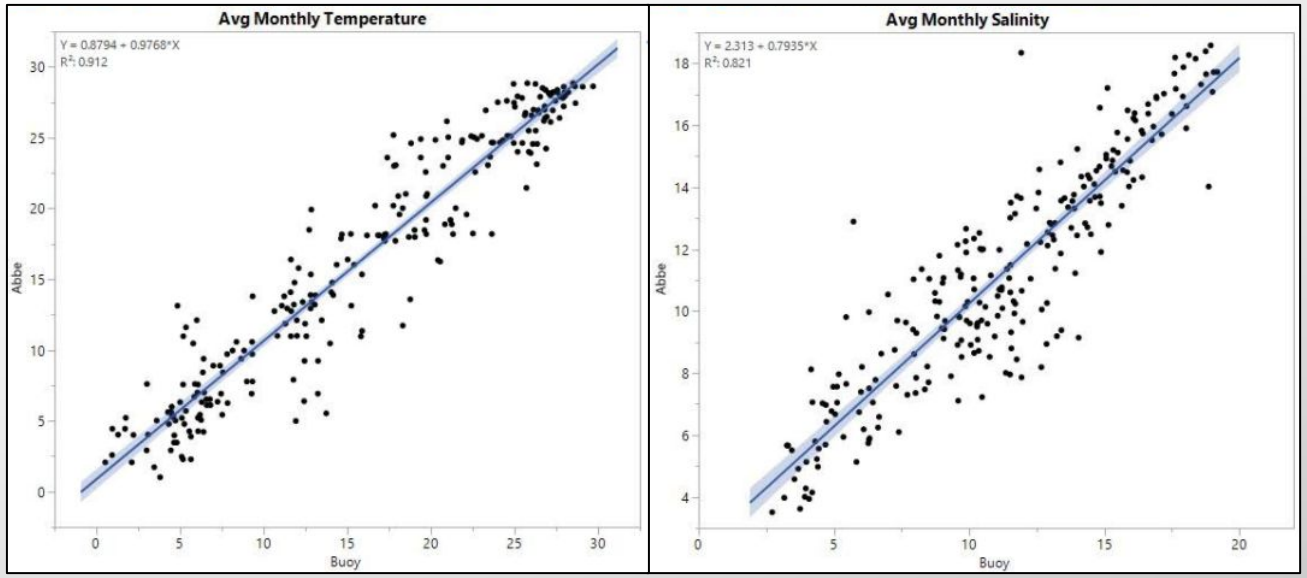
Results:
Water Quality Data

Correlation (R^2)

- Strength of relationship
- Salinity not exact ($R^2 = 0.821$)
- Temperature not exact ($R^2 = 0.912$)

Possible differences

- Interpolated data vs on-site data
- Water column average vs at-depth



(Abbe et al, 2010)

