2016

Effects of harmful algal blooms caused by Aureoumbra lagunensis (brown tide) on larval and juvenile life stages of the eastern oyster (Crassostrea virginica)

Panagiota Makris
University of Central Florida

EFFECTS OF HARMFUL ALGAL BLOOMS CAUSED BY *AUREOUMBRA LAGUNENSIS* (BROWN TIDE) ON LARVAL AND JUVENILE LIFE STAGES OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*)

by

PANAYIOTA MAKRIS
B.S. University of South Florida, 2012

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Spring Term
2016

Major Professor: Linda J. Walters

ABSTRACT

Harmful algal blooms caused by the marine microalga *Aureoumbra lagunensis* have been associated with negative impacts on marine fauna, both vertebrate and invertebrate. Within the Indian River Lagoon (IRL) estuary system along Florida's east coast, blooms of *A. lagunensis* in excess of 1×10^6 cells mL^{-1} have occurred along with higher than average salinities (>35 PSU) during times of peak reproduction and growth for the eastern oyster *Crassostrea virginica*. Field and laboratory studies were used to investigate the effects of *A. lagunensis* and high salinities on early life stages of eastern oysters, late pediveliger to early juvenile. Natural recruitment of *C. virginica* within Mosquito Lagoon (northern IRL) from 2013 to 2015 was negatively associated with blooms of *A. lagunensis* (>1×10^5 cells mL^{-1}) and high salinities (>35 PSU), but recruitment of barnacle competitors was not. Larval settlement, tested using recirculating raceway flumes, was affected both by *A. lagunensis* and high salinities. Additionally, survival and growth rates of juvenile *C. virginica* were tested following one-week laboratory exposure to *A. lagunensis* and subsequent transplantation to the field for four weeks. Survival of juvenile oysters was negatively correlated with *A. lagunensis* and time (80% survival for *A. lagunensis* exposure and 90% survival when exposed to *Isochrysis galbana* control). Our results indicated negative impacts of *Aureoumbra lagunensis* on larval and juvenile eastern oysters during the term of the experiment. Oyster recruitment and growth continued during and following exposure to bloom concentrations of *A. lagunensis*, but at reduced rates.

ACKNOWLEDGMENTS

Thank you to Dr. Linda Walters, Pedro F. Quintana-Ascencio, and Dr. John Scarpa for guidance on this project. Lacie Anderson, Donna Campbell, Lisa Field, Christina Makris, Paul Sacks, and CEElab members helped with fieldwork and experiments. Dr. Margaret Lasi and Dr. Edward Phlips provided water quality data. Dr. Christopher Gobler provided cultures of *Aureoumbra lagunensis*. The UCF Biology Department, the National Park Service, and the National Oceanic and Atmospheric Administration funded this work.

TABLE OF CONTENTS

LIST OF FIGURES vi
LIST OF TABLES vii
CHAPTER 1: INTRODUCTION 1
Aureococcus anophagefferens 2
Aureoumbra lagunensis 4
Crassostrea virginica 5
Mosquito Lagoon 7
Research Questions 10
CHAPTER 2: RECRUITMENT OF *CRASSOTREA VIRGINICA* 11
Methods 11
Results 15
Discussion 20
CHAPTER 3: SETTLEMENT OF *CRASSOSTREA VIRGINICA* 23
Methods 23
Algal culture 23
Experimental Design 23
Results 28
Discussion 30
CHAPTER 4: JUVENILE SURVIVAL & GROWTH 32
Methods 32
Results 37
Discussion 41
CHAPTER 5: CONCLUSIONS 43
APPENDIX A: RECRUITMENT 47
Oyster Recruitment 48
Barnacle Recruitment 50
R Code: Oyster Recruitment 52
R Code: Barnacle Recruitment 55

APPENDIX B: SETTLEMENT 58
R Code: Settlement 61
APPENDIX C: TRANSPLANT EXPERIEMNT 66
Oyster Survival 67
Oyster Growth 68
R Code: Oyster Survival 72
R Code: Oyster Growth 75
REFERENCES 81

LIST OF FIGURES

Figure 1: A) Differential interference microscopy image of *A. anophagefferens*
B) Transmission electron microscopy image of *A. anophagefferens* 3
Figure 2: A) Differential interference microscopy image of *Aureoumbra lagunensis*
B) Transmission electron microscopy image of *A. lagunensis* 5
Figure 3: Life cycle of *Crassostrea virginica* 7
Figure 4: Presence of phytoplankton in central Mosquito Lagoon.....9
Figure 5: Oyster shell lengths following initial 6-month deployment.....9
Figure 6: Oyster recruitment study sites in northern Mosquito Lagoon. 13
Figure 7: Locations of oyster reefs in northern Mosquito Lagoon 14
Figure 8: Recruitment of *Crassostrea virginica* as a function of *Aureoumbra lagunensis* and barnacle competitors 19
Figure 9: A) Recirculating raceway flume B) Simplified representation of raceway flume 26
Figure 10: Settlement of *Crassostrea virginica*.....30
Figure 11: A) Oyster mat with 10 oyster shells attached B) Deployed oyster mats.....36
Figure 12: Logistic regression predicting probability of survival of *Crassostrea virginica* as a function of algal species and time.....38
Figure 13: Interaction effects of algal concentration, algal species, and time on growth rates of *Crassostrea virginica* following acute laboratory exposure to algal species and subsequent transplantation in the field.....40
Figure 14: Histogram of oyster spat, barnacle, and *Aureoumbra lagunensis* density 48
Figure 15: Boxplot of oyster recruitment at each oyster reef within Mosquito Lagoon.....48
Figure 16: Residuals predicting oyster recruitment.....49
Figure 17: Boxplot of barnacle recruitment at each oyster reef within Mosquito Lagoon. 50
Figure 18: Residuals predicting barnacle recruitment 51
Figure 19: Histogram of oyster settlement.....59
Figure 20: Residuals predicting oyster settlement.....60
Figure 21: Residuals predicting probability of juvenile oyster survival.....67
Figure 22: Boxplots of oyster growth rates.....69
Figure 23: Residuals predicting growth rates of *Crassostrea virginica* 71

LIST OF TABLES

Table 1: Geographic coordinate locations of restored oyster reefs within Mosquito Lagoon chosen to monitor oyster recruitment....12
Table 2: Candidate fixed model set predicting juvenile oyster recruitment....15
Table 3: Mean monthly values for *C. virginica* and barnacle recruits, *Aureoumbra lagunensis* and salinity for Mosquito Lagoon....17
Table 4: AIC table predicting juvenile oyster density as a function of salinity, barnacle density and *Aureoumbra lagunensis* density. 18
Table 5: Parameter estimates predicting juvenile oyster density as a function of salinity, barnacle and *Aureoumbra lagunensis* density 18
Table 6: Parameter estimates predicting juvenile oyster density as a function of barnacle and *Aureoumbra lagunensis* density....18
Table 7: Model selection of optimal mixed model configurations for barnacle recruitment....19
Table 8: Parameter estimates of fixed effects predicting barnacle density as a function of salinity and *Aureoumbra lagunensis* density 20
Table 9: Parameter estimates of fixed effects predicting barnacle density as a function of salinity 20
Table 10: Settlement experimental treatments for each batch of purchased larvae....27
Table 11: AIC table predicting oyster settlement as a function of salinity, algal species, algal concentration, and replicate block 28
Table 12: Parameter estimates for fixed effects predicting settlement of *Crassostrea virginica* as a function of salinity....29
Table 13: AIC table predicting oyster settlement for replicate 2....29
Table 14: Parameter estimates predicting settlement of *Crassostrea virginica* for replicate 2....29
Table 15: Survival and growth experimental treatments 35
Table 16: AIC table predicting probability of survival of *Crassostrea virginica* 37
Table 17: Parameter estimates predicting probability of survival of *Crassostrea virginica*....37
Table 18: Model selection of optimal random factor configurations for oyster recruitment 49
Table 19: Variance for the random effect of site on the intercept 49
Table 20: Model selection of random factor configurations for barnacle recruitment determine.50
Table 21: Variance for the random effect of site on the intercept 50
Table 22: Configurations of random tank location for oyster settlement 59
Table 23: Model selection of optimal random factor configurations for oyster settlement 59
Table 24: Model selection of random factors for oyster survival models....67
Table 25: Configurations of random factors for oyster growth model 70
Table 26: Model selection of random factors for oyster growth models 70

CHAPTER 1: INTRODUCTION

Phytoplankton are microscopic organisms which form the base of the marine food web and account for 50% of global primary productivity (Longhurst et al. 1995, Hallegraeff 2010). However, when dense blooms of phytoplankton occur, they disrupt marine ecosystems and human activities (Shumway 1990, Glibert et al. 2005). Deleterious effects caused by certain marine microalgae include risks to human health, impacts on marine ecosystems such as mortality of marine species via toxins or anoxia, and impacts on the recreational use of coastal areas such as economic losses due to decreases in tourism (Zingone and Enevoldsen 2000). Dense blooms of algae, called harmful algal blooms (HABs), have been increasing in frequency, intensity, and global distribution (Hallegraeff 1993, Anderson et al. 2012). Although HABs are natural phenomena occurring throughout history, range expansions driven by climate change, eutrophication, and ship ballast water translocations continue to threaten new areas (Smayda 1990, Hallegraeff and Bolch 1991, Doblin et al. 2004, Hallegraeff 2010).

Novel HAB species are being discovered due to advances in detection and monitoring (Zingone and Enevoldsen 2000, Glibert et al. 2005). For example, brown tide algal blooms caused by the pelagophytes (class Pelagophyceae) *Aureococcus anophagefferens* Hargraves et Sieburth and *Aureoumbra lagunensis* Stockwell, DeYoe, Hargraves et Johnson are relatively recent (DeYoe et al. 1997, Gobler and Sunda 2012). Blooms of *A. anophagefferens* were detected for the first time in Long Island Sound in 1985 and *A. lagunensis* in the Laguna Madre, Texas in 1990 (Cosper et al. 1987, Buskey et al. 1998). Since their first occurrence, brown tide algal blooms have appeared along the east (*A. anophagefferens*) and Gulf coasts (*A. lagunensis*) of the United States as well as parts of South Africa (*A. anophagefferens*) and northeast China

(*A. anophagefferens*) (Bricelj and Lonsdale 1997, DeYoe et al. 1997, Zhang et al. 2012).

HABs caused by *A. anophagefferens* and *A. lagunensis* have occurred in shallow water estuaries and are associated with reduced flushing rates and elevated salinities (Bricelj and Lonsdale 1997, Gobler and Sunda 2012). Both species are capable of growing in low light and nutrient conditions and are generally preceded by blooms of high-nutrient adapted algae that reduce available inorganic nutrients (Gobler and Sunda 2012). The ability of these pelagophytes to use organic forms of nitrogen, phosphorus, and carbon further enhances their capacity to attain high biomass levels when inorganic nutrients are limited (Deyoe and Suttle 1994, Berg et al. 1997, Gobler and Sunda 2012). Although these genetically distinct algae share many similarities, *A. lagunensis* is not capable of using nitrates (NO_3^-), which can be used by *A. anophagefferens* (Deyoe and Suttle 1994, Berg et al. 1997). Additionally, *A. lagunensis* is capable of growing at higher salinities than *A. anophagefferens*; maximum growth rates are achieved at salinities ranging from 30-50 PSU and 28-31 PSU, respectively (Cosper et al. 1989, Buskey et al. 1998).

Aureococcus anophagefferens

Aureococcus anophagefferens is a 2 µm, spherical, non-motile pelagophyte (Sieburth et al. 1988, DeYoe et al. 1997; Fig 1). This unicellular, golden brown alga possesses an extracellular polymeric substance (EPS) (DeYoe et al. 1997). EPS is a thick polysaccharide mucus layer that surrounds cells and serves as a protective layer by helping cells survive under hypersaline conditions, by inhibiting grazing, and even allowing passage unharmed through the guts of grazers (Decho 1990, Liu and Buskey 2000a, b, Bersano et al. 2002). Toxic and non-toxic strains of *A. anophagefferens* exist, although the chemical composition of the toxin has not been characterized (Gainey and Shumway 1991, Bricelj et al. 2004). The bioactive compound believed to be associated with the EPS of *A. anophagefferens* has dopamine-mimetic effects

which inhibit gill lateral ciliary activity in bivalves, the physiological process involved in the capture of food particles (Aiello 1970, Gainey and Shumway 1991, Newell and Langdon 1996).

Negative impacts associated with blooms of *A. anophagefferens* on affected coastal ecosystems include widespread losses of seagrasses due to light attenuation, losses to shellfish fisheries such as the hard clam *Mercenaria mercenaria* and the bay scallop *Argopecten irridians* fisheries in New York, and mass mortalities of the blue mussel *Mytilus edulis* in Rhode Island (Cosper et al. 1987, Tracey 1988, Bricelj and Kuenstner 1989, Kraeuter et al. 2008). Blooms of *A. anophagefferens* have attained peak summer densities of 2.8 cells mL^{-1}. Controlled experimental studies have shown that three-week exposures to moderate concentrations ($\geq 4\times10^5$ cells mL^{-1}) of a toxic *A. anophagefferens* isolate (CCMP 1708) resulted in arrested shell growth and significant soft-tissue weight loss in juvenile *M. mercenaria* and *M. edulis* (Bricelj et al. 2004). In addition, shell growth of larvae of the hard clam *M. mercenaria* exposed to a toxic isolate of *A. anophagefferens* (8×10^5 cells mL^{-1}) for two weeks was approximately 90% less than larvae fed control algae (Bricelj and MacQuarrie 2007).

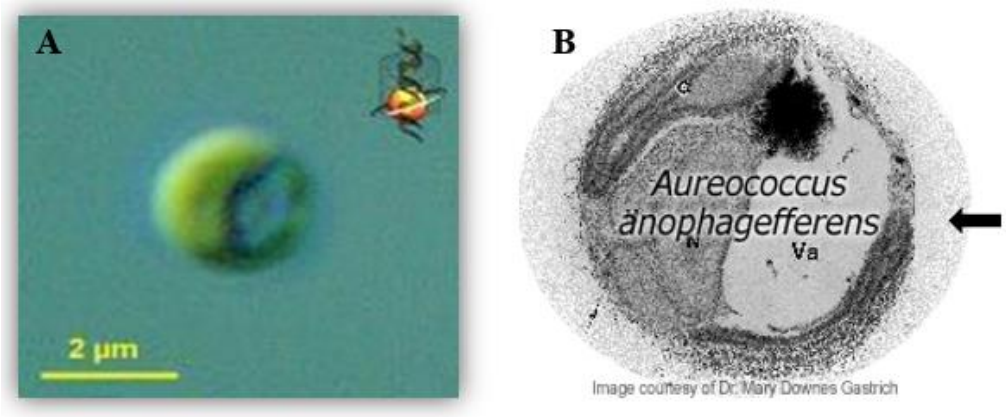


Figure 1: A) Differential interference microscopy image of *A. anophagefferens* (Image copyright: Bob Andersen and D. J. Patterson). B) Transmission electron microscopy image of *A. anophagefferens* showing the thick extracellular polymeric substance (EPS) indicated by the arrow.

Aureoumbra lagunensis

Aureoumbra lagunensis is a 4-5 µm, spherical, non-motile pelagophyte (DeYoe et al. 1997; Fig 2). Cells of *A. lagunensis* also possess an extracellular polymeric substance, a thick mucous layer (EPS) (DeYoe et al. 1997). Although a toxic strain of *A. lagunensis* has not been identified to date, the physical nature of the EPS may interfere with movement and feeding of ciliated grazers by coating and clogging feeding apparatuses, as was found with *A. anophagefferens* (Liu and Buskey 2000a).

Blooms of *A. lagunensis* occur when densities reach or exceed 100,000 cells ml^{-1}. In Mosquito Lagoon, blooms of *A. lagunensis* reached densities exceeding 3×10^6 cells ml^{-1} during the summer of 2012 (Phlips et al. 2015). Shading caused by dense blooms has led to the loss of seagrasses (*Halodule wrightii*) in both the Laguna Madre, Texas, and the Indian River Lagoon, Florida (Onuf 1996, Gobler et al. 2013). Decreases in abundance of benthic invertebrates such as the dwarf surf clam *Mulinea lateralis*, the hard clam *Mercenaria mercenaria*, and the eastern oyster *Crassostrea virginica* have been associated with blooms of *A. lagunensis* (Montagna et al. 1993, Buskey et al. 1997, Ward et al. 2000, Gobler et al. 2013). *Aureoumbra lagunensis* has also been shown to reduce feeding of planktonic grazers such as ciliate *Aspidisca* sp. (Liu and Buskey 2000a). Unfortunately, unlike *A. anophagefferens*, there have been limited controlled experimental studies to determine the effects of *A. lagunensis* on grazers and other organisms. Gobler et al. (2013) have shown that filtration rates of *M. mercenaria* and the eastern oyster *Crassostrea virginica* were significantly lower under both low (4×10^5 cells mL^{-1}) and high (1×10^6 cells mL^{-1}) concentrations of *A. lagunensis* compared to *Isochrysis galbana*, a microalga cultured as a food source in the bivalve aquaculture industry.

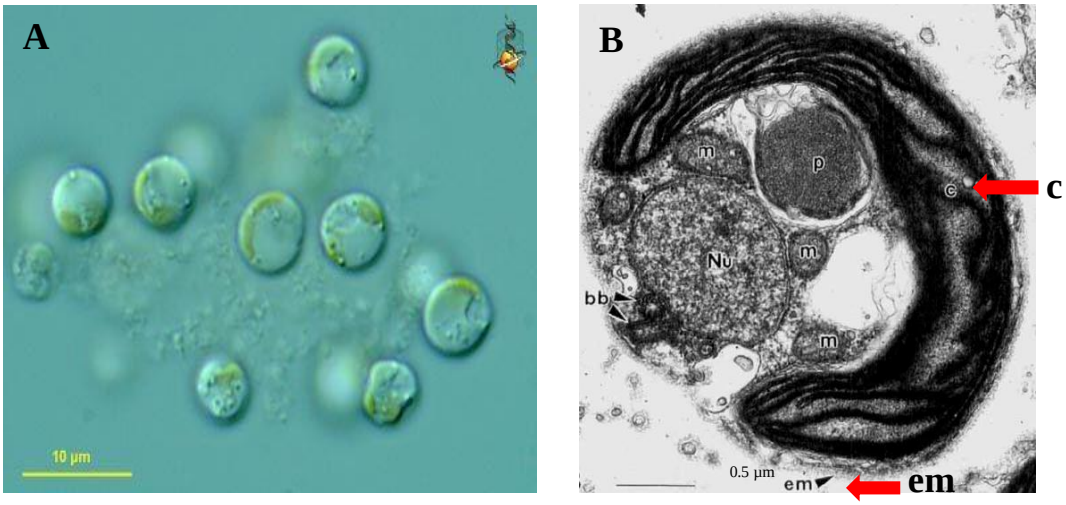


Figure 2: A) Differential interference microscopy image of *Aureoumbra lagunensis* (Photo credit: David Patterson and Bob Andersen). B) Transmission electron microscopy image of *A. lagunensis* showing cup-shaped chloroplast (c) and extracellular matrix (em) (Photo credit: DeYoe et al. 1997).

Crassostrea virginica

Oysters are ecologically and economically important, providing a variety of services including erosion protection, water filtration, habitat, and food (Newell and Langdon 1996, Cohen et al. 1999, Gutirrez et al. 2003, Grizzle et al. 2008). The eastern oyster *Crassostrea virginica* (phylum Mollusca, class Bivalvia, order Ostreoida, family Ostreidae) is particularly vulnerable in that this species has already suffered significant losses in its native ranges due to overharvesting, disease, and habitat degradation in the last century (Beck et al. 2011, Wilberg et al. 2011). Their global distribution is influenced by environmental factors including temperature, salinity, food availability, light, and pH (Shumway 1996). Adult oysters are euryhaline and eurythermal, occurring from the Gulf of St. Lawrence to the Gulf of Mexico (Reeb and Avise 1990). They are commonly found in salinities ranging from 0 to 42.5 PSU and temperatures ranging from -2℃ to 36℃ (Ingle and Dawson Jr 1950, Galstoff 1964).

Crassostrea virginica has a complex life history composed of a free-swimming larval stage and a sedentary juvenile through adult stage (Newell and Langdon 1996; Fig 3). The lifecycle of this benthic invertebrate filter-feeder begins when external fertilization produces a planktonic trochophore larva (Kennedy 1996). Two subsequent larval stages (swimming straight-hinge veliger and swimming late veliger) lead to the final larval stage called the pediveliger, which possesses a well-developed foot for crawling and cementing on hard substrates (Kennedy 1996). Settlement is followed by metamorphosis, an irreversible morphogenic step that begins with cementation to the substratum and absorption of larval structures (Bonar et al. 1990, Kennedy 1996). The process of metamorphosis, the reorganization of tissues and development of adult feeding structures, takes up to 6 days during which larvae rely mostly on lipid reserves for energy (Hickmann and Gruffydd 1971). Baker and Mann (1994) provide evidence that oysters have the ability to feed during all life stages including settlement and metamorphosis, although the mechanism of particle capture was unclear during metamorphosis.

Many external cues contribute to settlement and the induction of metamorphosis in oyster larvae. For example, preferred settlement surfaces are horizontal, rough, and covered by microbial films (Beiras and Widdows 1995). Abundance of food, illumination, high larval densities, and chemical cues also facilitate settlement (Cole and Jones 1939, Burke 1983, Bonar et al. 1990, Beiras and Widdows 1995). Furthermore, metamorphosis of oyster larvae without attachment to substrate can be induced in the presence of appropriate neuroactive compounds (Bonar et al. 1990, Beiras and Widdows 1995). These behavioral and developmental processes ultimately lead to the juvenile stage (Kennedy 1996).