Results: Modeling

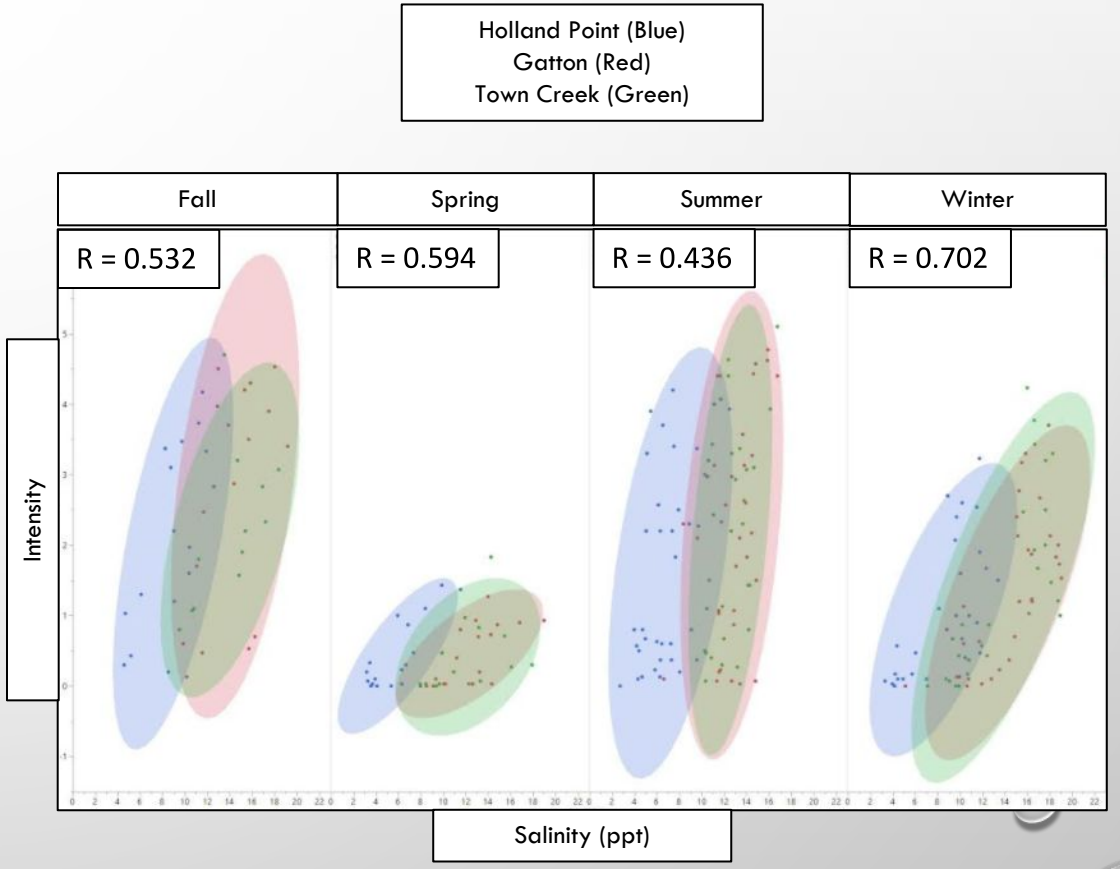
Correlation (R)

- Strength of relationship
- Overall low/moderate correlations across all parameters and time intervals
- Salinity stronger correlation than temperature
- Summer salinity R = 0.436 (low)
- Winter salinity R = 0.702 (high)

Abbe Findings

- Spring/summer salinity R = 0.99
- Summer temperature R = 0.22, P-value = 0.57
- Winter temperature R = -0.64, P-value = 0.06

(Abbe et al, 2010)



Results: Modeling

Aikike's Information Criterion

- Measure of model efficiency
- AIC 133 (best model, poor efficiency)

Generalized linear model (GLM) fit

- Probability we can guess intensity from environmental parameters (predicted vs actual)
- Whole model test: Probability model is not random (X^2, P-value < 0.05) (significant)

Effect tests

- Salinity alone (X^2, P-value = 0.08) (not significant)
- Temperature alone (X^2, P-value = 0.57) (not significant)
- Interaction (X^2, P-value < 0.05) (significant)

Generalized Linear Model Fit

Response: Intensity
Distribution: Binomial
Link: Log
Estimation Method: Maximum Likelihood
Observations (or Sum Wgts) = 130

Whole Model Test

Model	-LogLikelihood	L-R ChiSquare	DF	Prob>ChiSq
Difference	5.97372065	11.9474	3	0.0076*
Full	62.4870027			
Reduced	68.4607234			

Goodness Of Fit Statistic	ChiSquare	DF	Prob>ChiSq
Pearson	47.8813	126	1.0000
Deviance	53.7178	126	1.0000

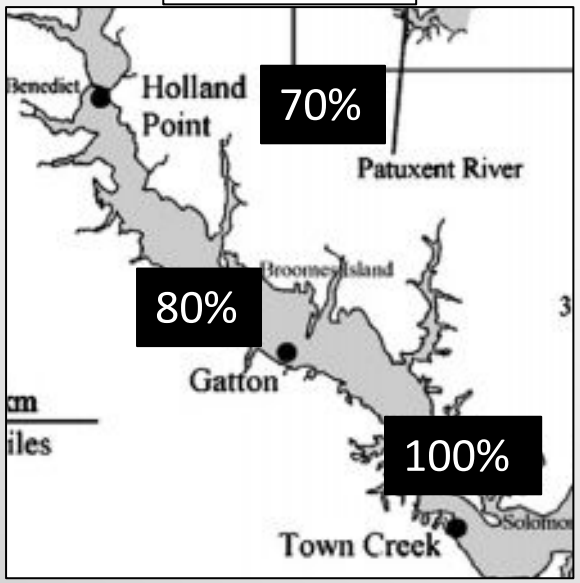
AICc
133.2940

Effect Tests

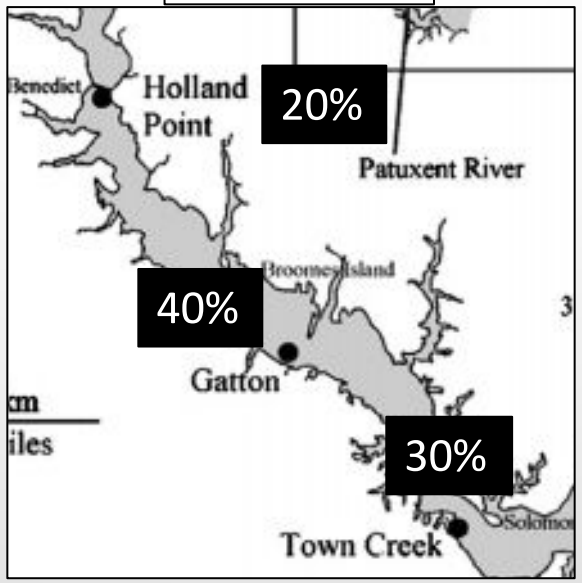
Source	DF	L-R ChiSquare	Prob>ChiSq
Avg Salinity	1	3.1473271	0.0761
Avg Temperature	1	0.3215171	0.5707
Avg Salinity*Avg Temperature	1	4.5110766	0.0337*

Results:
Question 1 Summary

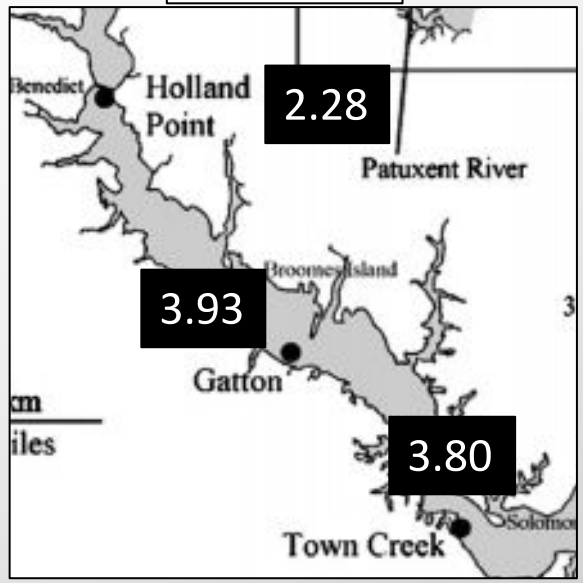
Total Prevalence



Lethal Prevalence



Mean Intensity

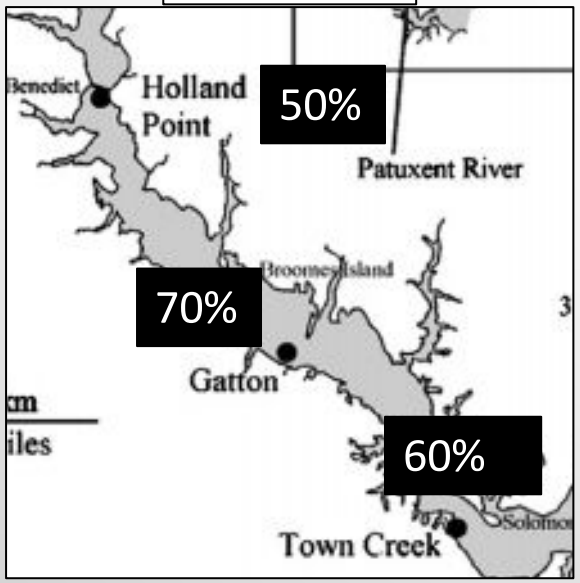


Question 1: Is there a difference in July 2017 disease status between sites?