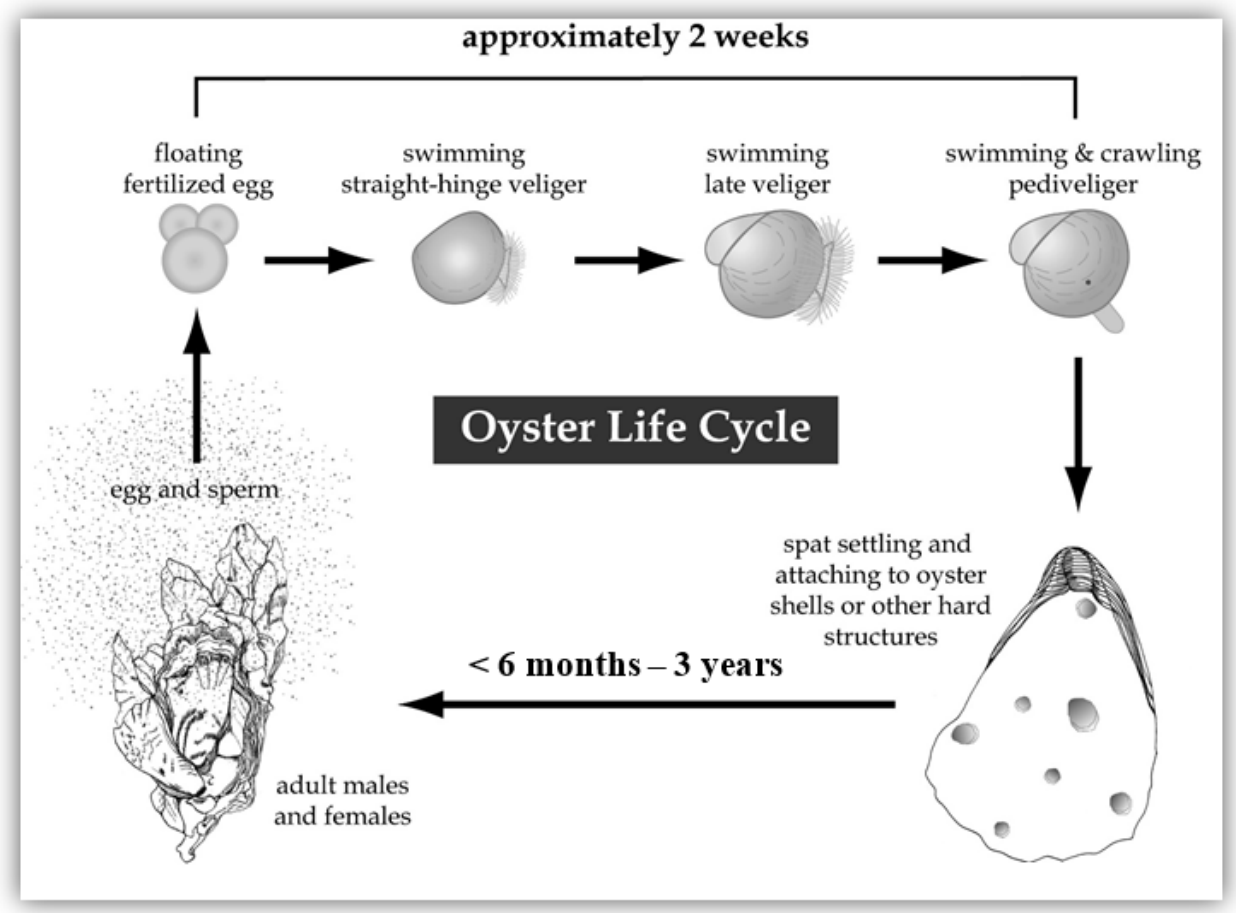


Figure 3: Image shows life cycle of *Crassostrea virginica* from release of gametes into the water column and fertilization through maturation to the adult stage. Modified from Karen R. Swanson/Consortium for Oceanic Science Exploration and Engagement, SouthEast/National Science Foundation.

Mosquito Lagoon

Florida's Indian River Lagoon (IRL) is a subtropical estuary system that is considered one of the most biologically diverse in the United States (Provancha et al. 1992). The IRL is comprised of three shallow-water estuaries: Mosquito Lagoon, Indian River, and Banana River. The northernmost estuary, Mosquito Lagoon, has an average depth of 1.7 m (Grizzle 1990, Walters et al. 2001). Currents are primarily wind-driven with an average mainstream flow rate of

5 cm s^{-1} (Boudreaux et al. 2009). Flushing rates in the northern IRL are very low and can exceed 1 year (Smith 1993). Due to drought, salinities became hypersaline throughout Mosquito Lagoon in 2011-2013, with values exceeding 35 PSU (Gobler et al. 2013). Blooms of *A. lagunensis* occurred during the summers of 2012 and 2013 (Phlips et al. 2015, Fig 4). Monthly monitoring has shown that *A. lagunensis* persists in Mosquito Lagoon at naturally low cell densities when not in bloom (Phlips and Badylak 2015).

The northern IRL is home to extensive intertidal reefs of *C. virginica* (Garvis et al. 2015). Ongoing monitoring of restored oyster reefs in the lagoon has shown that shell lengths after initial six month deployment were significantly smaller for the brown tide bloom years of 2012 and 2013 than previous years (Gobler et al. 2013; Fig 5). The potential of *A. lagunensis* to affect the most vulnerable life stages of eastern oysters, the larval and juvenile stages, is of great concern, but has not been studied extensively.

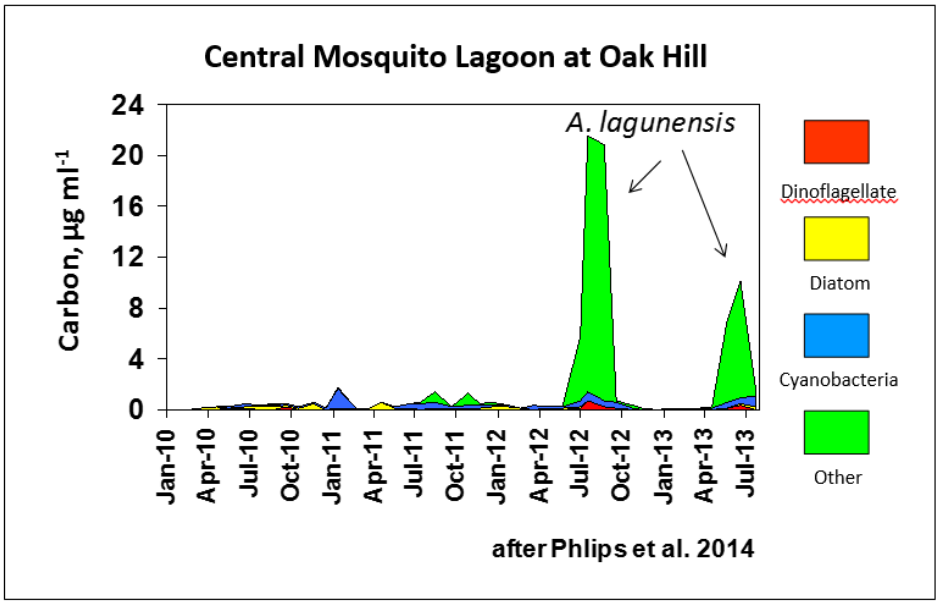


Figure 4: Presence of phytoplankton in central Mosquito Lagoon, FL; measured in terms of carbon. Dense blooms of *Aureoumbra lagunensis* during the summers of 2012 and 2013 can be seen in the two largest peaks categorized as other.

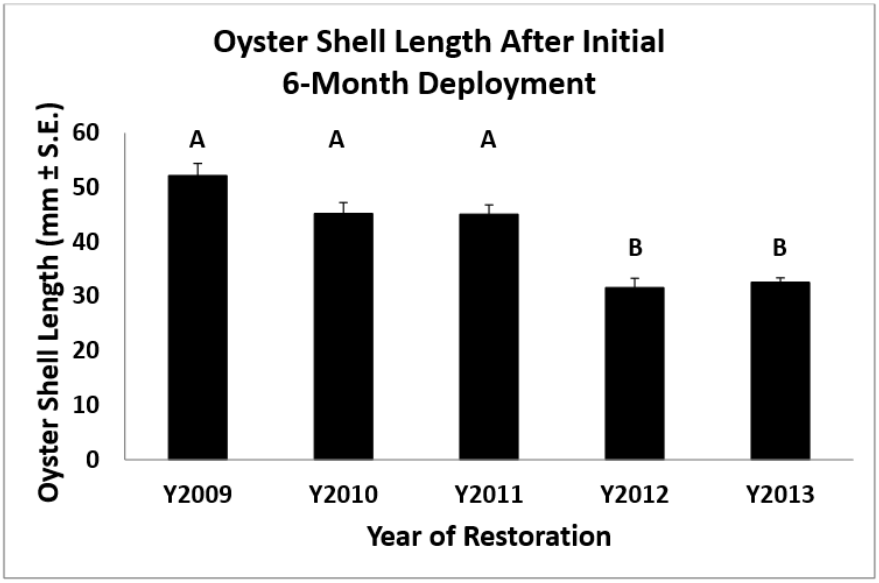


Figure 5: Oyster shell lengths following initial 6-month deployment of cultch (after Gobler et al. 2013 and L. Walters monitoring report). Means compared with ANOVA. Bars with different letters indicate significant differences at $p = 0.05$ when compared with Tukey's post hoc test. Oyster shell lengths for the brown tide bloom years of 2012 and 2013 were significantly lower than previous non-brown tide bloom years.

Research Questions

My research focused on the effects of *Aureoumbra lagunensis* and high salinities on the larval and juvenile life stages of *Crassostrea virginica*. My experimental studies aimed to uncouple any negative effects caused by *A. lagunensis* from high salinity. The specific questions tested are:

1) Is natural recruitment of *C. virginica* in Mosquito Lagoon, FL negatively correlated with the presence of *A. lagunensis* and high salinity?

2) Does *A. lagunensis*, high salinity, and their interaction affect settlement of *C. virginica*?

3) Does short-term exposure to *A. lagunensis*, high salinity, and their interaction affect survival and growth of juvenile *C. virginica*?

CHAPTER 2: RECRUITMENT OF *CRASSOTREA VIRGINICA*

Methods

This observational study evaluated the natural recruitment of juvenile eastern oysters (*Crassostrea virginica*) in Mosquito Lagoon, FL and how recruitment was affected by blooms of *Aureoumbra lagunensis*. Sampling occurred for a period of two years, beginning in May 2013 and ending in April 2015. The sampling unit consisted of a 0.25 m^2 mat made of aquaculture grade Vexar^{TM} mesh with 36 disarticulated (single) oyster shells attached via zip ties (Garvis et al. 2015). Oyster mats were deployed on the landward side of 10 successfully restored intertidal reefs of *Crassostrea virginica*, 1 mat per reef, covering a distance of approximately 2 Km (Table 1, Fig 6). Each month, deployed oyster mats were collected and replaced with new mats. Used mats were transported to the laboratory (under dry, non-climate controlled conditions) where oysters and barnacles (native ivory barnacle *Balanus eburneus* and the non-native purple-striped barnacle *Balanus amphitrite*) attached to disarticulated oyster shells were identified and counted with the aid of a dissecting microscope. Barnacles were included in the analysis as a covariate because they are known competitors of oysters and their recruitment is highly variable from shell to shell (Shumway 1996). Counts for barnacle species were combined because juveniles were too small to distinguish. Salinity data were obtained from St. Johns River Water Management District (SJRWMD). Brown tide data was collected from nearby Oak Hill in central Mosquito Lagoon (Phlips and Badylak 2015, Fig 7).

Recruitment of *Crassostrea virginica* in Mosquito Lagoon was analyzed using generalized linear mixed-effects models. Mixed-effects models were evaluated so that the potential random effect of site could be accounted for throughout the lagoon. The candidate

model set that includes only fixed effects is shown in Table 2. Predictor variables included *A. lagunensis* (cells mL^{-1}), salinity (PSU), and barnacle competitors (#/0.25m^2). Additionally, barnacle recruitment in Mosquito Lagoon, FL was modeled similarly as oyster recruitment, as a function of *A. lagunensis* and salinity. Cold-water months of December-March were omitted from the data analysis because settlement of oysters and barnacles was markedly depressed due to colder temperatures (Table 3). Omitting these months ensured that results would not be skewed by temperature. All analyses were conducted in *R* (R Core Team 2013). Data were modeled using the *R* function lm {stats} for linear models and the lmer function for linear mixed-effects models found in package lme4 (Bates et al. 2012). Model comparisons were made using Akaike Information Criterion (AIC).

Table 1: Geographic coordinate locations of restored oyster reefs within Mosquito Lagoon, FL chosen to monitor oyster recruitment.

	Oyster Reef	**Location**
1	Athena	28°56'22.38"N, 80°50'44.82"W
2	Diversity	28°56'39.07"N, 80°51'21.42"W
3	Everest	28°56'30.38"N, 80°51'54.21"W
4	Horizon	28°56'43.38"N, 80°52'20.92"W
5	Knightro	28°56'23.45"N, 80°51'35.88"W
6	Milk Chocolate	28°56'28.23"N, 80°52'10.54"W
7	Needlefish	28°56'25.61"N, 80°51'45.86"W
8	Picnic	28°56'33.56"N, 80°51'28.08"W
9	Poseidon	28°56'15.86"N, 80°50'48.19"W
10	Quiver	28°56'31.29"N, 80°51'90.64"W

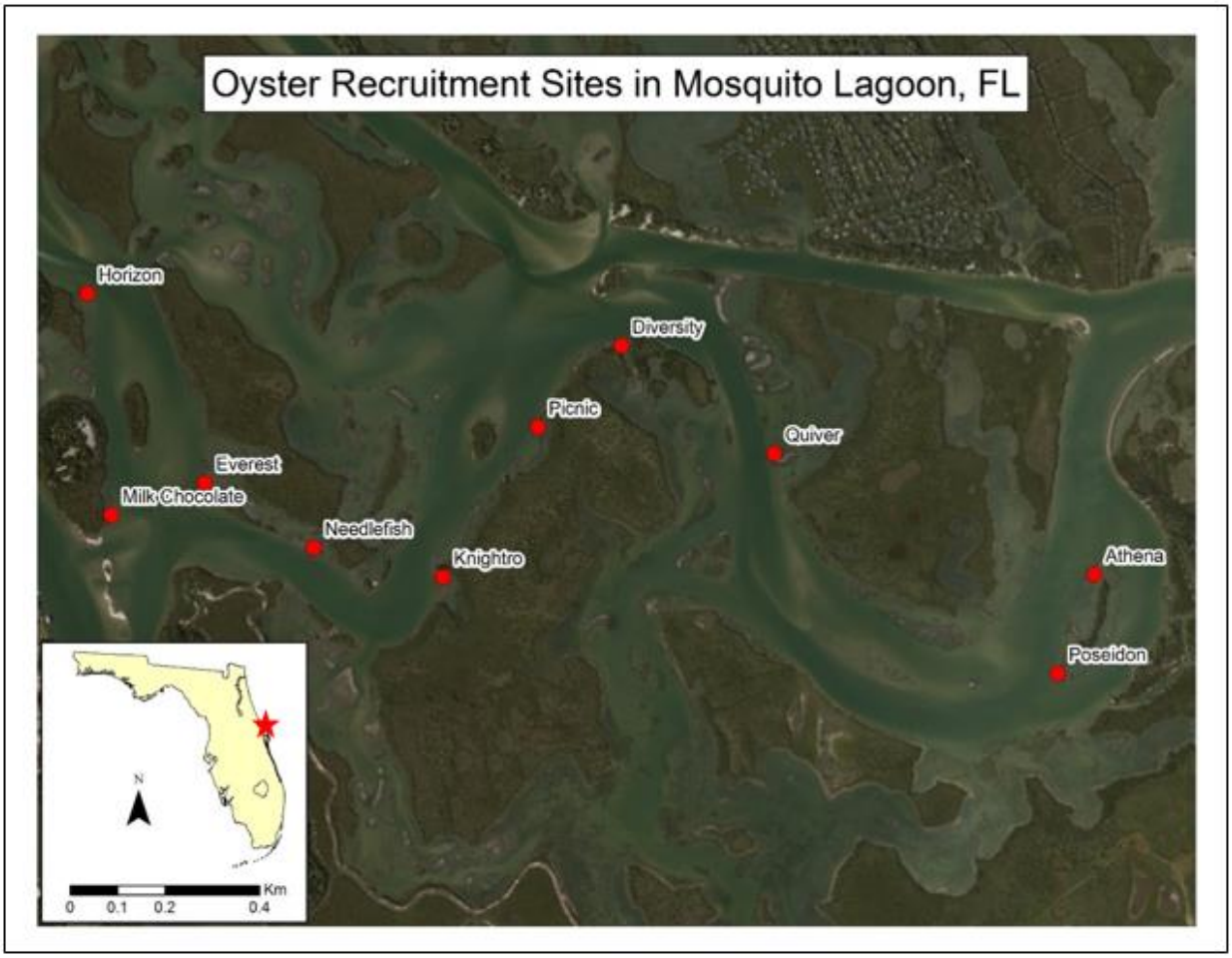


Figure 6: Oyster recruitment study sites in northern Mosquito Lagoon, FL.

Figure 7: Map depicts locations of oyster reefs in northern Mosquito Lagoon with area monitored for oyster recruitment indicated by polygon with diagonal stripes. Map also shows the location of monthly water sample testing for *Aureoumbra lagunensis* at Riverside Park in Oak Hill, Florida.

Table 2: Candidate fixed model set predicting juvenile oyster (spat) recruitment as a function of salinity (PSU), barnacle density and *Aureoumbra lagunensis* density.

Model	Response	Predictor
1	Recruitment	~ null
2	Recruitment	~ Salinity
3	Recruitment	~ Competitors
4	Recruitment	~ *A. lagunensis*
5	Recruitment	~ Salinity + Competitors
6	Recruitment	~ Salinity + *A. lagunensis*
7	Recruitment	~ Competitors + *A. lagunensis*
8	Recruitment	~ Salinity + Competitors + *A. lagunensis*
9	Recruitment	~ Salinity : Competitors
10	Recruitment	~ Salinity : *A. lagunensis*
11	Recruitment	~ Competitors : *A. lagunensis*
12	Recruitment	~ Salinity : Competitors : *A. lagunensis*

Results

Peak recruitment for both *Crassostrea virginica* and barnacles co-occur during warm water months of April through November (Table 3). Little to no recruitment occurred for both oysters and barnacles during winter when water temperatures dropped (Table 3). Recruitment of *C. virginica* in Mosquito Lagoon, FL was best described by the additive effects of *Aureoumbra lagunensis* and barnacle competitors. Models with random effects of intercept by site were less plausible (AIC) and were not included (Tables 18 and 19 in Appendix A). Model selection identified that the two most plausible models were within 2 AIC values (models 7 and 8) indicating no strong differences in evidence between them (Table 4). However, further inspection of model 8 indicated that the coefficient for the effects of salinity was not significantly different from zero (Table 5). Thus, model 7 was chosen as the optimal model (Table 6). *Aureoumbra lagunensis* was negatively associated with recruitment of eastern oysters, while presence of barnacles was positively associated with salinity (Table 6, Fig 8).

Barnacle recruitment in Mosquito Lagoon, FL was positively associated with salinity. Models with random effects of intercept by site added additional information and were included in the mixed effects models (Tables 20, 21 in Appendix A). The top two models (2 and 4) were within 2 AIC values indicating no strong evidence for different plausibility (Table 7). However, further inspection of model 4 indicated that the coefficient of *A. lagunensis* was not significantly different from zero (Table 8). Thus, model 2 was chosen as the optimal model (Table 9). Salinity had a positive effect on the recruitment of barnacles. Diagnostic plots, AIC tables, parameter estimates, residual plots, and *R* code for both oyster and barnacle recruitment are included in Appendix A.

Table 3: Mean monthly values for *Crassostrea virginica* and barnacle (*Balanus eburneus* and *Balanus amphitrite*) recruits, *Aureoumbra lagunensis* and salinity (PSU) for Mosquito Lagoon, FL.

Date	*C. virginica* (# / 0.25m^2)	Barnacles (# / 0.25m^2)	*A. lagunensis* (cells/mL^1)	Salinity (PSU)	Temperature (°C)
May-13	16	139	974810	33	27
Jun-13	18	435	1358094	38	28
Jul-13	40	2100	51234	34	28
Aug-13	41	612	24030	35	30
Sep-13	65	557	2520	34	29
Oct-13	98	367	0	32	26
Nov-13	57	209	43	35	23
Dec-13	5	50	78	34	14
Jan-14	0	0	91	34	14
Feb-14	0	1	181	31	16
Mar-14	0	1	151	31	23
Apr-14	9	19	165	33	20
May-14	149	126	706	33	26
Jun-14	51	1549	176	37	29
Jul-14	94	589	0	31	30
Aug-14	404	593	60	35	28
Sep-14	118	599	76	34	30
Oct-14	37	565	0	33	26
Nov-14	13	40	200	32	22
Dec-14	2	0	67	29	16
Jan-15	0	0	0	31	14
Feb-15	0	0	50	31	17
Mar-15	6	1	151	32	24
Apr-15	368	477	423	32	27
May-15	NA	NA	181	35	28
Jun-15	NA	NA	7857	37	32

Table 4: AIC table of top 5 models predicting log transformed juvenile oyster (spat) density per 0.25 m^2 as a function of salinity (PSU), log transformed barnacle density (logBar) and log transformed *Aureoumbra lagunensis* density (logAL).

#	Model	AIC	⊿AIC	AICWt
7	logSpat~logBar + logAL	491.9	0.0	0.690
8	logSpat~PSU + logBar + logAL	493.5	1.6	0.310
5	logSpat~PSU + logBar	510.3	18.4	<0.001
4	logSpat~logBar	517.2	25.3	<0.001
10	logSpat~PSU : logAL	521.9	30.0	<0.001

Table 5: Parameter estimates for model 8 predicting log transformed juvenile oyster (spat) density per 0.25 m^2 as a function of salinity (PSU), log transformed barnacle density (logBar) and log transformed *Aureoumbra lagunensis* density (logAL). The parameter estimate for salinity (PSU) was not found to be significant.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	4.086	2.069	1.975	0.050
logBar	0.343	0.057	5.968	<0.001
logAL	-0.115	0.026	-4.414	<0.001
PSU	*-0.042*	*0.065*	*-0.651*	*0.516*

Table 6: Parameter estimates for model 7 predicting log transformed juvenile oyster (spat) density per 0.25 m^2 as a function of log transformed barnacle density (logBar) and log transformed *Aureoumbra lagunensis* density (logAL).