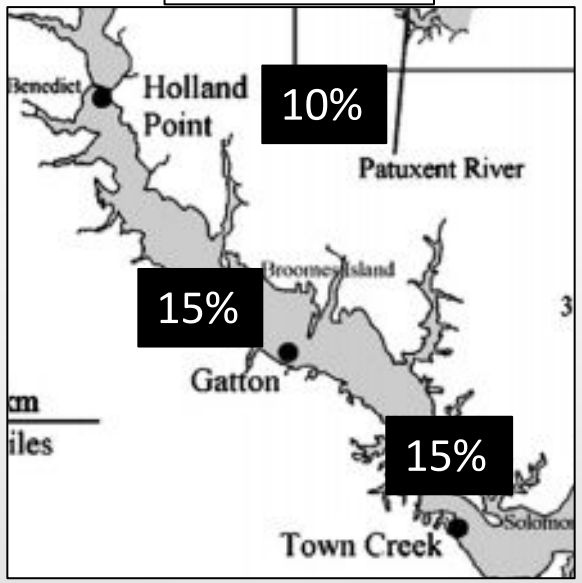
- $H1_o$: There is no difference in total prevalence, lethal prevalence, and intensity spread between sites
- **H1: There is a difference in total prevalence, lethal prevalence, and intensity spread between sites (X^2, P-value < 0.05)**

Results:
Question 2 Summary

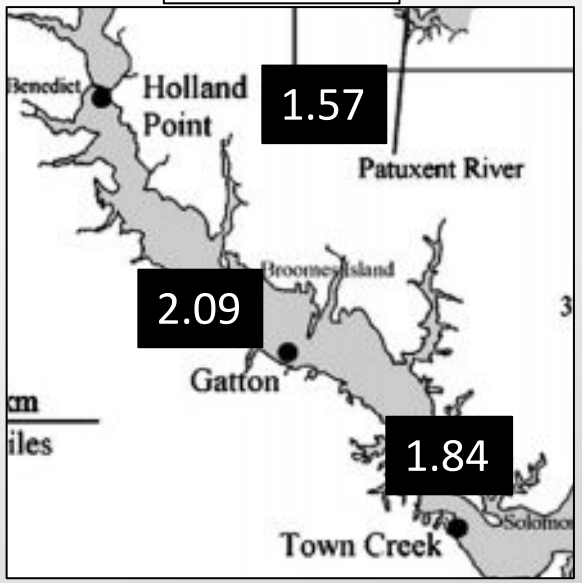
Total Prevalence



Lethal Prevalence



Mean Intensity



Question 2: How does July 2017 disease status compare to past years?

- $H2_o$: 2017 does not have higher disease presence than historical data in terms of prevalence, lethal prevalence or mean intensity
- **H2: 2017 has a higher disease presence than historical data in terms of prevalence and lethal prevalence (not significant) and no difference in mean intensity**

Results:
Question 3 Summary

Predictive modeling suggests

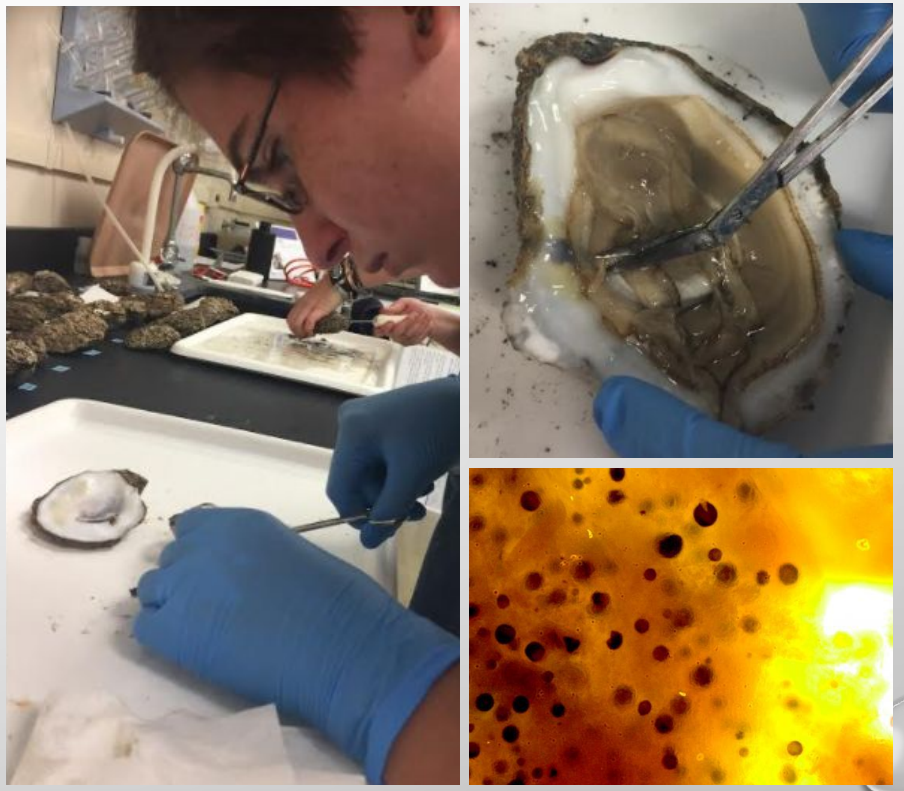
- No strong correlation between salinity or temperature on intensity
- Salinity alone has no significant effect on intensity (X^2, P-value = 0.08)
- Temperature alone has no significant effect on intensity (X^2, P-value = 0.57)
- Interaction between salinity and temperature affecting intensity (X^2, P-value < 0.05)