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2.76	0.35	7.89	<0.000
logBar	0.34	0.06	5.96	<0.000
logAL	-0.12	0.02	-5.40	<0.000

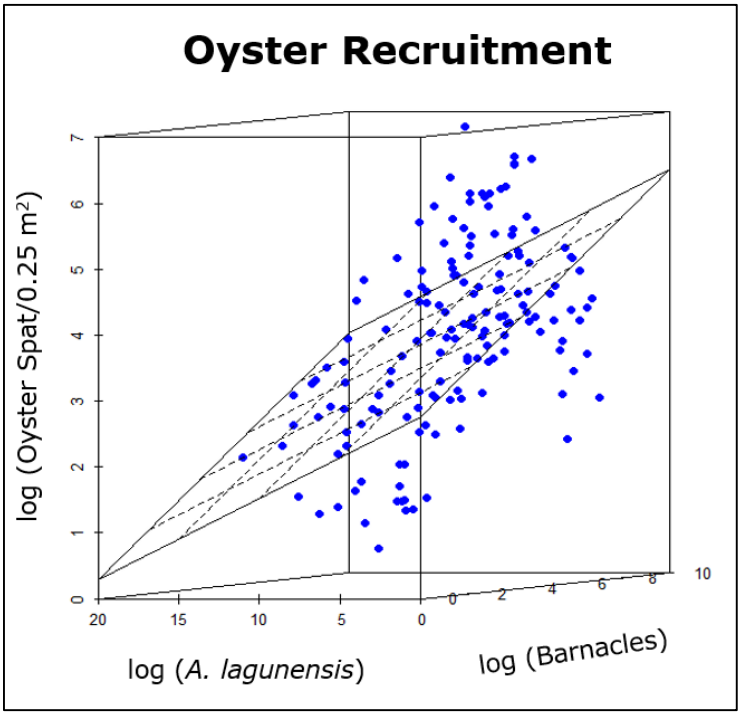


Figure 8: Recruitment of *Crassostrea virginica* as a function of *Aureoumbra lagunensis* and barnacle competitors.

Table 7: Model selection of optimal mixed model configurations for barnacle recruitment determined using AIC.

#	Model	AIC	ΔAIC	AICWt
2	logBar ~ PSU + (1|Site)	594.0	0.0	0.525
4	logBar ~ PSU + logAL + (1|Site)	595.3	1.3	0.276
1	logBar ~ 1 + (1|Site)	597.2	3.2	0.109
5	logBar ~ PSU * logAL + (1|Site)	598.8	4.8	0.047
3	logBar ~ logAL + (1|Site)	599.1	5.0	0.042

Table 8: Parameter estimates of fixed effects for model 4 predicting log transformed barnacle density per 0.25 m^2 as a function of salinity (PSU) and log transformed *Aureoumbra lagunensis* density (logAL). The parameter estimate for *A. lagunensis* was not found to be significant.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-1.243	2.746	-0.453	0.652
PSU	0.205	0.085	2.421	0.017
logAL	*-0.029*	*0.035*	*-0.847*	*0.398*

Table 9: Parameter estimates of fixed effects for models 2 predicting log transformed barnacle density per 0.25 m^2 as a function of salinity (PSU).

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.282	2.506	-0.112	0.911
PSU	0.171	0.074	2.291	0.023

Discussion

Although many different factors influence recruitment of eastern oysters in subtropical estuaries, the predictor variables chosen were intended to isolate novel pressures experienced by *Crassostrea virginica* in Mosquito Lagoon, FL. These pressures include higher than average salinities (>35 PSU) and the presence of *Aureoumbra lagunensis*. Additionally, the presence of barnacles was also included as a covariate in the model selection process because these competitors co-occur in Mosquito Lagoon during peak months of oyster recruitment in variable densities from reef to reef (Boudreaux et al. 2009).

Although Mosquito Lagoon experienced higher than average salinities during the brown tide bloom years of 2012 and 2013, salinity was not expected to have a significant impact on the recruitment of *C. virginica*. Historically, salinities in Mosquito Lagoon averaged near 30 PSU with summer values averaging 35 PSU (Gobler et al. 2013, Phlips et al. 2002). These salinities

are facilitated by low flushing rates and limited freshwater inputs (Phlips et al. 2002). Additionally, the northern portion of Mosquito Lagoon, where dense areas of oyster beds are found, is also located near the Ponce De Leon Inlet which allows tides to maintain fairly constant salinities similar to the open ocean (35 PSU) (Garvis et al. 2015, Phlips et al. 2002). Prior to and during the 2012 and 2013 blooms of *A. lagunensis*, low rainfall caused a rise in Mosquito Lagoon salinities with values exceeding 40 PSU in southern parts of the lagoon (Gobler et al. 2013). Oyster reefs in the northern portion of Mosquito Lagoon experience more stable salinities than southern parts of the lagoon where seagrass is dominant (Garvis et al. 2015). Location of the reef across the intertidal zones has been shown to affect oyster settlement, with abundant recruitment at areas with high salinities as is the case in Mosquito Lagoon (Menzel 1954). Oysters have wide salinity tolerances and can withstand large salinity fluctuations with the possibility of different salinity optima for different populations (Shumway 1996). *Crassostrea virginica* grows and survives well at high salinities ranging from 32-42 PSU (Breuer 1962, Shumway 1996). Thus, populations of *C. virginica* in Mosquito Lagoon are likely well adapted to higher salinities.

There was no evidence that barnacle densities have a negative impact on recruitment of *Crassostrea virginica*. Barnacles are known competitors of oysters for space and food as well as known predators of larval oysters (Shumway 1996, Osman et al. 1989). Boudreaux et al. (2009) showed that settlement, growth and survival of *C. virginica* was significantly reduced by the presence of *Balanus eburneus* and *Balanus amphitrite*. Alternatively, presence of the barnacle *Balanus improvisus* has been shown to facilitate settlement of *C. virginica* (Barnes et al. 2010). Despite known negative impacts on oysters, coincident setting of barnacles and oysters occurs during warm water months in Mosquito Lagoon. The nature of this observational study does not

imply any causation between predictors and oyster recruitment, but simply documents relationships. In order to compare these results with previous causational studies, a controlled experimental study would be required to determine if presence of barnacles is responsible for oyster recruitment, which was outside the realm of the experimental design utilized in the present study.

Aureoumbra lagunensis was negatively associated with recruitment of *Crassostrea virginica* in Mosquito Lagoon, although recruitment continued during blooms of *A. lagunensis*. Gobler et al. (2013) has shown that clearance rates of *C. virginica* are significantly lower when exposed to bloom densities of *A. lagunensis* as compared to a control of *Isochrysis galbana* (2013). Similarly, the brown tide species *Aureococcus anophagefferens* has been shown to significantly decrease clearance rates of suspension feeders such as the hard clam *Mercenaria mercenaria* (Harke et al. 2011).

Recruitment of *Crassostrea virginica* in Mosquito Lagoon was negatively associated with *Aureoumbra lagunensis*. There was minor evidence that decreased oyster recruitment during blooms of brown tide was associated with high salinities (Table 5). In order to provide causational data for such conclusions, an experimental study with oyster larvae was conducted examine separately the effects of salinity and *A. lagunensis*; these results are reported in the following chapter.

CHAPTER 3: SETTLEMENT OF *CRASSOSTREA VIRGINICA*

Methods

Algal culture

Aureoumbra lagunensis (CCMP 1510) was obtained from C. J. Gobler, Stony Brook University, Montauk, NY and *Isochrysis galbana* (Tahitian strain, 5-6 µm, spherical, flagellated) was obtained from J. Scarpa, Harbor Branch Oceanographic Institute at Florida Atlantic University, Fort Pierce, FL. *Isochrysis galbana* was used as a control species as it is known to support high growth and survival in *Crassostrea virginica* (Talmage and Gobler 2009). Algae were batch-cultured in aerated, 20 L, chemically sterilized, polycarbonate carboys (Parke 1949, DeYoe et al. 1997). Growth media was prepared using filtered, natural seawater with the addition of Guillard's f/2 for *I. galbana* and modified f/2 (addition of NH_4Cl) for *A. lagunensis* (Guillard and Ryther 1962, Deyoe and Suttle 1994). Algal cultures were grown at 35 PSU, 20-28°C and a 14/10 h light/dark illumination cycle using cool white fluorescent lamps (irradiance ≈ 150 µE m^{-2} s^{-1}).

Experimental Design

The effects of *Aureoumbra lagunensis* on settlement of *Crassostrea virginica* under moderate (25 PSU) and high (40 PSU) salinity conditions was evaluated with a regression experimental design. The salinities chosen represent normal conditions for many estuaries (25 PSU) as well as hypersaline conditions (40 PSU) associated with brown tides in Mosquito Lagoon (Phlips et al. 2014). Trials with no algae present served as negative controls, while

Isochrysis galbana acted as a positive control. Bloom thresholds for *A. lagunensis* are approximately 1×10^5 cells mL^{-1} with maximum *A. lagunensis* concentrations reaching 1,358,094 cells mL^{-1} in Mosquito Lagoon in June 2013 (Phlips and Badylak 2015). Algal concentrations chosen represent a wide range of possible natural conditions from non-bloom ($<1\times10^5$ cells mL^{-1}) to dense bloom densities (1×10^6 cells mL^{-1}), with emphasis on lower concentrations. This was accomplished by diluting algal cultures to different concentrations for each replicate, which also provided uniform coverage across the x-axis. Treatments are summarized in Table 10.

Settlement experiments occurred within replicate, recirculating, raceway flumes produced by Fish Tanks Direct (60.96 cm wide, 121.92 cm long with two semicircular ends modified from Tamburri et al. (1996; Fig 9). The settlement zone of the flow tank measured 25.4 cm wide by 55 cm long. A flow rate of 5 cm s^{-1}, representative of Mosquito Lagoon, was generated using a motor-driven paddle wheel (Boudreaux 2009). Water for treatments was prepared using Instant Ocean® seawater and added to flumes at a volume of 80 L. Oyster shell settlement substrate was allowed to develop a microbial biofilm via exposure to filtered Mosquito Lagoon seawater for approximately 1 week (Fitt et al. 1990). Ten disarticulated oyster shells were placed into the settlement zone of each tank with alternating orientation (out or inner shell facing up) following the addition of seawater. Next, algal treatments were added to specified densities. Competent pediveliger *C. virginica* larvae were added last. Replicates 1, 2, and 3 had larval densities of 0.31, 0.42, and 0.71 larvae mL^{-1}, respectively, which was based on larval availability. Larvae were obtained from different hatcheries via overnight mail: Florida Research Aquaculture, Inc. in Stuart, FL for replicate 1; Sea Grant Oyster Research Laboratory in Grand Isle, LA for replicate 2; and from Ward Oyster Co. in Gloucester, VA for replicate 3.

Settlement within flumes was allowed for 2 hours, after which, all shells were immediately removed and allowed to dry. Settled pediveligers attached to shells were counted with the aid of a dissecting microscope. Next, shells were photographed on a copy stand and surface area for each shell was calculated using ImageJ (Abràmoff et al. 2004). Oyster settlement for each shell was standardized by dividing number of settled larvae by available shell surface area. Then average settlement was calculated for all 10 shells in a tank.

Data were analyzed using linear mixed-effects models to assess the relative importance of salinity, algal species, algal concentration, tank location within laboratory, and replicate block (batch of larvae) to explain variation in oyster settlement. Mixed-effects models were used to estimate random variation in settlement due to location of tanks within the laboratory. Additionally, data were blocked by batch to account for expected differences in larvae acquired from different hatcheries. Batch was also treated as a random effect. All analyses were conducted using *R* (R Core Team 2013). Settlement was modeled using the *R* function lm {stats} for linear models and the lmer function for linear mixed-effects models found in package lme4 (Bates et al. 2012). Model comparisons were made using Akaike Information Criterion (AIC).

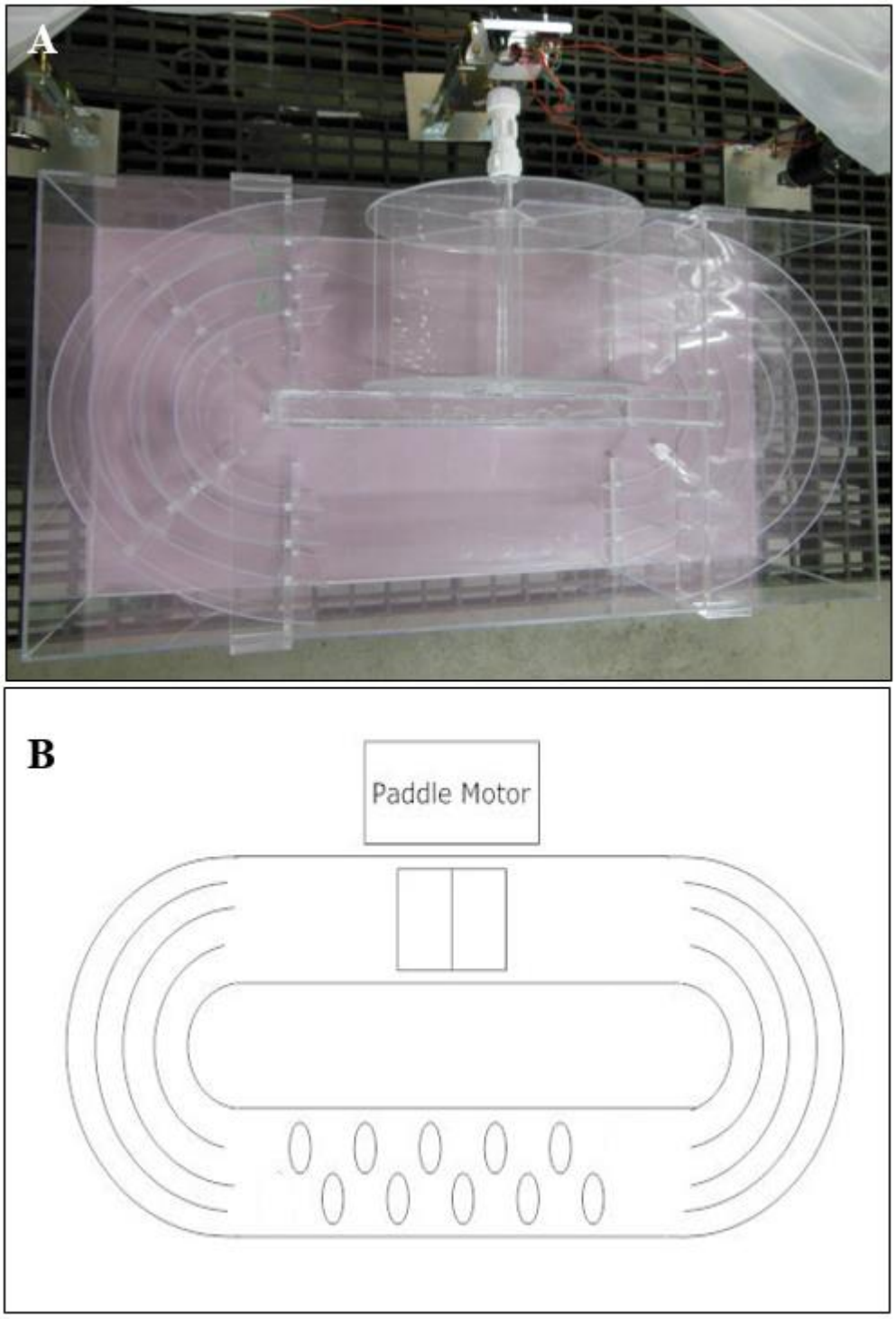


Figure 9: A) Recirculating raceway flume with attached motor. B) Simplified representation of raceway flume showing placement of oyster shells as represented by ovals.

Table 10: Settlement experimental treatments for each batch of purchased larvae

Trials	Salinity (PSU)	Algae	Concentration (cells/mL^{-1})
1	25	None	0
2	25	*Isochrysis galbana*	0-100
3	25	*Isochrysis galbana*	100-1,000
4	25	*Isochrysis galbana*	1,000-10,000
5	25	*Isochrysis galbana*	10,000-100,000
6	25	*Isochrysis galbana*	100,000-1,000,000
7	25	*Aureoumbra lagunensis*	0-100
8	25	*Aureoumbra lagunensis*	100-1,000
9	25	*Aureoumbra lagunensis*	1,000-10,000
10	25	*Aureoumbra lagunensis*	10,000-100,000
11	25	*Aureoumbra lagunensis*	100,000-1,000,000
12	40	None	0
13	40	*Isochrysis galbana*	0-100
14	40	*Isochrysis galbana*	100-1,000
15	40	*Isochrysis galbana*	1,000-10,000
16	40	*Isochrysis galbana*	10,000-100,000
17	40	*Isochrysis galbana*	100,000-1,000,000
18	40	*Aureoumbra lagunensis*	0-100
19	40	*Aureoumbra lagunensis*	100-1,000
20	40	*Aureoumbra lagunensis*	1,000-10,000
21	40	*Aureoumbra lagunensis*	10,000-100,000
22	40	*Aureoumbra lagunensis*	100,000-1,000,000

Results

There was no evidence of significant random effects for tank location within the laboratory on settlement (Tables 36 and 37 in Appendix B). There was no evidence that algal concentration influenced oyster settlement when all batches were included in the analysis (Table 11). The model that best described oyster settlement for all three replicates included the additive effects of salinity and replicate block (Table 11). Salinity had negative effects on settlement of *C. virginica* (Table 12, Fig 10). Batch one and three had almost no settlement in any condition, while settlement of batch two varied depending on the treatments. In order to account for the differences among batches, batch two was modeled separately. Oyster settlement for batch two was best described by the interaction effects of salinity, algal species, and algal concentration (Tables 13, 14). AIC table, residual plot, and *R* code are included in Appendix B.

Table 11: AIC table of top 5 models predicting oyster settlement (logPV) as a function of salinity (PSU), algal species, algal concentration (logCELL), and replicate block (REP).

#	Model	AIC	ΔAIC	AICWt
1	logPV ~ PSU + (1|REP)	-26.7	0.0	0.496
2	logPV ~ Algae + (1|REP)	-26.0	0.7	0.351
3	logPV ~ logCELL + (1|REP)	-23.9	2.8	0.122
4	logPV ~ PSU + Algae + (1|REP)	-20.0	6.7	0.018
5	logPV ~ logCELL + Algae + (1|REP)	-18.0	8.7	0.006

Table 12: Parameter estimates for fixed effects predicting settlement of *Crassostrea virginica* as a function of salinity (PSU).

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.313	0.100	3.123	0.005
PSU	-0.007	0.003	-2.506	0.019

Table 13: AIC table of top 5 models predicting oyster settlement (logPV) for replicate 2 as a function of salinity (PSU), algal species, and algal concentration (logCELL).

#	Model	AIC	ΔAIC	AICWt
1	logPV~ logCELL*PSU*Algae	-31.4	0.0	1
2	logPV ~ logCELL*PSU	5.1	36.6	<0.001
3	logPV ~PSU	5.9	37.3	<0.001
4	logPV ~ logCELL+PSU	6.0	37.5	<0.001
5	logPV ~ Algae*PSU	6.5	37.9	<0.001

Table 14: Parameter estimates predicting settlement of *Crassostrea virginica* as a function of salinity (PSU), algal species, and algal concentration (logCELL) for replicate 2. AL represents *Aureoumbra lagunensis*.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.703	0.120	-5.839	<0.001
logCELL	0.370	0.033	11.248	<0.001
PSU40	0.709	0.169	4.191	0.003
AL	1.256	0.167	7.520	<0.001
logCELL:PSU40	-0.369	0.046	-7.979	<0.001
logCELL:AL	-0.450	0.047	-9.632	<0.001
PSU40:AL	-1.138	0.235	-4.835	0.001
logCELL:PSU40:AL	0.428	0.066	6.515	<0.001

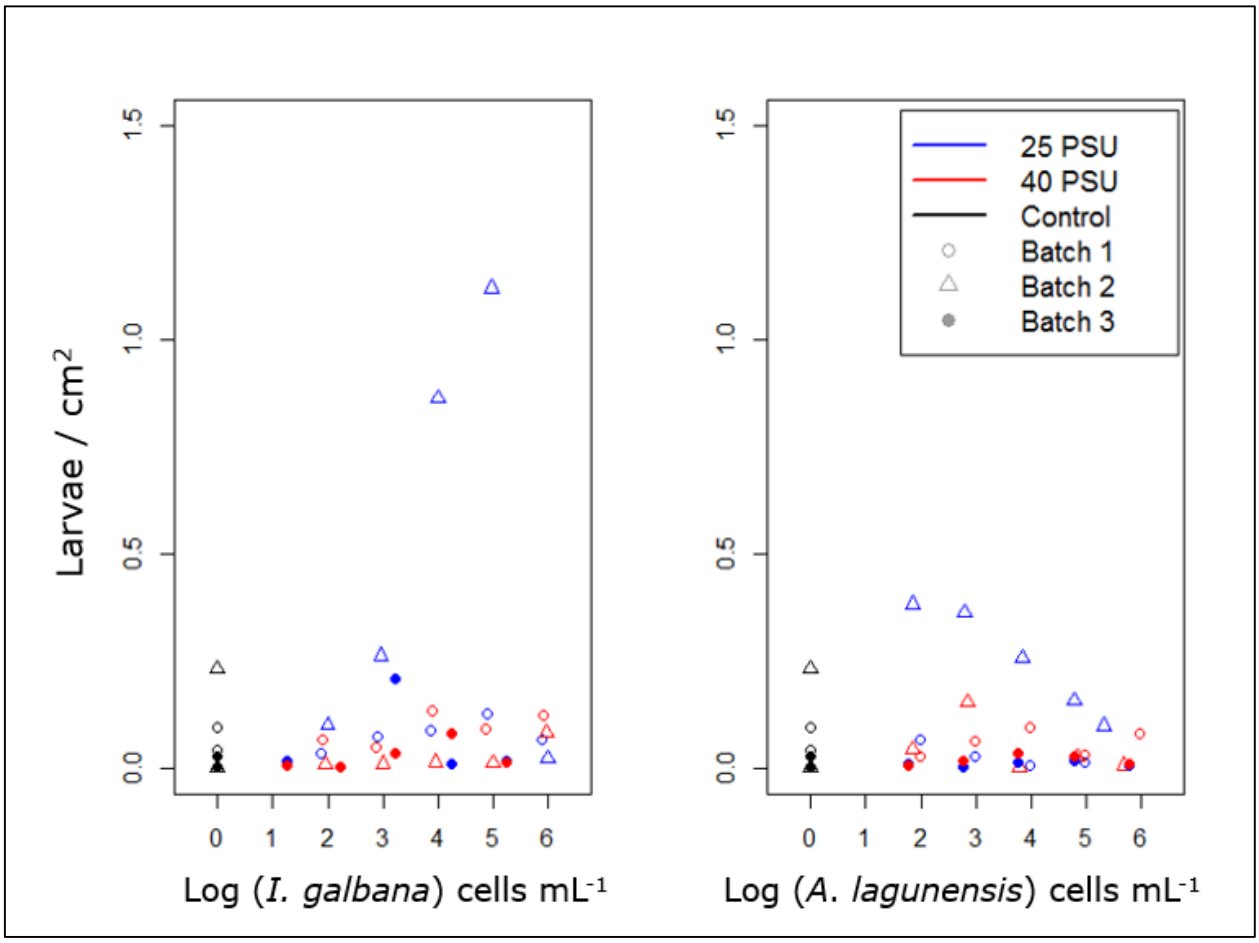


Figure 10: Scatterplot showing raw data for settlement of *Crassostrea virginica* as a function of salinity (PSU), algal species, and batch.

Discussion

Settlement of oyster larvae is influenced by many factors such as food quantity and quality, salinity, temperature, predators, and chemical cues (Kennedy 1996, Osman et al 1989, Thorson 1950, Bonar et al. 1990). We have limited evidence (from one of our batches) that the algal species *Aureoumbra lagunensis* influenced oyster settlement. Although there have been no studies on the effects of *A. lagunensis* on the larvae of *Crassostrea virginica*, studies have shown that *Aureococcus anophagefferens*, a similar species of brown tide forming microalga, does

significantly depress rates of survival, development, and growth of larval *C. virginica* by decreasing lipid content and delaying metamorphosis (Talmage and Gobler 2012). It is important to note that toxic strains of *A. anophagefferens* have been identified, while no toxin has been identified for *A. lagunensis* (Gainey and Shumway 1991, Bricelj et al. 2004).

We present evidence for the negative effects of high salinity on oyster settlement, although salinity effects were not consistent across replicates. Larvae for different replicates were obtained from different hatcheries based on availability. The culture conditions for the larval *C. virginica* were different for each hatchery with salinities ranging from ≈ 32 PSU, 13-25 PSU, to ≈ 22 PSU, for replicates 1-3 respectively. Prior rearing condition appeared to affect settlement and future studies should attempt to use larvae reared under similar conditions if possible.

The condition of adult oysters affects fecundity, egg quality, and ultimately, larval quantity and viability (Thompson et al. 1996). *Aureoumbra lagunensis* has the ability to negatively impact adult oysters by reducing filtration rates (Gobler et al. 2013). It can be hypothesized that reduced feeding rates may lead to reduction in nutrient uptake and the subsequent deterioration of oyster condition. Thus, *A. lagunensis* may also be affecting settlement and recruitment of *C. virginica* via top-down controls that reduce the quantity and viability of larvae.

CHAPTER 4: JUVENILE SURVIVAL & GROWTH

Methods

Survival and growth of juvenile *Crassostrea virginica* (spat) were monitored following exposure to *Aureoumbra lagunensis* and high salinities to determine the ability of eastern oysters to recover from these pressures. This study consisted of acute, laboratory exposure of juvenile eastern oysters to *A. lagunensis* and subsequent transplantation into the field. Juvenile oysters for the study were collected as described below in May 2015 from Mosquito Lagoon. Water quality monthly averages for May 2015 were: water temperature of 28 degrees Celsius, salinity 34 PSU, and 182 cells mL^{-1} background concentrations of *A. lagunensis*. Oyster mats (described in Chapter 2) were set out on reefs (Horizon, Milk Chocolate, Knightro, Quiver) and allowed to recruit oysters for a period of 2 weeks. Disarticulated oyster shells with attached spat were clipped off mats and transported to the University of Central Florida in aerated Mosquito Lagoon water in a climate-controlled vehicle. Shells with live, undamaged spat were cataloged via photographs. Images were analyzed using ImageJ to measure the initial sizes (mm) of individual spat (Abràmoff et al. 2004).

Aquaria to hold the spat with algal and salinity treatments were prepared as described in Table 15. Algal concentrations consisted of 1×10^5 cells mL^{-1} and 1×10^6 cells mL^{-1} for both *Isochrysis galbana* (control species) and *A. lagunensis*. Moderate (25 PSU) and high (40 PSU) salinities were also included as treatment variables. Water used in the study was prepared using Instant Ocean® salts. Three-liter, aerated aquaria were filled with 2 L of water, and 3 disarticulated oyster shells with attached spat were added to each tank. Each treatment exposure was replicated four times and tanks were arranged in an interspersed manner to minimize any

random effects of location within the laboratory. Spat were exposed to treatments for 1 week. Algal densities and salinities were monitored daily and adjusted as needed to maintain specified concentrations.

Aureoumbra lagunensis occurs naturally at low cell densities throughout Mosquito Lagoon (Phlips and Badylak 2015). To eliminate the possible introduction of a new strain of *A. lagunensis* to Mosquito Lagoon, a 100% identical isolate (CCMP 1510) was used (Gobler et al. 2013). Furthermore, shells were removed from aquaria following treatment exposure and rinsed with Instant Ocean® seawater to ensure excess *A. lagunensis* would not be transferred to the field. Spat on shells were re-cataloged via photographs and tagged with flagging tape to indicate treatment and replicate, and held in aerated 5 gallon buckets for 24 hours in filtered Mosquito Lagoon water to clear oyster spat gut contents (Laabir et al. 2007).

Shells with attached oysters were transported in 5-gallon buckets with aerated seawater to reefs in Mosquito Lagoon where they were attached to 0.25 m^2 mats made of aquaculture grade Vexar™ mesh via zip ties (Garvis et al. 2015). Each mat contained 1 shell from each treatment for a total of 10 shells, with an average of 2 spat per shell (Fig 11A). There were three replicate mats on each of four replicate reefs (Reefs: Horizon, Everest, Knightro, Quiver; Fig 11B). Shells were monitored weekly for a total of 4 weeks. Photographs were taken (with the use of a camera stand and ruler to maintain consistent scale) in the field each week by removing shells from mats and then reattaching them with zip ties. Spat growth was determined using ImageJ. Only spat that could be clearly followed throughout the experiment were included in the study.

Survival was analyzed using generalized linear mixed-effects models to assess the relative importance of salinity, algal species, algal concentration, time since deployment, and site. Mixed-effects models were chosen so that the random effect of site (oyster reefs at which

replicates were deployed) could be assessed (Casas et al. 2015). Survival (logistic regression) was modeled using the *R* function glmer found in package lme4 (Bates et al. 2012). Examination of the data revealed that there was complete survival for the zero control treatment at one of the replicate reefs. Thus, the zero control was dropped in this analysis since the logistic function is undefined for survival probabilities of 0 or 1 (Gotelli and Ellison 2004). All analyses were conducted using *R* (R Core Team 2013).

Oyster growth rates were analyzed using linear mixed-effects models to assess the relative importance of salinity, algal species, algal concentration, time since deployment, and site. The nature of the data did not allow for a full-rank matrix; thus, the zero control was excluded to allow *Isochrysis galbana* to be directly compared to *Aureoumbra lagunensis*. Rank deficiency occurred because of insufficient information in the data due to the experimental design. Since zero concentrations for *I. galbana* and *A. lagunensis* were identical treatments, the duplicate was not included in the experimental design; however, this resulted in a rank deficient matrix and the duplicate should have been included (Table 15). After determining that the random effect of oyster reef sites did not provide significant additional information (as indicated by AIC), growth rate was modeled as linear regression (Bates et al. 2012). Model comparisons for all model selection were made using Akaike Information Criterion (AIC).

Table 15: Survival and growth experimental treatments.

Treatment	Salinity	Algae	Concentration (cells/mL^{-1})
1	25	None	0
2	25	*Isochrysis galbana*	100,000
3	25	*Isochrysis galbana*	1,000,000
4	25	*Aureoumbra lagunensis*	100,000
5	25	*Aureoumbra lagunensis*	1,000,000
6	40	None	0
7	40	*Isochrysis galbana*	100,000
8	40	*Isochrysis galbana*	1,000,000
9	40	*Aureoumbra lagunensis*	100,000
10	40	*Aureoumbra lagunensis*	1,000,000

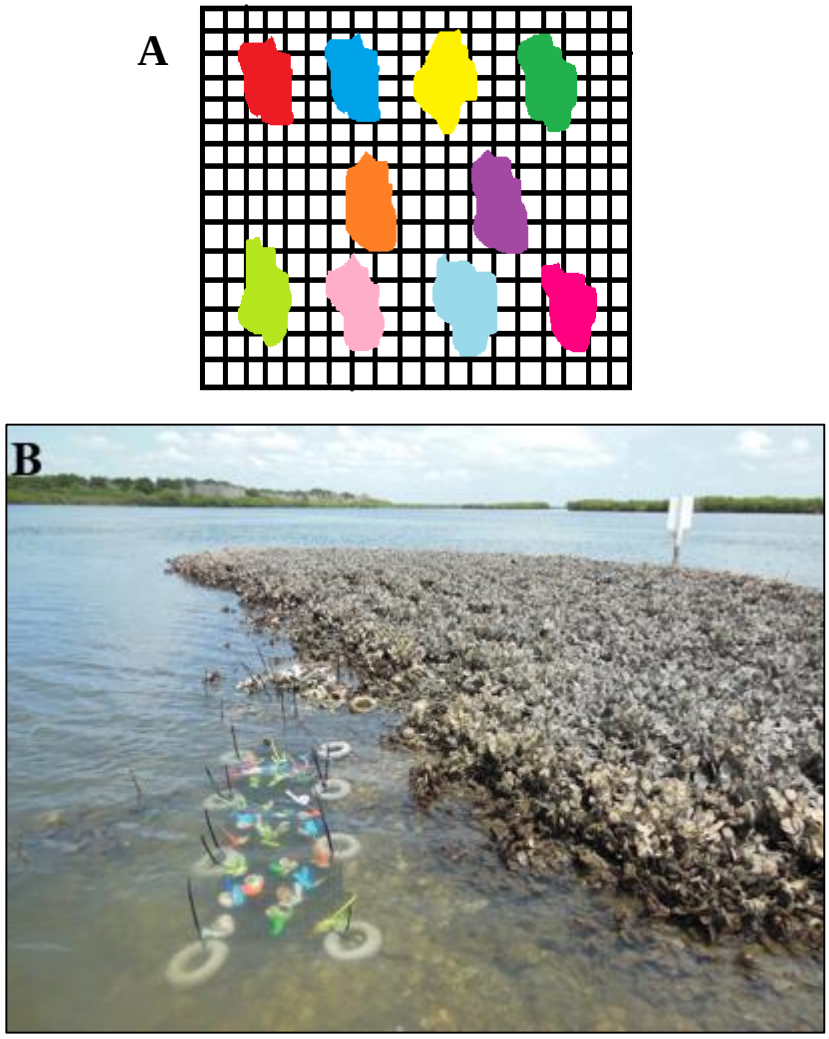


Figure 11: A) Mesh mat with 10 oyster shells attached. Each colored shell represents a different treatment (described in Table 16). B) Three replicate mats separated by 10 cm were deployed on the intertidal, landward side of each of four restored reefs.

Results

Survival was best described by the additive effects of algal species and time since transplantation (Table 16). The random effect of oyster reef location (SITE) provided additional information and was included in the models (Table 24 in Appendix C). Three-way interactions and the full interaction of all predictors could not be evaluated due to survival probabilities of zero or one for some combinations. Both *Aureoumbra lagunensis* and time since transplantation had significant negative effects on survival of *Crassostrea virginica* (Table 17, Fig 12). Diagnostic plot, AIC tables, residual plots, and *R* code are included in Appendix C.

Table 16: AIC table predicting probability of survival of *Crassostrea virginica* as a function of algal concentration (logCELL), algal species (Algae), time, salinity (PSU), and the random effect of site.

	Model	**AIC**	**ΔAIC**	**weight**
1	SURV~Algae+TIME+(1|SITE)	264.1	0.0	0.70
2	SURV~Algae*TIME+(1|SITE)	266.0	1.9	0.27
3	SURV~TIME+(1|SITE)	270.4	6.3	0.03
4	SURV~Algae+(1|SITE)	283.4	19.3	<0.001
5	SURV~logCELL+(1|SITE)	288.9	24.8	<0.001
6	SURV~PSU+(1|SITE)	289.2	25.1	<0.001

Table 17: Parameter estimates predicting probability of survival of *Crassostrea virginica* as a function of algal species and time since transplantation in the field. AL indicates *Aureoumbra lagunensis*.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.466	0.427	8.114	0.000
AL	-0.894	0.327	-2.733	0.006
TIME	-0.516	0.122	-4.214	0.000

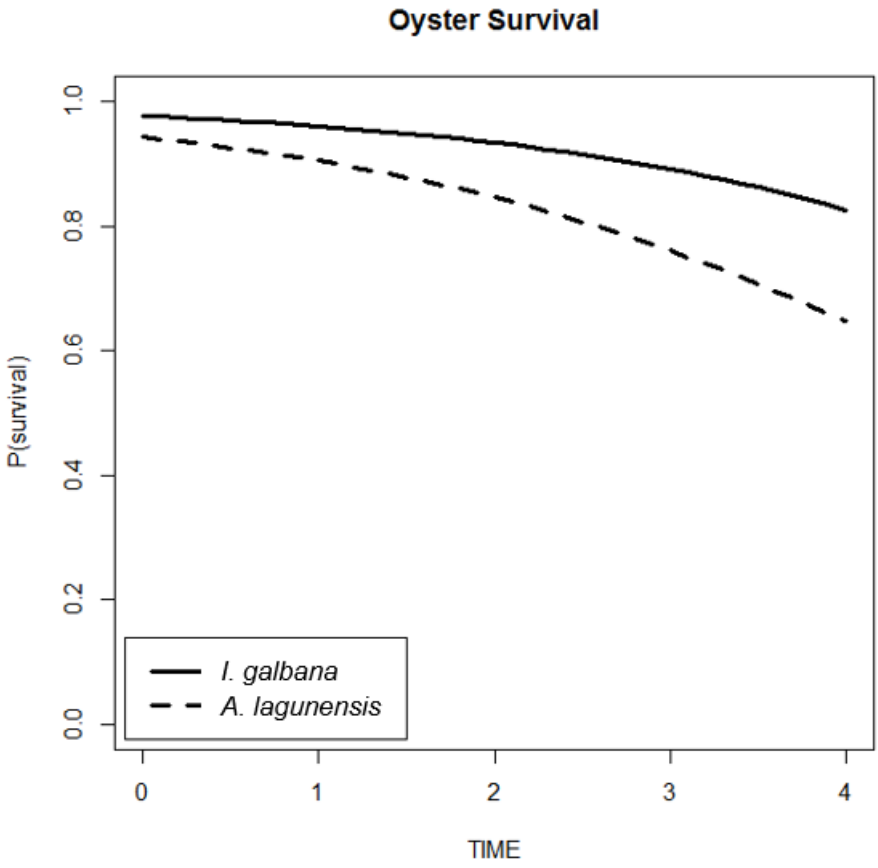


Figure 12: Logistic regression predicting probability of survival of *Crassostrea virginica* as a function of algal species and time.

Growth of juvenile *Crassostrea virginica* was best described by time since deployment in the field (Tables 20, 21). Both the random effects of site on the intercept (1|SITE) as well as spat-specific growth rates on the slope and intercept (1+TIME|spatID) were not found to be significant and thus were not included (Appendix C). Although salinity, algal concentration, and algal species are not predictors included in the top model, they must not be discounted. Model selection found all top five models to be within three AIC values; thus, there was limited evidence that algal species, salinity, and algal concentration may affect growth of juvenile

C. virginica. Diagnostic plots, AIC table, residual plot, and *R* code are included in Appendix C.

Table 20: AIC table predicting log transformed growth rates (mm^2) of *Crassostrea virginica* as a function of algal concentration (logCELL), algal species (Algae), time, and salinity (PSU). The top five models are shown.

	Model	**AIC**	**ΔAIC**	**weight**
1	Growth~Time	181.8	0.0	0.23
2	Growth ~Algae + Time	183.1	1.2	0.12
3	Growth ~ PSU + Time	183.6	1.8	0.10
4	Growth ~logCELL + Time	183.8	2.0	0.09
5	Growth ~logCELL * Time	184.0	2.2	0.08

Table 21: Parameter estimates for model 1 predicting growth rate (mm^2) of *Crassostrea virginica* as a function of time.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.665	0.059	11.312	<0.001
TIME	-0.051	0.025	-2.044	0.042

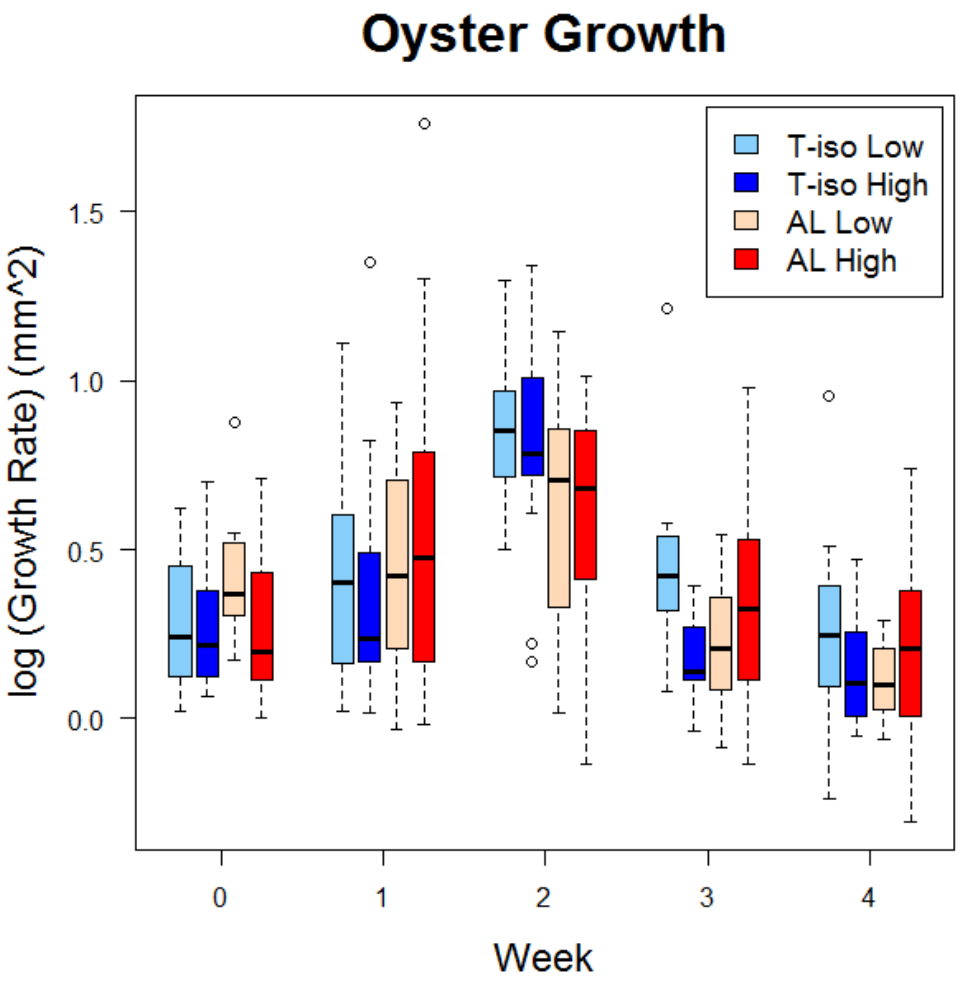


Figure 13: Boxplot for model 1 showing the interaction effects of algal concentration, algal species, and time on growth rates (mm^2) of *Crassostrea virginica* following acute laboratory exposure to algal species and subsequent transplantation in the field. Week zero indicates initial shell sizes following one week laboratory exposure. The control species *Isochrysis galbana* is denoted by T-iso and *Aureoumbra lagunensis* is denoted by AL. Low concentration refers to 1×10^5 cells mL^{-1} and high concentration refers to 1×10^6 cells mL^{-1}.

Discussion

This study demonstrated that survival of juvenile eastern oysters over a four-week period was affected by acute and transient exposure to *Aureoumbra lagunensis* compared to oysters exposed to *Isochrysis galbana*. Growth rates for surviving juvenile eastern oysters did not appear to be greatly affected by brief exposure to *A. lagunensis.* Oyster mortality over the 28-day period of this study was approximately 10% for juveniles exposed to *I. galbana* and 20% for *A. lagunensis*. Kennedy (1996) emphasized the limited amount of quantitative information on the mortality of oyster spat, but that mortality estimates for young oysters are high. For example, Loosanoff and Engle (1940) found mortality to range between 86 and 100% for spat over a 52-day period in summer. Despite the positive implications of these low mortality rates for Mosquito Lagoon, low variation in survival amongst treatments did not allow for differences to be assessed comprehensively. It is important to note that only individuals that could be followed throughout the study were included in the analysis. Identification of individual spat in the photographs taken was difficult in some cases due to their small size in the beginning of the study. Furthermore, signs of predation were minimal and limited to non-lethal crab damage.

There was little evidence for effects of *Aureoumbra lagunensis* and high salinities on juvenile eastern oyster growth. Unfortunately, there have been no previous studies on how *A. lagunensis* affects growth rates of juvenile eastern oysters. The closest study that may be used for comparison was conducted by Gobler et al. (2013) which found filtration rates of *C. virginica* to be significantly lower under both 4×10^5 cells mL^{-1} and 1×10^6 cells mL^{-1} concentrations of *A. lagunensis* as compared to *I. galbana.* His and Seaman (1992) found that lack of food following spawning impairs subsequent digestive ability of *Crassostrea gigas*. Interestingly,

1×10^5 and 1×10^6 cells mL^{-1} of *A. lagunensis* did not appear to negatively impact growth of juvenile oysters as compared to *I. galbana*.

Furthermore, we do not have evidence that the salinity levels studied had an effect on growth and survival of juvenile eastern oysters in Mosquito Lagoon. Salinities in Mosquito Lagoon have historically averaged near 30 PSU with summer values averaging 35 PSU (Gobler et al. 2013, Phlips et al. 2002). For example, during transplantation for the survival and growth experiment, salinities in northern Mosquito Lagoon ranged from 35 to 37 PSU for the months of May and June, respectively. Wide salinity tolerances and the ability of *C. virginica* to grow and survive well at high salinities ranging from 32-42 PSU suggests that populations of this species in Mosquito Lagoon are likely well adapted to higher salinity pressures, and as such, are not greatly affected by periods of high salinity (Shumway 1996, Breuer 1962).

Despite the negative impacts of *Aureoumbra lagunensis* on juvenile eastern oysters, growth continued for individuals exposed for one week to both harmful and control algal species. Additionally, mortality rates of juvenile eastern oysters in Mosquito Lagoon exposed briefly to *A. lagunensis* were low. There is only limited evidence that current extreme dense blooms of *A. lagunensis* and high salinity conditions in Mosquito Lagoon will effect eastern oyster persistence.