Question 3: Can environmental data predict July 2017 infection intensity?

- **$H3_o$: We cannot predict intensity based on temperature and salinity data**
- H3: We can predict intensity based on temperature and salinity data

Conclusion

- Town Creek displays highest prevalence, Gatton displays highest intensity
  - Inconsistent with temperature and salinity gradient
  - Possible driving factors (small size, dredging)
- Current prevalence higher than historic data, but no difference in infection intensity
  - Possible driving factors (climate change)
- No strong correlation between salinity or temperature on infection intensity
  - Interaction between salinity and temperature
  - Construct functioning prediction model



Dermo

Can’t Live With It

- ABBE, G.R., C. B. MCCOLLOUGH, L. S. BARKER, AND C. F. DUNGAN. 2010. Performance of disease-tolerant strains of Eastern Oyster (*Crassostra virginica*) in the Patuxent River, Maryland, 2003 to 2007. *Journal of Shellfish Research* 29-1: 161-175.
- CHESAPEAKE BAY PROGRAM. 2017. Water quality database. Chesapeake Bay Program, MD, USA. Retrieved from http://www.chesapeakebay.net/what/downloads/cbp_water_quality_database_1984_present.
- DUNGAN, C. 2016. Ray’s fluid thioglycollate medium (RFTM) assays for dermo disease in oysters, and *Perkinsus sp*. infections in other molluscs. Cooperative Oxford Laboratory, MD, USA.
- ENVIRONMENTAL PRTOECTION AGENCY. 2017. Climate change adaptation and estuaries. Environmental Protection Agency, USA. Retrieved from https://www.epa.gov/arc-x/climate-adaptation-and-estuaries.
- ENVIRONMENTAL PROTECTION AGENCY. 2017. Climate change indicators: Sea surface temperature. Environmental Protection Agency, USA. Retrieved from https://www.epa.gov/climate-indicators/climate-change-indicators-sea-surface-temperature.
- EYES ON THE BAY. 2017. Eyes on the Bay station map. Eyes on the Bay, MD, USA. Retrieved from http://eyesonthebay.dnr.maryland.gov/.
- GREER, J. AND M. W. FINCHAM. 2006. The culture of disease. *Chesapeake Quarterly Online*. Retrieved from http://www.chesapeakequarterly.net/V05N2/side1/.
- MARYLAND DEPARTMENT OF NATURAL RESOURCES. 2016. 2015 Maryland FMP report (September 2016) Section 14. Eastern Oyster (*Crassostra virginica*). Maryland Department of Natural Resources, MD, USA. Retrieved from http://www.dnr.state.md.us/fisheries/Documents/Section_14_Oyster.pdf.
- VIRGINIA INSTITUTE OF MARINE SCIENCE. Oyster diseases of the Chesapeake Bay. Virginia Institute of Marine Science, VA, USA. Retrieved from http://www.vims.edu/_docs/oysters/oyster-diseases-CB.pdf.