CHAPTER 5: CONCLUSIONS

Eastern oysters currently exist at 15% of historical populations globally with many populations in North America, Australia, and Europe remaining at 1% of prior abundances (Beck et al. 2011). Further population declines will only exacerbate the loss of ecosystem functions, especially water filtration and nutrient cycling. Fortunately, populations of eastern oysters in Mosquito Lagoon have fared comparatively well, experiencing losses less than 50% (Garvis et al. 2015). However, the ability of *Aureoumbra lagunensis* to negatively impact early life stages of *Crassostrea virginica* has many implications for the stability of already threatened populations, and losses can be expected to increase with the expanding threats of harmful algal blooms (HABs).

Global increases in the frequency and intensity of HABs have been associated with changes in climatic conditions and are expected to experience range expansions, putting more habitats and coastal communities at risk (Glibert et al. 2005, Hallegraeff 1993, Havens 2015, Hallegraeff 2010). For example, sea surface temperatures are expected to increase by 1 to 3 degrees Celsius for the state of Florida by the end of this century (IPCC 2014). With temperatures being one of the most important factors influencing algal bloom formation, it can be predicted that increases in phytoplankton abundance will occur (Havens 2015). In the case of *A. lagunensis*, changes in salinity regimes also play a major role in the initiation of blooms (Buskey et al. 1998, Phlips et al. 2015). Blooms of *A. lagunensis* in Texas and Florida occurred following drought years resulting in hypersaline conditions (Buskey et al. 1998, Phlips et al. 2015). Climate models run by Wuebbles et al. (2014) and Sheffield and Wood (2008) predict increases in the duration, intensity, and frequency of droughts. This suggests that more habitat will be at risk of suffering from blooms of high-salinity tolerant species such as *A. lagunensis*,

and that the return rate and duration of blooms in already affected areas will also increase. While eastern oysters in Mosquito Lagoon do not appear to be strongly affected by high salinities tested in this study, hypersaline conditions are conducive to bloom development of *A. lagunensis*, which does negatively impact eastern oysters.

Although changes in climate and rising sea surface temperatures are difficult to control, steps can be taken to reduce the risks of HABs. A major source of nitrogen to marine environments, a nutrient necessary for the formation of algal blooms, is derived from the combustion of fossil fuels (Castro and Huber 2010). Lifestyle choices to reduce energy consumption and greenhouse gas emissions can be implemented by individuals to help stabilize the global nitrogen cycle (Havens 2015). Additionally, nutrient pollution can be controlled to prevent eutrophication (excessive nutrient enrichment) and the initiation of HABs (Cloern 2001, Nixon 1995). Nutrient rich effluents to water bodies resulting from sewage, storm water, and agricultural run-off are major sources of nitrate and phosphorous (Nixon 1995). For example, Lapointe et al. (2015) found that sewage-driven eutrophication is a major driver of HAB formation in the Indian River Lagoon system, and argue that inadequate sewage treatment facilities should be at the forefront of nutrient reduction management for the IRL. As blooms of *A. lagunensis* occur following blooms of high-nutrient adapted species, eliminating or minimizing preceding algal blooms may also help prevent blooms of *A. lagunensis*.

Damage to local economies and public health are major concerns of HABs. For example, a red-tide event caused by *Karenia digitata* in Hong Kong in 1998 led to aquaculture losses totaling HK$250 million (Yin et al. 1999). Additionally, blooms of *Pyrodinium* spp. in the Philippines have been responsible for >2,000 human illnesses and >100 deaths (Hallegraeff and Maclean 1989). In Mosquito Lagoon, cultured populations of the hard clam *Mercenaria*

mercenaria and *C. virginica* experienced mortalities, and over 30 fish kills were reported during the 2012 bloom of *A. lagunensis* (Gobler et al. 2013). This study showed that natural recruitment of eastern oysters in Mosquito Lagoon was negatively impacted by *A. lagunensis.* Estimated economic impacts of the brown tides caused by *A. lagunensis* in 2012 and 2013 were $197 million loss/year for the Indian River Lagoon system, FL (Lapointe et al. 2015). With a large percentage of populations living near coastal areas globally and deriving livelihoods from aquatic resources, economies will continue to be negatively impacted and losses can be predicted to increase with increases in the duration, frequency, and intensity of HABs.

Economic losses are not the only negative impacts to warrant concern. Blooms of *A. lagunensis* have led to decreases in the diversity and abundance of marine flora and fauna (Montagna et al. 1993, Buskey et al. 1997, Ward et al. 2000, Gobler et al. 2013, Onuf 1996). For example, blooms of *A. lagunensis* in Baffin Bay, Texas, have resulted in the suppression of zooplankton grazers (Buskey 200). Sunda and Shertzer (2012) showed that bloom formation of ecosystem disruptive algal bloom (EDAB) species such as *A. lagunensis* require low grazing pressures. Loss of grazers lowers nutrient recycling, decreasing nutrient availability, which facilitates bloom development of EDAB species that are adapted to nutrient-limited environments (Sunda et al. 2006, Buskey 2008). This positive feedback mechanism predicts that harmful blooms should persist once formed, as exemplified by the persistent eight-year bloom of *A. lagunensis* that occurred in Texas (Sunda and Shertzer 2012, Buskey et al. 1998). With ecological theory emphasizing the importance of bottom-up controls, any loss in abundance and diversity in lower trophic levels may have major implications for the stability of higher trophic levels such as commercially important species (Cushing 1974, Petchey 1999).

Changes in community structure and introductions of new species are part of the dynamic nature of coastal habitats (Smayda 1980, Castro and Huber 2010, Hallegraeff and Bolch 1991). However, unexpected shifts in phytoplankton composition driven by uncharacteristic climatic conditions leading to HABs will continue to pose a threat to ecosystems, human health, and economic development (Havens 2015). Multidisciplinary efforts to better understand bloom dynamics and to predict HAB formation will be essential in preparing for the negative impacts on ecosystems and society. Although individuals may feel that their actions do not significantly influence algal bloom formation, one of the most important steps in preventing and minimizing HABs is to educate the public and community leaders about the steps that can be taken, and that prevention begins at the level of individuals.

APPENDIX A:
RECRUITMENT

Oyster Recruitment

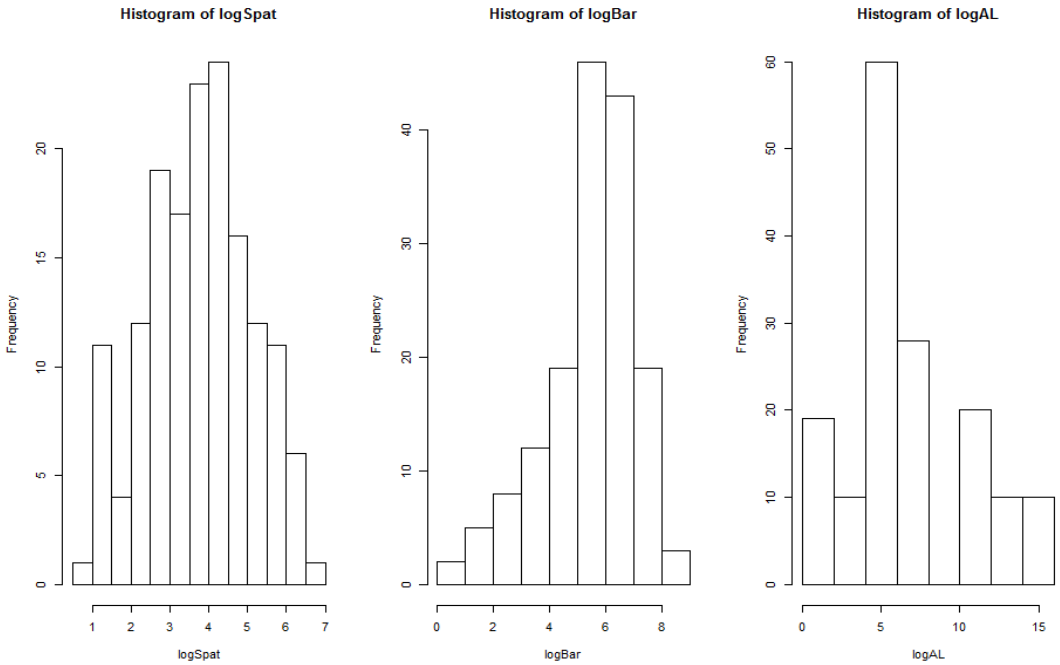


Figure 14: Histogram of log transformed oyster spat, barnacle, and *Aureoumbra lagunensis* density.

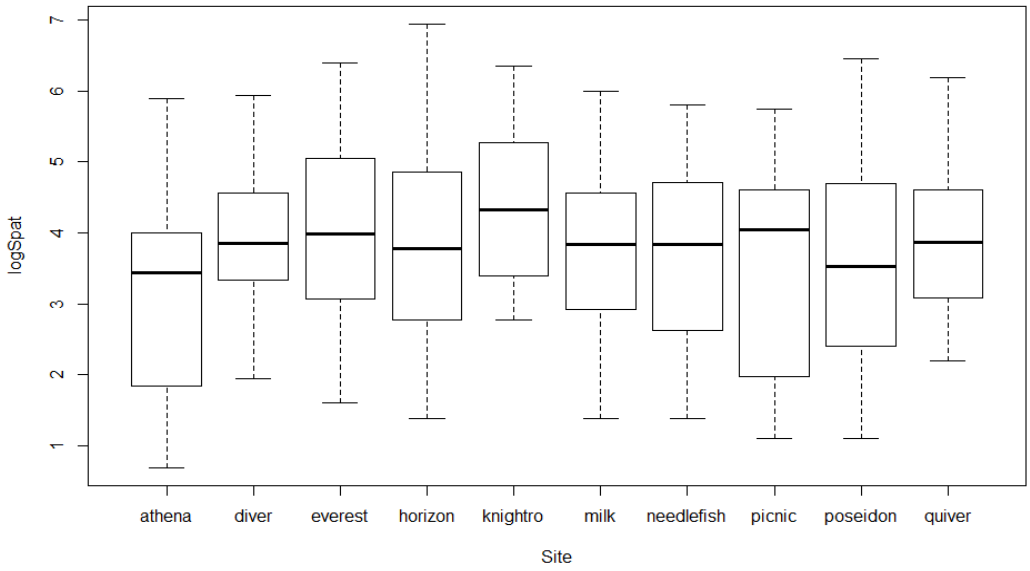


Figure 15: Boxplot of oyster recruitment at each oyster reef within Mosquito Lagoon, FL.

Table 18: Model selection of optimal random factor configurations for oyster recruitment determined using AIC.

#	Model	AIC	ΔAIC	AICWt
1	logSpat ~ PSU*logBar*logAL	490.1	0	1
2	logSpat ~ PSU*logBar*logAL + (1|Site)	532.2	42.1	<0.001

Table 19: Variance for the random effect of site on the intercept.

Groups	Name	Variance	Std. Dev.
Site	(Intercept)	1.38E-13	3.71E-07
Residual		1.25E+00	1.12E+00

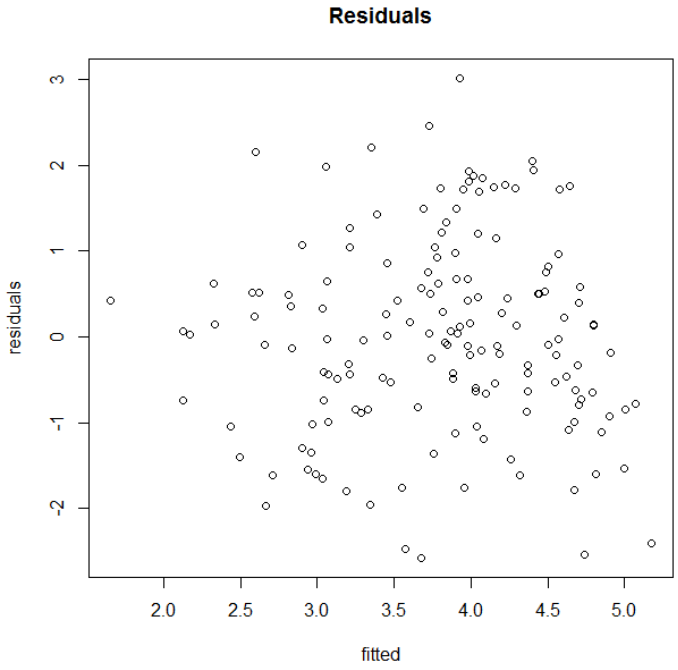


Figure 16: Residuals of model 7 predicting log transformed oyster recruitment per 0.25 m^2.

Barnacle Recruitment

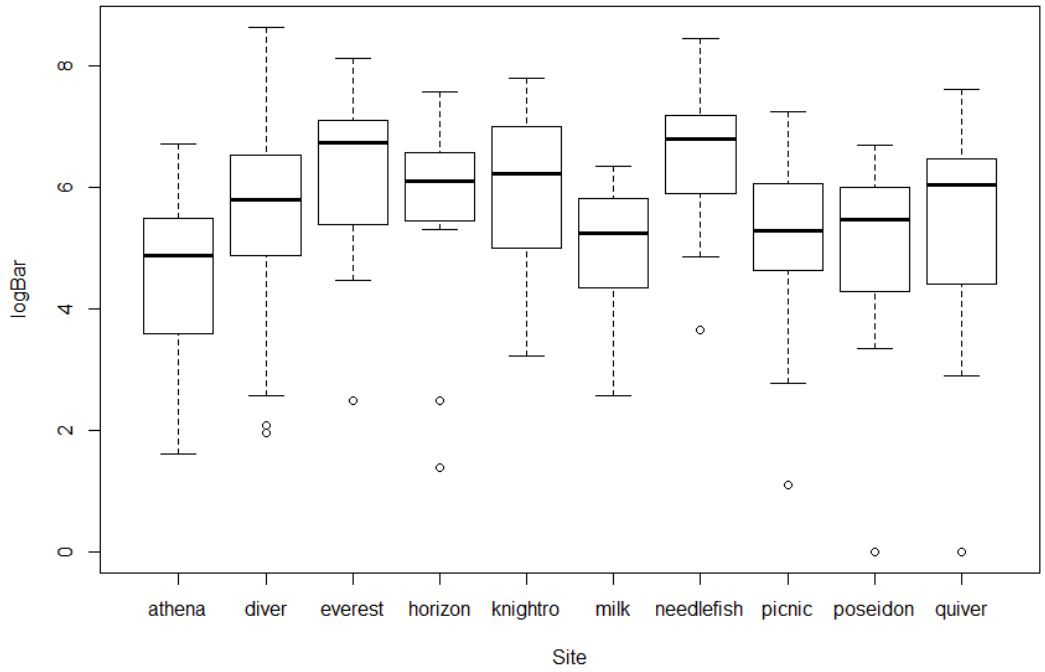


Figure 17: Boxplot of barnacle recruitment at each oyster reef within Mosquito Lagoon, FL.

Table 20: Model selection of random factor configurations for barnacle recruitment determined using AIC.

#	Model	AIC	ΔAIC	AICWt
1	logBar ~ PSU*logAL	600.4	0.0	0.998
2	logBar ~ PSU *logAL + (1|Site)	613.1	12.7	0.002

Table 21: Variance for the random effect of site on the intercept.

Groups	Name	Variance	Std. Dev.
Site	(Intercept)	0.258	0.508
Residual		2.343	1.531

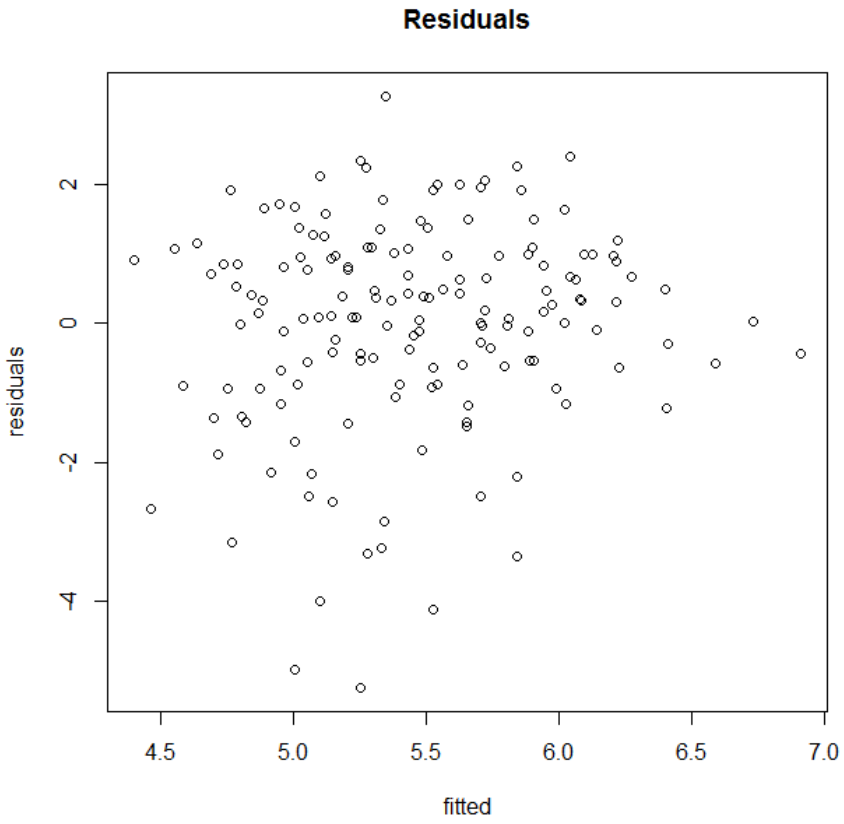


Figure 18: Residuals of model 2 predicting log transformed barnacle recruitment per 0.25 m^2.

R Code: Oyster Recruitment

```
rm(list=ls())

  library(nlme)
  library(bbmle)
  library(lme4)     # for fitting GLMMs
  library(lattice)  # for the xyplot function
  library(MuMIn)    # for the r.squaredGLMM function

setwd("F:/Grad Work/Thesis/Stats")

orig_data<-read.table("Spat_AL_psu_minus winter.txt", header=T)
attach(orig_data)
names(orig_data)

# use factor function to specify categorical variables
orig_data$Site<-factor(orig_data$Site)

################## Data Diagnostics ###################

# plot data
par(mfrow=c(2,2))
plot(Spat)
plot(Bar)
plot(AL)
plot(PSU)
par(mfrow=c(1,1))

# plot histograms
par(mfrow=c(2,2))
hist(Spat,10)
hist(Bar,10)
hist(AL,10)
hist(PSU,10)
par(mfrow=c(1,1))

# log transform variables
logSpat<-log(orig_data$Spat+1)
logBar<-log(orig_data$Bar+1)
logAL<-log(orig_data$AL+1)

par(mfrow=c(1,3))
hist(logSpat,10)
hist(logBar,10)
hist(logAL,10)
```

```
par(mfrow=c(3,1))
plot(logBar,logSpat)
plot(logAL,logSpat)
plot(PSU,logSpat)

par(mfrow=c(1,1))
plot(logSpat~Site)

# assess collinearity: create data subset containing only predictors
subset_data <- data.frame(logBar, logAL, PSU)

# use pairs() function to plot all the variables against each other
pairs(subset_data,panel=panel.smooth)

# generate a correlation matrix using the cor() function
cor(subset_data)

## create a full regression model and summarize the results
model <- lm(logSpat~PSU + logBar + logAL)
summary(model)

## use the vif() function to calculate the variance inflation factors
library(car)
vif(model)

####### Random Models #############

m1 <-lm(logSpat~PSU * logBar * logAL,data = orig_data)
m2 <-lmer(logSpat~PSU * logBar * logAL+(1|Site),data = orig_data)#Site not significant

AICtab(m1,m2,weights=TRUE,base = TRUE)
summary(m1)
summary(m2)


############ Without Site #############

M1 <-lm(logSpat~1, data = orig_data)
M2 <-lm(logSpat~logAL,data = orig_data)
M3 <-lm(logSpat~PSU,data = orig_data)
M4 <-lm(logSpat~logBar,data = orig_data)
M5 <-lm(logSpat~PSU + logBar,data = orig_data)
M6 <-lm(logSpat~PSU + logAL,data = orig_data)
M7 <-lm(logSpat~logBar + logAL,data = orig_data)
M8 <-lm(logSpat~PSU + logBar + logAL,data = orig_data)
M9 <-lm(logSpat~PSU : logBar,data = orig_data)
```

```
M10 <-lm(logSpat~PSU : logAL, data = orig_data)
M11 <-lm(logSpat~logBar : logAL,data = orig_data)
M12 <-lm(logSpat~PSU : logBar : logAL,data = orig_data)

AICtab(M1,M2,M3,M4,M5,M6,M7,M8,M9,M10,M11,M12,weights=TRUE,base = TRUE)

summary(M7)
summary(M8)

# Plot Residuals
fitted <- fitted(M7)
residuals <- resid(M7)
par(mfrow=c(1,1))
plot(fitted, residuals,main="Residuals")
abline (h=0, col="red")

########## Plot Data #############

library(scatterplot3d)

 h =0
 x <- seq(min(logBar),9,0.25)
 y <- seq(min(logAL),16,0.50)
datp <- array(0,c(length(x)*length(y),3))
for (i in 1:length(x)){
 for (j in 1:length(y)){
  h <- h+1
  datp[h,1] <- x[i]
  datp[h,2] <- y[j]
  datp[h,3] <- predict(M7, list(logBar=x[i], logAL=y[j]))
   }}

s3d <- scatterplot3d(datp, highlight.3d=TRUE,type="n",
angle=190, scale.y=0.7,pch=16, zlim=c(0,7), main="Oyster Recruitment", cex.main=2,
ylabgrid=FALSE)

# Add points to the "scatterplot3d"
s3d$points3d(logBar, logAL, logSpat,
col="blue", type="p", pch=16)

# Add a regression plane to the "scatterplot3d"
my.lm <- lm(logSpat~logBar + logAL,data = orig_data)
s3d$plane3d(my.lm, lty.box = "solid")
```

R Code: Barnacle Recruitment

```
rm(list=ls())

  library(nlme)
  library(bbmle)
  library(lme4)     # for fitting GLMMs
  library(lattice)  # for the xyplot function
  library(MuMIn)    # for the r.squaredGLMM function

setwd("F:/Grad Work/Thesis/Stats")

orig_data<-read.table("Spat_AL_psu_minus winter.txt", header=T)
attach(orig_data)
names(orig_data)

# use factor function to specify categorical variables
orig_data$Site<-factor(orig_data$Site)

################## Data Diagnostics ###################

# plot data
par(mfrow=c(2,2))
plot(Spat)
plot(Bar)
plot(AL)
plot(PSU)

# plot histograms
par(mfrow=c(2,2))
hist(Spat,10)
hist(Bar,10)
hist(AL,10)
hist(PSU,10)

# log transform variables
logSpat<-log(orig_data$Spat+1)
logBar<-log(orig_data$Bar+1)
logAL<-log(orig_data$AL+1)

par(mfrow=c(2,2))
hist(logSpat,10)
hist(logBar,10)
hist(logAL,10)
hist(PSU,10)
```

```
par(mfrow=c(3,1))
plot(logBar,logSpat)
plot(logAL,logSpat)
plot(PSU,logSpat)

par(mfrow=c(1,1))
plot(logBar~Site)

# assess collinearity: create data subset containing only predictors
subset_data <- data.frame(logBar, logAL, PSU)

# use pairs() function to plot all the variables against each other
pairs(subset_data,panel=panel.smooth)

# generate a correlation matrix using the cor() function
cor(subset_data)

## create a full regression model and summarize the results
model <- lm(logBar ~ PSU + logAL)
summary(model)

## use the vif() function to calculate the variance inflation factors
library(car)
vif(model)

####### Random Models #############

m1 <-lm(logBar~PSU * logAL,data = orig_data)
m2 <-lmer(logBar~PSU * logAL + (1|Site),data = orig_data)

AICtab(m1,m2,weights=TRUE,base = TRUE)
summary(m1)
summary(m2)

####### Mixed Models #############

library(lme4)
library(AICcmodavg)

M1 <-lmer(logBar ~ 1 + (1|Site), data = orig_data, REML = F)
M2 <-lmer(logBar ~ PSU + (1|Site), data = orig_data, REML = F)
M3 <-lmer(logBar ~ logAL + (1|Site), data = orig_data, REML = F)
M4 <-lmer(logBar ~ PSU + logAL + (1|Site), data = orig_data, REML = F)
M5 <-lmer(logBar ~ PSU : logAL + (1|Site), data = orig_data, REML = F)

AICtab(M1,M2,M3,M4,M5,weights=TRUE,base = TRUE)
```

```
summary(M2)
summary(M4)

##### Post-hoc coefficient analysis #####

library(pbkrtest)

# get the KR-approximated degrees of freedom
df.KR <- get_ddf_Lb(M2, fixef(M4))

# get p-values from the t-distribution using the t-values and approximated
# degrees of freedom
coefs <- data.frame(coef(summary(M4)))
coefs$p.KR <- 2 * (1 - pt(abs(coefs$t.value), df.KR))
coefs

#################### Residuals ####################

# Plot Residuals
fitted <- fitted(m2)
residuals <- resid(m2)
par(mfrow=c(1,1))
plot(fitted, residuals,main="Residuals")
abline (h=0, col="red")

detach(orig_data)
```

APPENDIX B:
SETTLEMENT

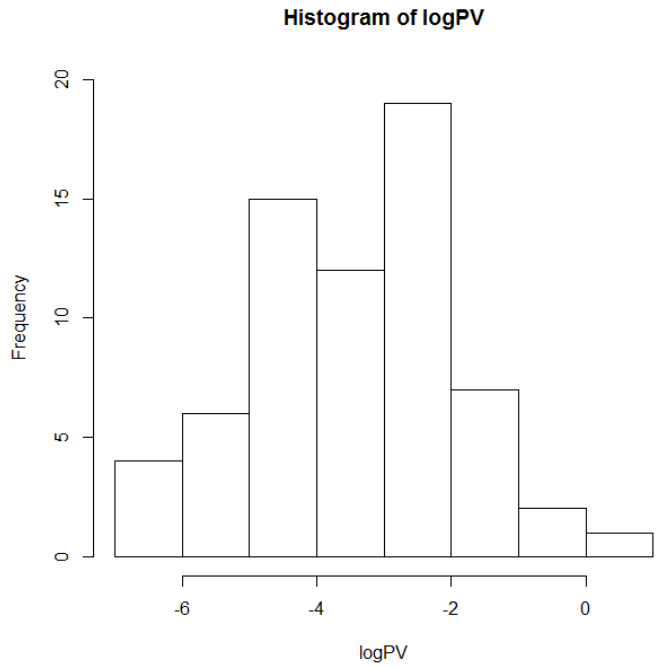


Figure 19: Histogram of log transformed oyster settlement.

Table 22: Configurations of random tank location factors for oyster settlement keeping all fixed effects constant.

1	logPV~logCELL*PSU*Algae+(1|REP)
2	logPV~logCELL*PSU*Algae+(1|REP)+(1|X)+(1|Y)
3	logPV~logCELL*PSU*Algae+(1|REP)+(1|X)
4	logPV~logCELL*PSU*Algae+(1|REP)+(1|Y))

Table 23: Model selection of optimal random factor configurations for oyster settlement determined using AIC.

	AIC	ΔAIC	Weight
1	273.2	0.0	0.534
3	275.2	2.0	0.197
4	275.2	2.0	0.197
2	277.2	4.0	0.072

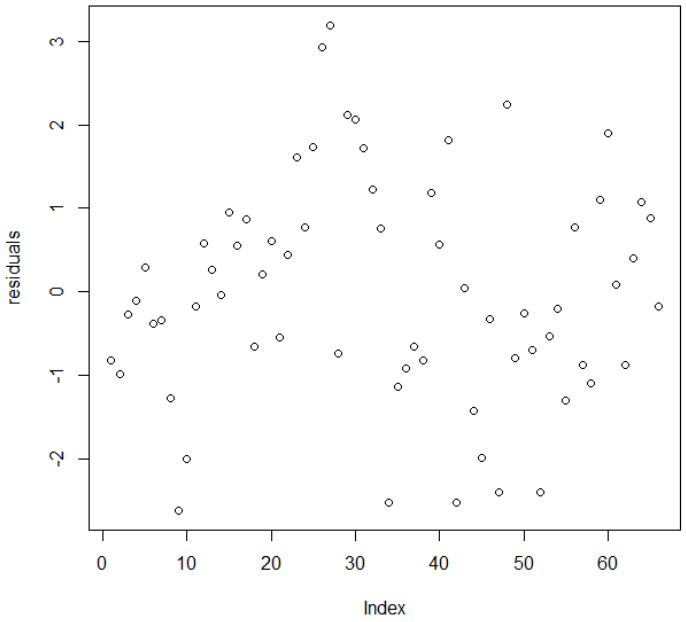


Figure 20: Residuals of model 1 predicting log transformed oyster settlement per cm^2.

R Code: Settlement

```
rm(list=ls())

  library(nlme)
  library(bbmle)
  library(lme4)     # for fitting GLMMs
  library(lattice)  # for the xyplot function
  library(MuMIn)    # for the r.squaredGLMM function
  library(MASS)

setwd("F:/Grad Work/Thesis/Stats")

orig_data<-read.table("Settlement.txt", header=T)

orig_data$Algae <- 1
orig_data$Algae[orig_data$algae=="T"] <-2
orig_data$Algae[orig_data$algae=="AL"] <-3
Algae <- orig_data$Algae
Algae <-factor(Algae)

attach(orig_data)
names(orig_data)

REP<-factor(orig_data$REP)

par(mfrow=c(2,2))
plot(orig_data$PV.cm2[Algae==2]~orig_data$logCELL[Algae==2], ylab="Larvae/cm^2")
plot(orig_data$PV.cm2[Algae==3]~orig_data$logCELL[Algae==3], ylab="Larvae/cm^2")
plot(orig_data$PV.cm2~orig_data$algae, ylab="Larvae/cm^2")
plot(orig_data$PV.cm2~orig_data$PSU, ylab="Larvae/cm^2")

par(mfrow=c(1,3))
plot(orig_data$PV.cm2~orig_data$X, ylab="Larvae/cm^2")#tank location
plot(orig_data$PV.cm2~orig_data$Y, ylab="Larvae/cm^2")#tank location
plot(orig_data$PV.cm2~orig_data$REP, ylab="Larvae/cm^2")

par(mfrow=c(1,2))
hist(orig_data$PV.cm2)
logPV <- log(orig_data$PV.cm2)
hist(logPV,ylim=c(0,20))

par(mfrow=c(1,2))
plot(logPV[Algae==2]~orig_data$logCELL[Algae==2], ylab="Larvae/cm^2")
plot(logPV[Algae==3]~orig_data$logCELL[Algae==3], ylab="Larvae/cm^2")
```

```
par(mfrow=c(1,3))
plot(logPV~orig_data$X, ylab="Larvae/cm^2")#tank location
plot(logPV~orig_data$Y, ylab="Larvae/cm^2")#tank location
plot(logPV~orig_data$REP, ylab="Larvae/cm^2")

par(mfrow=c(1,2))
plot(orig_data$logCELL[PSU=="25" & Algae==2 & REP
=="1"],orig_data$PV.cm2[PSU=="25"& Algae==2 & REP =="1"],
ylab=expression(Larvae/cm^2),
xlab="I. galbana",col="blue",pch=1,xlim=c(-0.5,6.5),ylim=c(0,1.5))
points(orig_data$logCELL[PSU=="40" & Algae==2 & REP
=="1"],orig_data$PV.cm2[PSU=="40" & Algae==2 & REP =="1"], col="red",pch=1)
points(orig_data$logCELL[PSU=="25" & Algae==2 & REP
=="2"],orig_data$PV.cm2[PSU=="25"& Algae==2 & REP =="2"], col="blue",pch=2)
points(orig_data$logCELL[PSU=="40" & Algae==2 & REP
=="2"],orig_data$PV.cm2[PSU=="40" & Algae==2 & REP =="2"], col="red",pch=2)
points(orig_data$logCELL[PSU=="25" & Algae==2 & REP
=="3"],orig_data$PV.cm2[PSU=="25"& Algae==2 & REP =="3"], col="blue",pch=16)
points(orig_data$logCELL[PSU=="40" & Algae==2 & REP
=="3"],orig_data$PV.cm2[PSU=="40" & Algae==2 & REP =="3"], col="red",pch=16)
points(orig_data$logCELL[PSU=="40" & Algae==1 & REP
=="3"],orig_data$PV.cm2[PSU=="40" & Algae==1 & REP =="3"], col="black",pch=16)
points(orig_data$logCELL[PSU=="25" & Algae==1 & REP
=="3"],orig_data$PV.cm2[PSU=="25" & Algae==1 & REP =="3"], col="black",pch=16)
points(orig_data$logCELL[PSU=="40" & Algae==1 & REP
=="2"],orig_data$PV.cm2[PSU=="40" & Algae==1 & REP =="2"], col="black",pch=2)
points(orig_data$logCELL[PSU=="25" & Algae==1 & REP
=="2"],orig_data$PV.cm2[PSU=="25" & Algae==1 & REP =="2"], col="black",pch=2)
points(orig_data$logCELL[PSU=="40" & Algae==1 & REP
=="1"],orig_data$PV.cm2[PSU=="40" & Algae==1 & REP =="1"], col="black",pch=1)
points(orig_data$logCELL[PSU=="25" & Algae==1 & REP
=="1"],orig_data$PV.cm2[PSU=="25" & Algae==1 & REP =="1"], col="black",pch=1)

plot(orig_data$logCELL[PSU=="25" & Algae==3 & REP
=="1"],orig_data$PV.cm2[PSU=="25"& Algae==3 & REP =="1"], ylab=NA,
xlab="A. lagunensis",col="blue",pch=1,xlim=c(-0.5,6.5),ylim=c(0,1.5))
points(orig_data$logCELL[PSU=="40" & Algae==3 & REP
=="1"],orig_data$PV.cm2[PSU=="40" & Algae==3 & REP =="1"],col="red",pch=1)
points(orig_data$logCELL[PSU=="25" & Algae==3 & REP
=="2"],orig_data$PV.cm2[PSU=="25"& Algae==3 & REP =="2"], col="blue",pch=2)
points(orig_data$logCELL[PSU=="40" & Algae==3 & REP
=="2"],orig_data$PV.cm2[PSU=="40" & Algae==3 & REP =="2"], col="red",pch=2)
points(orig_data$logCELL[PSU=="25" & Algae==3 & REP
=="3"],orig_data$PV.cm2[PSU=="25"& Algae==3 & REP =="3"], col="blue",pch=16)
points(orig_data$logCELL[PSU=="40" & Algae==3 & REP
=="3"],orig_data$PV.cm2[PSU=="40" & Algae==3 & REP =="3"], col="red",pch=16)
```

```
points(orig_data$logCELL[PSU=="40" & Algae==1 & REP
=="3"],orig_data$PV.cm2[PSU=="40" & Algae==1 & REP =="3"], col="black",pch=16)
points(orig_data$logCELL[PSU=="25" & Algae==1 & REP
=="3"],orig_data$PV.cm2[PSU=="25" & Algae==1 & REP =="3"], col="black",pch=16)
points(orig_data$logCELL[PSU=="40" & Algae==1 & REP
=="2"],orig_data$PV.cm2[PSU=="40" & Algae==1 & REP =="2"], col="black",pch=2)
points(orig_data$logCELL[PSU=="25" & Algae==1 & REP
=="2"],orig_data$PV.cm2[PSU=="25" & Algae==1 & REP =="2"], col="black",pch=2)
points(orig_data$logCELL[PSU=="40" & Algae==1 & REP
=="1"],orig_data$PV.cm2[PSU=="40" & Algae==1 & REP =="1"], col="black",pch=1)
points(orig_data$logCELL[PSU=="25" & Algae==1 & REP
=="1"],orig_data$PV.cm2[PSU=="25" & Algae==1 & REP =="1"], col="black",pch=1)

legend("topright",inset=0.014,cex= 1.2,
   c("25 PSU","40 PSU","Control","Batch 1","Batch 2","Batch 3"),col =
c("blue","red","black","grey60","grey60","grey60"), lty = c(1, 1, 1,NA,NA,NA),
lwd=c(2,2,2,1,1,1), pch = c(NA,NA,NA,1,2,16))

################ Random Effects ################

m1 <- lmer(logPV~logCELL*PSU*Algae+(1|REP),data=orig_data)
m2 <- lmer(logPV~logCELL*PSU*Algae+(1|REP)+(1|X)+(1|Y),data=orig_data)
m3 <- lmer(logPV~logCELL*PSU*Algae+(1|REP)+(1|X),data=orig_data)
m4 <- lmer(logPV~logCELL*PSU*Algae+(1|REP)+(1|Y),data=orig_data)

AICtab(m1,m2,m3,m4,weights=TRUE,base = TRUE)
summary(m2)

#Tank location not more informative. Mixed-effects modeling not needed.


################ Fixed-effects Models ################

M3 <- lmer(PV.cm2~logCELL*PSU*Algae+(1|REP),data=orig_data)
m3 <- lmer(PV.cm2~logCELL+PSU+Algae+(1|REP),data=orig_data)
M2 <- lmer(PV.cm2~logCELL*Algae+(1|REP),data=orig_data)
M2.1 <- lmer(PV.cm2~logCELL*PSU+(1|REP),data=orig_data)
M2.2 <- lmer(PV.cm2~Algae*PSU+(1|REP),data=orig_data)
m2 <- lmer(PV.cm2~logCELL+Algae+(1|REP),data=orig_data)
m2.1 <- lmer(PV.cm2~logCELL+PSU+(1|REP),data=orig_data)
m2.2 <- lmer(PV.cm2~Algae+PSU+(1|REP),data=orig_data)
m1.1 <- lmer(PV.cm2~PSU+(1|REP),data=orig_data)
m1.2 <- lmer(PV.cm2~Algae+(1|REP),data=orig_data)
m1.3 <- lmer(PV.cm2~logCELL+(1|REP),data=orig_data)

AICtab (M3,m3,M2,M2.1,M2.2,m2,m2.1,m2.2,m1.1,m1.2,m1.3,
```

```
weights=TRUE,base = TRUE)
summary(m1.1)

##### Post-hoc coefficient analysis #####

library(pbkrtest)

# get the KR-approximated degrees of freedom
df.KR <- get_ddf_Lb(m1.1, fixef(m1.1))

# get p-values from the t-distribution using the t-values and approximated
# degrees of freedom
coefs <- data.frame(coef(summary(m1.1)))
coefs$p.KR <- 2 * (1 - pt(abs(coefs$t.value), df.KR))
coefs

############################## Models for REP 2 Only ##############################

rep2 <- subset(orig_data,REP==2 & Algae !=1 & logCELL <5)
rep2$PSU <- factor(rep2$PSU)
rep2$Algae <- factor(rep2$Algae)

M3 <- lm(PV.cm2~logCELL*PSU*Algae,data=rep2)
m3 <- lm(PV.cm2~logCELL+PSU+Algae,data=rep2)
M2 <- lm(PV.cm2~logCELL*Algae,data=rep2)
M2.1 <- lm(PV.cm2~logCELL*PSU,data=rep2)
M2.2 <- lm(PV.cm2~Algae*PSU,data=rep2)
m2 <- lm(PV.cm2~logCELL+Algae,data=rep2)
m2.1 <- lm(PV.cm2~logCELL+PSU,data=rep2)
m2.2 <- lm(PV.cm2~Algae+PSU,data=rep2)
m1.1 <- lm(PV.cm2~PSU,data=rep2)
m1.2 <- lm(PV.cm2~Algae,data=rep2)
m1.3 <- lm(PV.cm2~logCELL,data=rep2)

AICtab (M3,m3,M2,M2.1,M2.2,m2,m2.1,m2.2,m1.1,m1.2,m1.3,
weights=TRUE,base = TRUE)
summary(M3)


########### Best Model Plots ##########

#When inluding all batches

########### Best Model Plots ##########

par(mfrow=c(1,1))
```

```
boxplot(logPV~PSU+REP,
main="Oyster Settlement", cex.main= 2, xlab="Replicate", cex.lab = 1.5, ylab=expression(log
(Spat/cm^2)),
par(mar = c(5, 5, 4, 2)+ 0.1),las = 2,xaxt='n',
col = c("blue","red","blue","red","blue","red"),
at =c(1,2, 5,6, 9,10),
names = c("25 PSU","40 PSU","25 PSU","40 PSU","25 PSU","40 PSU"))
axis(1,at=c(1.5, 5.5, 9.5),labels=c("1","2","3"),cex=2)

legend("topright",inset=0.014,cex= 1.3,
   c("25 PSU","40 PSU"), ,fill=c("blue","red"))


##################### Checking assumptions #################

yhat <- fitted(m1.1)
summary(yhat)

residuals <- resid(m1.1)
summary(residuals)

par(mfrow=c (1,1))
plot(residuals)

detach(orig_data)
```

APPENDIX C:
TRANSPLANT EXPERIEMNT

Oyster Survival

Table 24: Model selection of optimal configuration for random factors for oyster survival models determined using AIC.

	Model	AIC	*Δ*AIC	weight
1	SURV~Algae*TIME+(1|SITE)	266.0	0.0	0.985
2	SURV~Algae*TIME	274.3	8.3	0.015

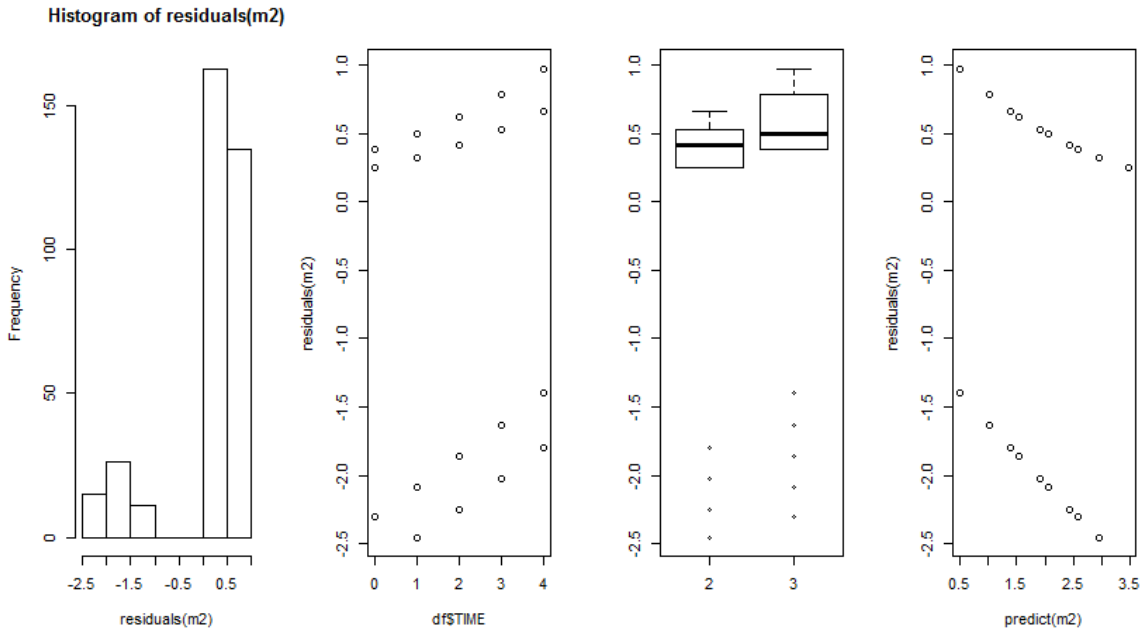
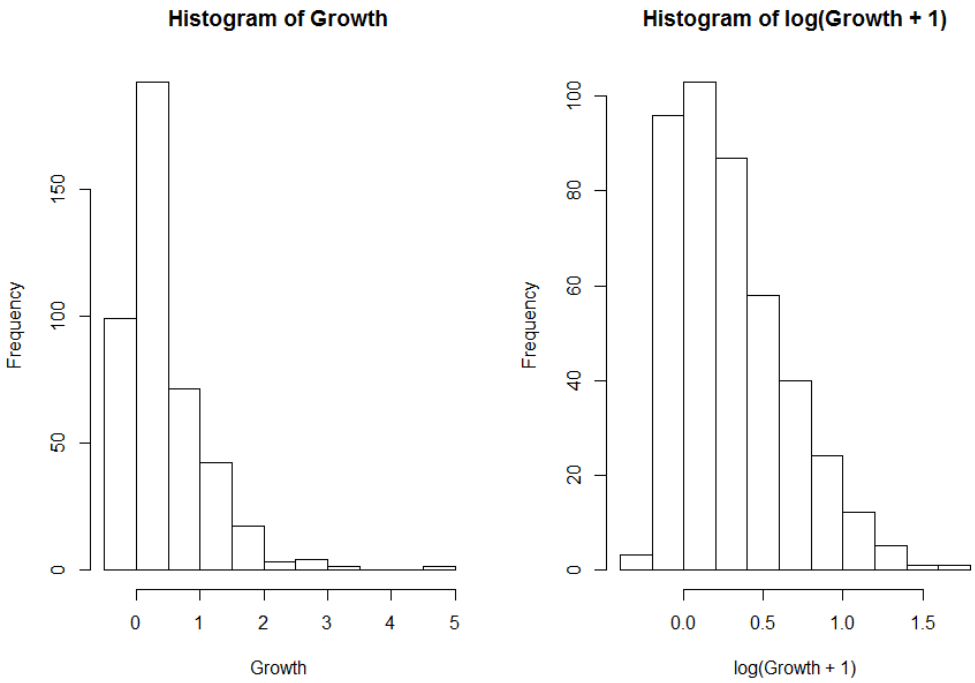


Figure 21: Residuals of best model predicting probability of juvenile oyster survival after 1-week exposure to algal treatments and subsequent transplantation to the field for 4-weeks.

Oyster Growth



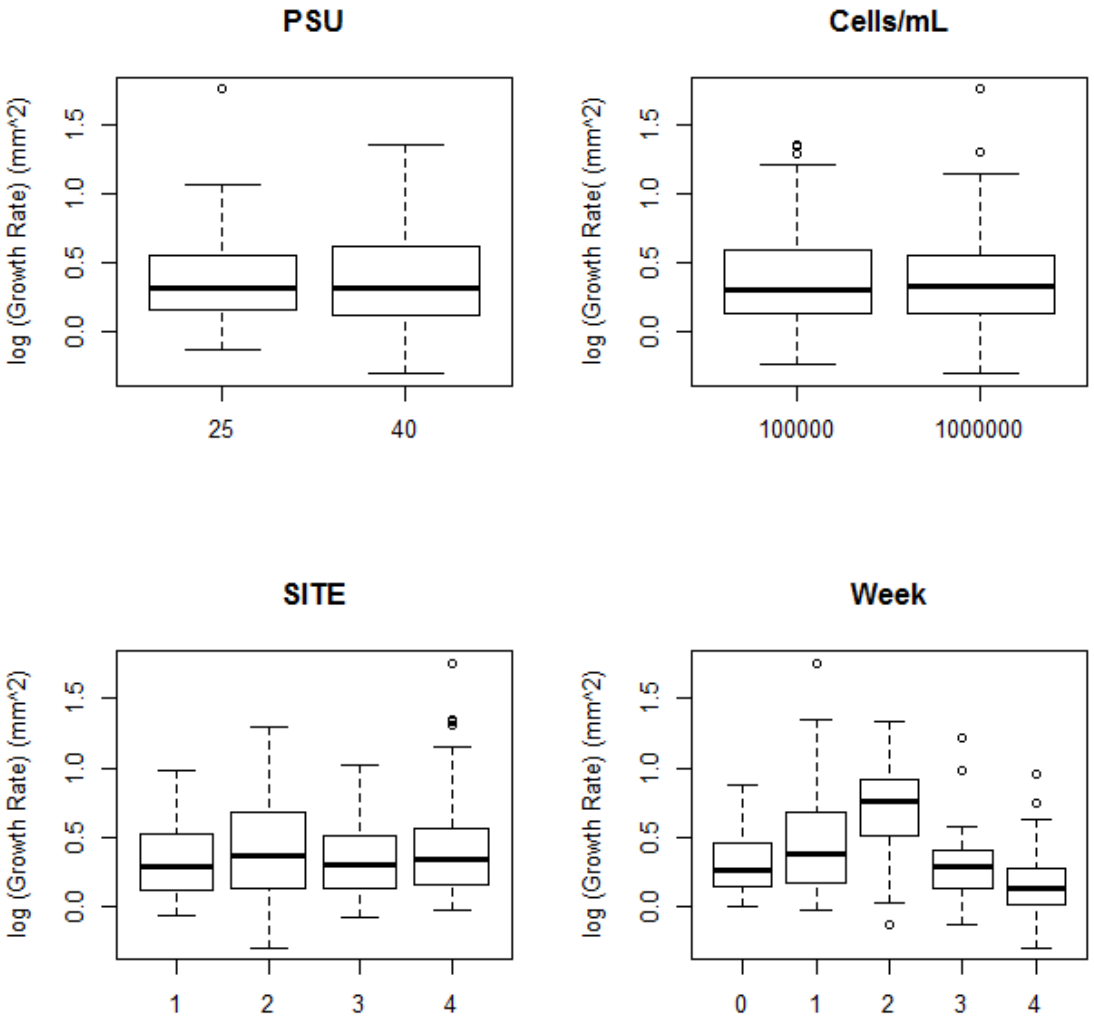


Figure 22: Boxplots of oyster log transformed growth rates (mm^2) in response to predictor variables.

Table 25: Configurations of random factors for oyster growth model keeping all fixed effects constant. Random factors include site and spat-specific growth rate.

1	Growth~Algae*logCELL*PSU*TIME
2	Growth ~Algae*logCELL*PSU*TIME+(1|SITE)
3	Growth ~Algae*logCELL*PSU*TIME+(1+TIME|spatID)
4	Growth ~Algae*logCELL*PSU*TIME+(1+TIME|spatID)+(1|SITE)

Table 26: Model selection of optimal configuration for random factors for oyster growth models determined using AIC.

	AIC	**ΔAIC**	**df**	**weight**
1	198.3	0.0	17	1
2	325.8	127.5	18	<0.001
3	330.3	132.0	20	<0.001
4	331.8	133.5	21	<0.001

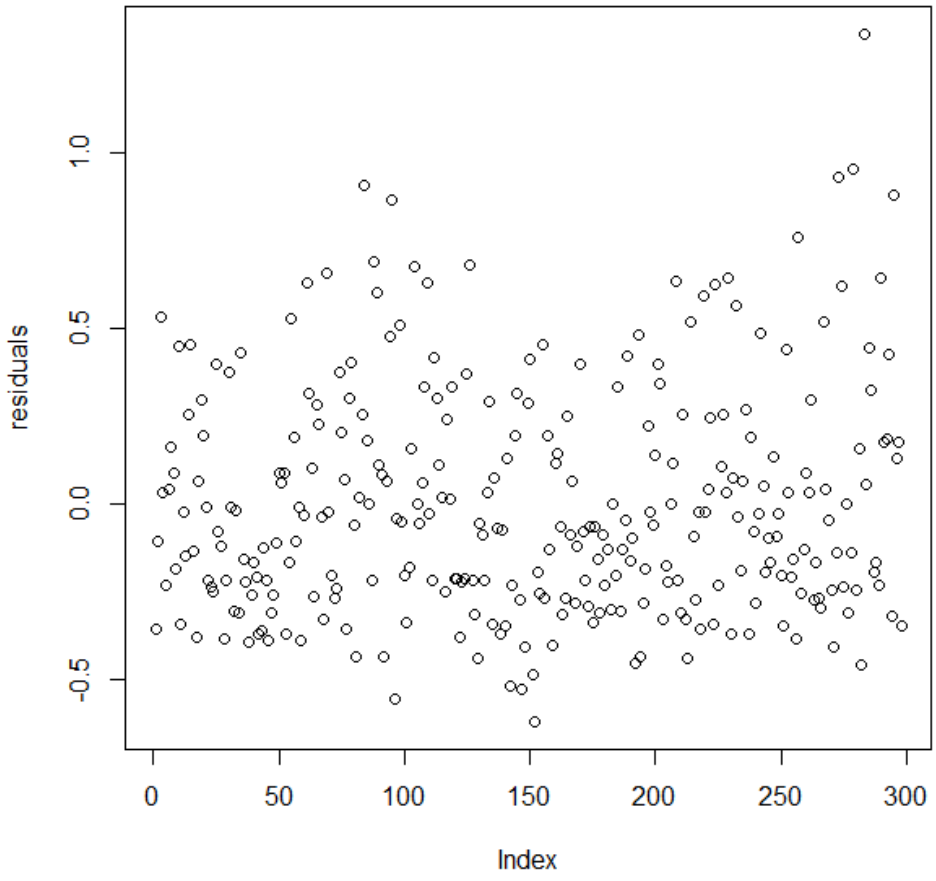


Figure 23: Residuals of optimal model predicting log transformed growth rates (mm^2) of *Crassostrea virginica*.

R Code: Oyster Survival

```
rm(list=ls())

  library(nlme)
  library(bbmle)
  library(lme4)     # for fitting GLMMs
  library(lattice)  # for the xyplot function
  library(MuMIn)    # for the r.squaredGLMM function

setwd("F:/Grad Work/Thesis/Stats")

df<-read.table("Transplant.txt", header=T)

##Remove Zero Control
df <- subset(df, Algae != 1)

attach(df)
names(df)

df$SITE<-factor(df$SITE)
df$Algae<-factor(df$Algae)
df$logCELL<-log(df$CONCEN+1)

#Overall survival between T-iso and AL is significanlty different
table(df$SURV,df$Algae)
t<-table(df$SURV,df$Algae)
chisq.test(t)

#Determine which variables and interations have survival probabilities of 0 or 1
table(df$SURV,df$TIME)
table(df$SURV,df$logCELL)
table(df$SURV,df$PSU)

table(df$SURV,df$Algae,df$PSU)
table(df$SURV,df$Algae,df$TIME)#
table(df$SURV,df$PSU,df$TIME)

table(df$SURV,df$Algae,df$PSU,df$TIME)#
table(df$SURV,df$SITE,df$Algae)#
table(df$SURV,df$SITE,df$PSU)#
table(df$SURV,df$SITE,df$logCELL)#


############################################################
#Full interaction does not work
```

```
#THREE-WAY INTERACTION
M3 <- glm(SURV~TIME*Algae*PSU,data=df, family="binomial")#Does not work as predicted from table above

################ Random Effects Models #################

m1 <- glm(SURV~Algae*TIME,data=df, family="binomial")#not significant
m2 <- glmer(SURV~Algae*TIME+(1|SITE),data=df, family="binomial")#not significant

AICtab(m1,m2,weights=TRUE,base = TRUE)
summary(m2)

################ Mixed Effects Models ##################

#TWO-WAY INTERACTION

M2 <- glmer(SURV~Algae*TIME+(1|SITE),data=df, family="binomial")#not significant

#TWO-WAY ADDITIVE
m2 <- glmer(SURV~Algae+TIME+(1|SITE),data=df, family="binomial")

#ONE-WAY
m1 <- glmer(SURV~TIME+(1|SITE),data=df, family="binomial")
m1.2 <- glmer(SURV~Algae+(1|SITE),data=df, family="binomial")
m1.3 <- glmer(SURV~PSU+(1|SITE),data=df, family="binomial")#not significant
m1.4 <- glmer(SURV~logCELL+(1|SITE),data=df, family="binomial")#not significant

AICtab(m1,m1.2,m1.3,m1.4,m2,M2,weights=TRUE,base = TRUE)

summary(m2)
summary(M2)
summary(m1.3)
summary(m1.4)

##Best model m2


############ Best Model Plots ############

m2 <- glmer(SURV~Algae+TIME+(1|SITE),data=df, family="binomial")
summary(m2)

x<- seq(0, 4, 0.1)
par(mfrow=c (1,1))
```

```
plot(df$TIME,df$SURV,type="n",ylim=c(0,1),xlim=c(0,4),main="Oyster
Survival",ylab="P(survival)",xlab="TIME")
cfm <- fixef(m2)
odds_ratio3m <- cfm[1]+cfm[2]+cfm[3]*x
odds_ratio2m <- cfm[1]+cfm[3]*x

  prob3m <- 1/(1 + (1/exp(odds_ratio3m)))
  prob2m <- 1/(1 + (1/exp(odds_ratio2m)))

lines(x, prob3m,lwd=3,lty=2)
lines(x, prob2m,lwd=3)

legend("bottomleft",inset=0.014,cex= 1.2,
   c("I. galbana","A. lagunensis"), lty = c(1,2),lwd= c(3,3))

#################### Residuals ####################

par(mfrow=c (1,4))
hist(residuals(m2))
plot(df$TIME,residuals(m2))
plot(df$Algae,residuals(m2))
plot(predict(m2),residuals(m2))

detach(df)
```

R Code: Oyster Growth

```
rm(list=ls())

  library(nlme)
  library(bbmle)
  library(lme4)     # for fitting GLMMs
  library(lattice)  # for the xyplot function
  library(MuMIn)    # for the r.squaredGLMM function

setwd("F:/Grad Work/Thesis/Stats")

orig_data<-read.table("Transplant_Darea.txt", header=T)

# remove NA AREA values (dead) and zero concentration control (to correct rank deficiency)
area_data <- subset(orig_data,!is.na(AREA))
area_data <- subset(area_data, Algae != 1)

names(orig_data)
attach(orig_data)

## use factor function to specify categorical variables
area_data$SITE<-factor(area_data$SITE)
area_data$TREAT<-factor(area_data$TREAT)
area_data$ALGAE<-factor(area_data$ALGAE)
area_data$Algae<-factor(area_data$Algae)

area_data$logAREA<-log(area_data$AREA+1)
area_data$logCELL<-log(area_data$CONCEN+1)

#Data transformations
par(mfrow=c(1,2))
hist(Growth)
hist(log(Growth+1))

area_data$logGrowth<-log(area_data$Growth+1)

###Part 1: Lab exposure
#area_data <- subset(area_data, TIME < 0)

table(area_data$ALGAE)

#Test difference in initial shell sizes between AL and Tiso
anova(lm(logAREA~ALGAE,data=area_data))
#significant differences between two algal species initial sizes
#using growth rate accounts for this (change in area rather than total area)
```

```
###Part 2: Field transplant (must detach data and reattach without Part 1 subset)
area_data <- subset(area_data, TIME != -1)

## boxplots
par(mfrow=c(2,2))
boxplot(area_data$logGrowth~area_data$PSU,main="PSU", ylab="log (Growth Rate) (mm^2)")
boxplot(area_data$logGrowth~area_data$CONCEN,main="Cells/mL", ylab="log (Growth Rate(
(mm^2)")
boxplot(area_data$logGrowth~area_data$SITE,main="SITE", ylab="log (Growth Rate)
(mm^2)")
boxplot(area_data$logGrowth~area_data$TIME,main="Week", ylab="log (Growth Rate)
(mm^2)")

######## Full plot with logCELL x-axis
par(mfrow=c(1,2))
plot(area_data$logCELL[area_data$PSU=="25" & area_data$Algae==2
],area_data$logAREA[area_data$PSU=="25"& area_data$Algae==2 ],
ylab="logAREA",xlab="I. galbana",col="red",pch=1,xlim=c(-1,14),ylim=c(0,6))
points(area_data$logCELL[area_data$PSU=="40" & area_data$Algae==2
],area_data$logAREA[area_data$PSU=="40" & area_data$Algae==2 ], col="red")
points(area_data$logCELL[area_data$PSU=="25" & area_data$Algae==2
],area_data$logAREA[area_data$PSU=="25"& area_data$Algae==2 ], col="blue")
points(area_data$logCELL[area_data$PSU=="40" & area_data$Algae==2
],area_data$logAREA[area_data$PSU=="40" & area_data$Algae==2 ], col="red")
points(area_data$logCELL[area_data$PSU=="40" & area_data$Algae==1
],area_data$logAREA[area_data$PSU=="40" & area_data$Algae==1], col="black")

plot(area_data$logCELL[area_data$PSU=="25" & area_data$Algae==3
],area_data$logAREA[area_data$PSU=="25"& area_data$Algae==3 ],
ylab="logAREA",xlab="A. lagunensis",col="red",pch=1,xlim=c(-1,14),ylim=c(0,6))
points(area_data$logCELL[area_data$PSU=="40" & area_data$Algae==3
],area_data$logAREA[area_data$PSU=="40" & area_data$Algae==3 ], col="red")
points(area_data$logCELL[area_data$PSU=="25" & area_data$Algae==3
],area_data$logAREA[area_data$PSU=="25"& area_data$Algae==3 ], col="blue")
points(area_data$logCELL[area_data$PSU=="40" & area_data$Algae==3
],area_data$logAREA[area_data$PSU=="40" & area_data$Algae==3 ], col="red")
points(area_data$logCELL[area_data$PSU=="40" & area_data$Algae==1
],area_data$logAREA[area_data$PSU=="40" & area_data$Algae==1], col="black")
```

```
######## Full plot with Time x-axis
par(mfrow=c(1,2))
plot(area_data$TIME[area_data$PSU=="25" & area_data$Algae==2
],area_data$logGrowth[area_data$PSU=="25"& area_data$Algae==2 ], ylab="Growth Rate
(mm)",xlab="I. galbana",col="red",pch=1,xlim=c(0,4),ylim=c(-0.5,2))
points(area_data$TIME[area_data$PSU=="40" & area_data$Algae==2
],area_data$logGrowth[area_data$PSU=="40" & area_data$Algae==2 ], col="red")
points(area_data$TIME[area_data$PSU=="25" & area_data$Algae==2
],area_data$logGrowth[area_data$PSU=="25"& area_data$Algae==2 ], col="blue")
points(area_data$TIME[area_data$PSU=="40" & area_data$Algae==2
],area_data$logGrowth[area_data$PSU=="40" & area_data$Algae==2 ], col="red")
points(area_data$TIME[area_data$PSU=="40" & area_data$Algae==1
],area_data$logGrowth[area_data$PSU=="40" & area_data$Algae==1], col="black")

plot(area_data$TIME[area_data$PSU=="25" & area_data$Algae==3
],area_data$logGrowth[area_data$PSU=="25"& area_data$Algae==3 ], ylab="Growth Rate
(mm)",xlab="A. lagunensis",col="red",pch=1,xlim=c(0,4),ylim=c(-0.5,2))
points(area_data$TIME[area_data$PSU=="40" & area_data$Algae==3
],area_data$logGrowth[area_data$PSU=="40" & area_data$Algae==3 ], col="red")
points(area_data$TIME[area_data$PSU=="25" & area_data$Algae==3
],area_data$logGrowth[area_data$PSU=="25"& area_data$Algae==3 ], col="blue")
points(area_data$TIME[area_data$PSU=="40" & area_data$Algae==3
],area_data$logGrowth[area_data$PSU=="40" & area_data$Algae==3 ], col="red")
points(area_data$TIME[area_data$PSU=="40" & area_data$Algae==1
],area_data$logGrowth[area_data$PSU=="40" & area_data$Algae==1], col="black")


######################  RANDOM MODELS ############################

m1 <-
lm(area_data$logGrowth~area_data$Algae*logCELL*PSU*area_data$TIME,data=area_data)
m2 <-
lmer(area_data$logGrowth~area_data$Algae*logCELL*PSU*area_data$TIME+(1|SITE),data=a
rea_data)
m3 <-
lmer(area_data$logGrowth~area_data$Algae*logCELL*PSU*area_data$TIME+(1+TIME|spatI
D),data=area_data)
m4<-
lmer(area_data$logGrowth~area_data$Algae*logCELL*PSU*area_data$TIME+(1+TIME|spatI
D)+(1|SITE),data=area_data)

AICtab(m1,m2,m3,m4,weights=TRUE,base = TRUE)

summary(m4)
```

```
############################## FIXED MODELS ################################

#FOUR-WAY INTERACTION
M4 <-
lm(area_data$Growth~logCELL*area_data$TIME*area_data$Algae*PSU,data=area_data)

#THREE-WAY INTERACTION
M3 <- lm(area_data$logGrowth~logCELL*area_data$TIME*Algae,data=area_data)
M3.2 <- lm(area_data$logGrowth~area_data$TIME*area_data$Algae*PSU,data=area_data)
M3.3 <- lm(area_data$logGrowth~area_data$Algae*PSU*logCELL,data=area_data)
M3.4 <- lm(area_data$logGrowth~PSU*logCELL*area_data$TIME,data=area_data)

#TWO-WAY INTERACTION
M2 <- lm(area_data$logGrowth~area_data$Algae*area_data$TIME,data=area_data)
M2.2 <- lm(area_data$logGrowth~area_data$Algae*PSU,data=area_data)
M2.3 <- lm(area_data$logGrowth~area_data$Algae*logCELL,data=area_data)
M2.4 <- lm(area_data$logGrowth~PSU*area_data$TIME,data=area_data)
M2.5 <- lm(area_data$logGrowth~PSU*logCELL,data=area_data)
M2.6 <- lm(area_data$logGrowth~logCELL*area_data$TIME,data=area_data)

#FOUR-WAY ADDITIVE
m4 <-
lm(area_data$logGrowth~logCELL+area_data$TIME+area_data$Algae+PSU,data=area_data)

#THREE-WAY ADDITIVE
m3 <- lm(area_data$logGrowth~logCELL+area_data$TIME+area_data$Algae,data=area_data)
m3.2 <- lm(area_data$logGrowth~area_data$TIME+area_data$Algae+PSU,data=area_data)
m3.3 <- lm(area_data$logGrowth~area_data$Algae+PSU+logCELL,data=area_data)
m3.4 <- lm(area_data$logGrowth~PSU+logCELL+area_data$TIME,data=area_data)

#TWO-WAY ADDITIVE
m2 <- lm(area_data$logGrowth~area_data$Algae+area_data$TIME,data=area_data)
m2.2 <- lm(area_data$logGrowth~area_data$Algae+PSU,data=area_data)
m2.3 <- lm(area_data$logGrowth~area_data$Algae+logCELL,data=area_data)
m2.4 <- lm(area_data$logGrowth~PSU+area_data$TIME,data=area_data)
m2.5 <- lm(area_data$logGrowth~PSU+logCELL,data=area_data)
m2.6 <- lm(area_data$logGrowth~logCELL+area_data$TIME,data=area_data)

#ONE-WAY
m1 <- lm(area_data$logGrowth~area_data$TIME,data=area_data)
m1.2 <- lm(area_data$logGrowth~area_data$Algae,data=area_data)
m1.3 <- lm(area_data$logGrowth~PSU,data=area_data)
m1.4 <- lm(area_data$logGrowth~logCELL,data=area_data)

#Mixed
```

```
Ma <-
lm(area_data$logGrowth~logCELL*area_data$TIME*area_data$Algae+PSU,data=area_data)#a
ddition of salinity beneficial

AICtab (M4,M3,M3.2,M3.3,M3.4,M2,M2.2,M2.3,M2.4,M2.5,M2.6,m4,m3,m3.2,m3.3,m3.4,
m2,m2.2,m2.3,m2.4,m2.5,m2.6,m1,m1.2,m1.3,m1.4,Ma,weights=TRUE,base = TRUE)

summary(m1)

########### Best Model Plots ###########

m1 <- lm(area_data$logGrowth~area_data$TIME,data=area_data)

par(mfrow=c(1,1))
boxplot(area_data$logGrowth~area_data$Algae*area_data$logCELL*area_data$TIME,
main="Oyster Growth", cex.main= 2, xlab="Week", cex.lab = 1.5, ylab="log (Growth Rate)
(mm^2)",
par(mar = c(5, 5, 4, 2)+ 0.1),las = 2,xaxt='n',
col =
c("lightskyblue","blue","peachpuff","red","lightskyblue","blue","peachpuff","red","lightskyblue"
,"blue","peachpuff","red",
"lightskyblue","blue","peachpuff","red","lightskyblue","blue","peachpuff","red"),
at =c(1,2,3,4, 7,8,9,10, 13,14,15,16, 19,20,21,22, 25,26,27,28),
names = c("Low","High","Low","High","Low","High","Low","High","Low","High",
"Low","High","Low","High","Low","High","Low","High","Low","High"))
axis(1,at=c(2.5, 8.5, 14.5, 20.5, 26.5),labels=c("0","1","2","3","4"))

legend("topright",inset=0.014,cex= 1.2,
   c("T-iso Low","T-iso High","AL Low","AL High"),
,fill=c("lightskyblue","blue","peachpuff","red"))

##################### Checking assumptions #################

yhat <- fitted(m1)
summary(yhat)

residuals <- resid(m1)
summary(residuals)

par(mfrow=c (1,1))
plot(residuals)



par(mfrow=c (1,3))
#get unstandardized predicted and residual values
```

```
unstandardizedPredicted <- predict(m1)
unstandardizedResiduals <- resid(m1)
#get standardized values
standardizedPredicted <- (unstandardizedPredicted - mean(unstandardizedPredicted)) /
sd(unstandardizedPredicted)
standardizedResiduals <- (unstandardizedResiduals - mean(unstandardizedResiduals)) /
sd(unstandardizedResiduals)
#create standardized residuals plot
plot(standardizedPredicted, standardizedResiduals, main = "Standardized Residuals Plot", xlab =
"Standardized Predicted Values", ylab = "Standardized Residuals")
#add horizontal line
abline(0,0, col="red")

#create residuals histogram
hist(standardizedResiduals, freq = FALSE,,ylim=c(0,0.5))
#add normal curve
curve(dnorm, add = TRUE)

#get probability distribution for residuals
probDist <- pnorm(standardizedResiduals)
#create PP plot
plot(ppoints(length(standardizedResiduals)), sort(probDist), main = "PP Plot", xlab = "Observed
Probability", ylab = "Expected Probability")
#add diagonal line
abline(0,1)


detach(orig_data)
```

REFERENCES

Abràmoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with ImageJ Biophotonics International 11:36-43.

Aiello, E. 1970. Nervous and chemical stimulation of gill cilia in bivalve mollusks. Physiological Zoology 43.

Anderson, D. M., A. D. Cembella, and G. M. Hallegraeff. 2012. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. Annual Review of Marine Science 4:143-176.

Baker, S.M., and R. Mann. 1994. Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on post-settlement ingestion rates. Journal of Experimental Marine Biology and Ecology 181:239-253.

Barnes, B. B., M. W. Luckenbach, P. R. Kingsley-Smith. 2010. Oyster reef community interactions: The effect of resident fauna on oyster (*Crassostrea* spp.) larval recruitment. Journal of Experimental Marine Biology and Ecology 391:169–177.

Bates, D. M., M. Maechler, and B. Bolker. 2012. lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0.

Beck, M. W., R. D. Brumbaugh, L. Airoldi, A. Carranza, L. D. Coen, C. Crawford, O. Defeo, G. J. Edgar, B. Hancock, and M. C. Kay. 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. Bioscience 61:107-116.

Beiras, R., and J. Widdows. 1995. Induction of metamorphosis in larvae of the oyster *Crassostrea gigas* using neuroactive compounds. Marine Biology 123:327-334.

Berg, G., P. Glibert, M. Lomas, and M. Burford. 1997. Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. Marine Biology 129:377-387.

Bersano, J. G. F., E. J. Buskey, and T. A. Villareal. 2002. Viability of the Texas brown tide alga, *Aureoumbra lagunensis*, in fecal pellets of the copepod *Acartia tonsa*. Plankton Biology and Ecology 49:88-92.

Bonar, D. B., S. L. Coon, M. Walch, R. M. Weiner, and W. Fitt. 1990. Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. Bulletin of Marine Science 46:484-498.

Boudreaux, M. L., L. J. Walters, and D. Rittschof. 2009. Interactions between native barnacles, non-native barnacles, and the eastern oyster *Crassostrea virginica*. Bulletin of Marine Science 84:43-57.

Breuer, J. P. 1962. An ecological survey of the lower Laguna Madre of Texas, 1953-1959. Publications of the Institute of Marine Science, University of Texas, 8(15): 3-183.

Bricelj, V. M., and S. H. Kuenstner. 1989. Effects of the brown tide on the feeding and growth of bay scallops and mussels. In: Cosper, E. M., V. M. Bricelj, and E. J. Carpenter, (Eds.), Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms, Springer Berlin.491-509.

Bricelj, V. M., and D. J. Lonsdale. 1997. *Aureococcus anophagefferens*: Causes and ecological consequences of brown tides in US mid-Atlantic coastal waters. Limnology and Oceanography 42:1023-1038.

Bricelj, V. M., and S. P. MacQuarrie. 2007. Effects of brown tide (*Aureococcus anophagefferens*) on hard clam *Mercenaria mercenaria* larvae and implications for benthic recruitment. Marine Ecology Progress Series 331:147-159.

Bricelj, V. M., S. P. MacQuarrie, and R. Smolowitz. 2004. Concentration-dependent effects of toxic and non-toxic isolates of the brown tide alga *Aureococcus anophagefferens* on growth of juvenile bivalves. Marine Ecology Progress Series 282:101-114.

Burke, R. D. 1983. The induction of metamorphosis of marine invertebrate larvae: stimulus and response. Canadian Journal of Zoology 61:1701-1719.

Buskey, E. J., and C. J. Hyatt. 1995. Effects of the Texas (USA) " brown tide" alga on planktonic grazers. Marine Ecology Progress Series 126:285-292.

Buskey, E. J., P. A. Montagna, A. F. Amos, and T. E. Whitledge. 1997. Disruption of grazer populations as a contributing factor to the initiation of the Texas brown tide algal bloom. Limnology and Oceanography 42:1215-1222.

Buskey, E. J., B. Wysor, and C. Hyatt. 1998. The role of hypersalinity in the persistence of the Texas 'brown tide' in the Laguna Madre. Journal of Plankton Research 20:1553-1565.

Buskey. E. J. 2008. How does eutrophication affect the role of grazers in harmful algal bloom dynamics? Harmful Algae 8: 152–157.

Casas, S. M., J. La Peyre, M. K. La Peyre. 2015. Restoration of oyster reefs in an estuarine lake: Population dynamics and shell accretion. Marine Ecology Progess Series 524:171-184.

Castro, P., and M. E. Huber. 2010b. The Impacts of Humans on the Marine Environment. Marine Biology. 8th ed. McGraw-Hill, New York, NY.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series 210: 223–253.

Cohen, L. D., M. W. Luckenbach, and D. L. Breitberg. 1999. The role of oyster reefs as essential fish habitat: a review of current knowledge and some new perspectives. American Fisheries Society Symposium 22:438-454.

Cole, H., and E. K. Jones. 1939. Some observations and experiments on the setting behaviour of larvae of *Ostrea edulis*. Journal du Conseil 14:86-105.

Cosper, E. M., W. Dennison, A. Milligan, E. J. Carpenter, C. Lee, J. Holzapfel, and L. Milanese. 1989. An examination of the environmental factors important to initiating and sustaining "brown tide" blooms. Coastal and Estuarine Studies 35:317-340.

Cosper, E. M., W. C. Dennison, E. J. Carpenter, V. M. Bricelj, J. G. Mitchell, S. H. Kuenstner, D. Colflesh, and M. Dewey. 1987. Recurrent and persistent brown tide blooms perturb coastal marine ecosystem. Estuaries 10:284-290.

Cushing, D. H. 1974. The natural regulation of fish populations. In Harden-Jones, F. R. (ed) Sea Fisheries Research. Elek Science, London, 399–412.

Decho, A. W. 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanography and Marine Biology: An Annual Review 28:73-153.

DeYoe, H. R., D. A. Stockwell, R. R. Bidigare, M. Latasa, P. W. Johnson, P. E. Hargraves, and C. A. Suttle. 1997. Description and characterization of the algal species *Aureoumbra lagunensis* gen. et sp. nov. and referral of *Aureoumbra* and *Aureococcus* to the Pelagophyceae. Journal of Phycology 33:1042-1048.

Deyoe, H. R., and C. A. Suttle. 1994. The inability of the Texas "brown-tide'' alga to use nitrate-nitrogen and the role of nitrogen in the initiation of a persistent bloom of this organism. Journal of Phycology 30:800-806.

Doblin, M. A., L. C. Popels, K. J. Coyne, D. A. Hutchins, S. C. Cary, and F. C. Dobbs. 2004. Transport of the harmful bloom alga *Aureococcus anophagefferens* by oceangoing ships and coastal boats. Applied and Environmental Microbiology 70:6495-6500.

Fitt, W., S. Coon, M. Walch, R. Weiner, R. Colwell, and D. Bonar. 1990. Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. Marine Biology 106:389-394.

Gainey, L. F., and S. E. Shumway. 1991. The physiological effects of *Aureococcus anophgefferens* ('brown tide') on the lateral cilia of bivalve mollusks. Biological Bulletin (Woods Hole) 181:298-306.

Galstoff, P. 1964. The American oyster *Crassotrea virginica* Gmelin. Fishery Bulletin 64:1-480.

Garvis, S. K., P. E. Sacks, and L. J. Walters. 2015. Formation, Movement, and Restoration of Dead Intertidal Oyster Reefs in Canaveral National Seashore and Mosquito Lagoon, Florida. Journal of Shellfish Research 34(2): 251–258.

Glibert, P. M., D. M. Anderson, P. Gentien, E. Graneli, and K. G. Sellner. 2005. The global, complex phenomena of harmful algal blooms. Oceanography 18:136-147.

Gobler, C. J., F. Koch, Y. Kang, D. L. Berry, Y. Z. Tang, M. Lasi, L. Walters, L. Hall, and J. D. Miller. 2013. Expansion of harmful brown tides caused by the pelagophyte, *Aureoumbra lagunensis* DeYoe et Stockwell, to the US east coast. Harmful Algae 27:29-41.

Gobler, C. J., and W. G. Sunda. 2012. Ecosystem disruptive algal blooms of the brown tide species, *Aureococcus anophagefferens* and *Aureoumbra lagunensis*. Harmful Algae 14:36-45.

Gotelli, N., and A. M. Ellison. 2004. A Primer of Ecological Statistics. Sinauer Associates, Sunderland, Massachusetts.

Grizzle, R. 1990. Distribution and abundance of *Crassostrea virginica* (Gmelin, 1791) (eastern oyster) and *Mercenaria* spp.(quahogs) in a coastal lagoon. Journal of Shellfish Research 9:347-358.

Grizzle, R. E., J. K. Greene, and L. D. Coen. 2008. Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: a comparison with previous laboratory studies, and the value of in situ methods. Estuaries and Coasts 31:1208-1220.

Guillard, R. R. L., and L. H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonella confervacea* (Cleve). Gran. Can J Microbiol 8:229-239.

Gutirrez, L. J., C. J. Jones, D. L. Strayer, and O. O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101:79-90.

Hallegraeff, G. M., and C. J. Bolch. 1991. Transport of toxic dinoflagellate cysts via ships' ballast water. Marine Pollution Bulletin 22(1): 27-30.

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99.

Hallegraeff, G. M. 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: A formidable predictive challenge. Journal of Phycology 46:220-235.

Hallegraeff, G. M., and C. J. Bolch. 1991. Transport of toxic dinoflagellate cysts via ships' ballast water. Marine Pollution Bulletin 22:27-30.

Hallegraeff, G. M., and J. L. Maclean. 1989. Biology, Epidemiology and Management of Pyrodinium Red Tides. International Centre for Living Aquatic Resources Management, Manila, Conf. Proc. 21, 286.

Harke M. J., C. J. Gobler, S. E. Shumway. 2011. Suspension feeding by the Atlantic slipper limpet (*Crepidula fornicata*) and the northern quahog (*Mercenaria mercenaria*) in the presence of cultured and wild populations of the harmful brown tide alga, *Aureococcus anophagefferens*. Harmful Algae 10:503–511.

Havens, K. E. 2015. Climate change and the occurrence of harmful microorganisms in Florida's ocean and coastal waters. Florida Sea Grant Program, University of Florida Institute of Food and Agricultural Sciences. http://edis.ifas.ufl.edu/sg136.

Hickmann, R. W., and L. D. Gruffydd. 1971. The histology of larva of *Ostrea edulis* during metamorphosis. Pages 281-294 in D. J. Crisp (ed). Fourth European Marine Biology Symposium, Cambridge University Press, Cambridge.

His, E. and M. N. L. Seaman. 1992. Effects of temporary starvation on the survival, and on subsequent feeding and growth, of oyster (*Crassostrea gigas*) larvae. Marine Biology 114: 277-279.

Ingle, R. M., and C. Dawson Jr. 1951. Variation in salinity and its relation to the Florida oyster. Salinity variation in Apalachicola Bay. Proceeding of the Gulf and Caribean Fisheries Institute 3: 35-42.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Core Writing Team, R.K. Pachauri and L.A. Meyer (ed), IPCC, Geneva, Switzerland.

Kennedy, V. S. 1996. Biology of larvae and Spat. In: V.S. Kennedy, R.I.E. Newell and A.F. Eble, (ed). The Eastern Oyster *Crassostrea virginica* 371-421.

Kraeuter, J. N., J. M. Klinck, E. N. H. Powell, E. E., S. C. Buckner, R. E. Grizzle, and V. M. Bricelj. 2008. Effects of the fishery on the northern quahog (=hard clam, *Mercenaria*

mercenaria L.) population in Great South Bay, New York: A modeling study. Journal of Shellfish Research 27:653-666.

Laabir, M., Z. Amzil, P. Lassus, E. Masseret, Y. Tapilatu, R. De Vargas, and D. Grzebyk. 2007. Viability, growth and toxicity of *Alexandrium catenella* and *Alexandrium minutum* (Dinophyceae) following ingestion and gut passage in the oyster *Crassostrea gigas*. Aquatic Living Resources 20:51-57.

Lapointe, B. E., L. W. Herren, D. D. Debortoli, and M. A. Vogel. 2015. Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. Harmful Algae 43: 82–102.

Liu, H. B., and E. J. Buskey. 2000a. The exopolymer secretions (EPS) layer surrounding *Aureoumbra lagunensis* cells affects growth, grazing, and behavior of protozoa. Limnology and Oceanography 45:1187-1191.

Liu, H. B., and E. J. Buskey. 2000b. Hypersalinity enhances the production of extracellular polymeric substance (EPS) in the Texas brown tide alga, *Aureoumbra lagunensis* (Pelagophyceae). Journal of Phycology 36:71-77.

Longhurst, A., S. Sathyendranath, T. Platt, and C. Caverhill. 1995. An estimate of global primary production in the ocean from satellite radiometer data. Journal of Plankton Research 17:1245-1271.

Loosanoff, V. L., and J. B. Engle. 1940. Spawning and setting of oysters in Long Island Sound in 1937, and discussion of the method for predicting the intensity and time of oyster setting. Bulletin of the Bureau of Fisheries. 49(33):217-255.

Menzel, R. W. 1954. The prodissoconchs and the setting behavior of three species of oysters. Proceedings of the National Shellfish Association. 44:104-112.

Montagna, P. A., D. A. Stockwell, and R. D. Kalke. 1993. Dwarf surfclam *Mulinia lateralis* (Say, 1822) populations and feeding during the Texas brown tide event. Journal of Shellfish Research 12:433-442.

Newell, R. I. E., and C. J. Langdon. 1996. Mechanisms and physiology of larval and adult feeding. In: V.S. Kennedy, R.I.E. Newell and A.F. Eble, (eds). The Eastern Oyster *Crassostrea virginica* Maryland Sea Grant College, University of Maryland, College Park, Maryland.

Nixon, S.W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41:199–219.

Onuf, C. P. 1996. Seagrass responses to long-term light reduction by brown tide in upper Laguna Madre, Texas: distribution and biomass patterns. Marine Ecology Progress Series 138:219-231.

Osman, R. W., R. B. Whitlatch, and R. N. Zajac. 1989. Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. Marine Ecology Progress Series 54:61-73.

Parke, M. 1949. Studies on marine flagellates. Journal of the Marine Biological Association of the United Kingdom 28:255-286.

Petchey O., P. McPhearson, T. Casey, and P. Morin. 1999. Environmental warming alters food-web structure and ecosystem function. Nature 402: 69–72.

Phlips E. J., S. Badylak, and T. Grosskopf. 2002. Factors Affecting the Abundance of Phytoplankton in a Restricted Subtropical Lagoon, the Indian River Lagoon, Florida, USA. Estuarine, Coastal and Shelf Science 55:385–402.

Phlips E. J., S. Badylak & M. A. Lasi, R. Chamberlain,W. C. Green, L. M. Hall, J. A. Hart, J. C. Lockwood, J. D. Miller, L. J. Morris, J. S. Steward. 2015. From Red Tides to Green and Brown Tides: Bloom Dynamics in a Restricted Subtropical Lagoon Under Shifting Climatic Conditions. Estuaries and Coasts 38: 886–904.

Phlips E. J. and S. Badylak. 2015. Phytoplankton abundance and composition in the Indian River Lagoon. St. Johns River Water Management District Annual Report.

Provancha, J. A., C. R. Hall, and D. M. Oddy. 1992. Mosquito Lagoon environmental resources inventory. NASA Technical Memorandum 107548. The Bionetics Corporation. Kennedy Space Center, Florida.

R Core Team. 2013. R: A language and environment for statistical computing. Vienna, Austria: Foundation for Statistical Computing.

Reeb, C. A., and J. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics 124:397-406.

Sheffield, J., and E. F. Wood. 2008. Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 simulations.Climate Dynamics 31: 79–105.

Shumway, S. E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. Journal of the World Aquaculture Society 21:65-104.

Shumway, S. E. 1996. Natural Environmental Factors. In: V.S. Kennedy, R.I.E. Newell and A.F. Eble, (eds). The Eastern Oyster *Crassostrea virginica*.

Sieburth, J. M., P. W. Johnson, and P. E. Hargraves. 1988. Ultrastructure and ecology of *Aureococcus anophageferens* gen. et sp. nov. (Chrysophyceae): the dominant

picoplankter during a bloom in Narragansett Bay, Rhode Island, summer 1985. Journal of Phycology 24:416-425.

Smayda, T. J. 1980. Phytoplankton species succession. The Physiological Ecology of Phytolankton, I. Morris (ed), Berkeley: University of California Press.

Smayda, T. J. 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. Toxic Marine Phytoplankton, E. Graneli, B. Sundstrom, L. Edler, and D. M. Anderson (eds), Elsevier Science Publishing Company, New York, pp. 29-40.

Smith, N. P. 1993. Tidal and nontidal flushing of Florida's Indian River Lagoon. Estuaries 16:739-746.

Sunda, G. W., E. Graneli, and C. J. Gobler. 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. Journal of Phycology 42: 963–97.

Sunda, G. W., and K. W. Shertzer. 2012. Modeling ecosystem disruptive algal blooms: positive feedback mechanisms. Marine Ecology Progress Series 447: 31-47.

Talmage, S. C., and C. J. Gobler. 2009. The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and eastern oysters (*Crassostrea virginica*). Limnology and Oceanography 54:2072-2080.

Talmage, S. C., and C. J. Gobler. 2012. Effects of CO2 and the harmful alga *Aureococcus anophagefferens* on growth and survival of oyster and scallop larvae. Marine Ecology Progress Series 464:121–134.

Tamburri, M. N., C. M. Finelli, D. S. Wethey, and R. K. Zimmer-Faust. 1996. Chemical induction of larval settlement behavior in flow. Biological Bulletin 191:367-373.

Thompson, R. J., R. I. E. Newell, V. S. Kennedy, and R. Mann. 1996. Reproduction and larval development. In: V. S. Kennedy, R.I.E. Newell, and A.F. Eble (eds). The eastern oyster, *Crassostrea virginica*. Maryland Sea Grant Publication, College Park, Maryland, pp. 335-370.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biological Review 25:l-45.

Tracey, G. A. 1988. Feeding reduction, reproductive failure, and mortality in *Mytilus edulis* during the 1985 " brown tide" in Narragansett Bay, Rhode Island. Marine Ecology Progress Series 50:73-81.

Turner, R. E. (2006). Will lowering estuarine salinity increase Gulf of Mexico oyster landings? Estuaries and Coasts, 29(3), 345-352.

Walters, L. J., A. Roman, J. Stiner, and D. Weeks. 2001. Water Resource Management Plan, Canaveral National Seashore. Titusville, Florida. National Park Service, Canaveral National Seashore.

Ward, L. A., P. A. Montagna, R. D. Kalke, and E. J. Buskey. 2000. Sublethal effects of Texas brown tide on *Streblospio benedicti* (Polychaeta) larvae. Journal of Experimental Marine Biology and Ecology 248:121-129.

Wilberg, M. J., M. E. Livings, J. S. Barkman, B. T. Morris, and J. M. Robinson. 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Marine Ecology Progress Series 436:131-144.

Wuebbles, D., G. Meehl, K. Hayhoe, T. R. Karl, K. Kunkel, B. Santer, M. Wehner, B. Colle, E.M. Fischer, R. Fu, A. Goodman, E. Janssen, V. Kharin, H. Lee, W. Li, L. N. Long, S. C. Olsen, Z. Pan, A. Seth, J. Sheffield, and L. Sun. 2014. CMIP5 climate model analysis:

climate extremes in the United States. Bulletin of the American Meteorological Society 95:571–583.

Zhang, Q. C., L. M. Qiu, R. C. Yu, F. Z. Kong, Y. F. Wang, T. Yan, C. J. Gobler, and M. J. Zhou. 2012. Emergence of brown tides caused by *Aureococcus anophagefferens* Hargraves et Sieburth in China. Harmful Algae 19:117-124.

Zingone, A., and H. O. Enevoldsen. 2000. The diversity of harmful algal blooms: challenge for science and management. Ocean and Coastal Management 43:725-748.