# PREDICTING HARMFUL ALGAL BLOOMS: A CASE STUDY WITH *DINOPHYSIS OVUM* IN THE GULF OF MEXICO

A Thesis

by

LAURA BROOKE HARRED

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| Chair of Committee, | Lisa Campbell |
| Committee Members, | George Jackson |
| | Heidi Sosik |
| | Masami Fujiwara |
| | Steve DiMarco |
| Head of Department, | Debbie Thomas |

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ABSTRACT

Blooms of *Dinophysis ovum* and *Mesodinium spp.* have been observed in the Gulf of Mexico since 2007 using Imaging FlowCytobot technology. Bloom dynamics of these two organisms in conjunction with ancillary environmental data for a 5 year period were analyzed to identify the conditions necessary for bloom initiation or presence with the goal of predicting future blooms of *Dinophysis*. I determined that a narrow range of temperature and salinity may be necessary for bloom initiation of *Dinophysis* and *Mesodinium* in the Gulf of Mexico. Using time series analysis, I observed a positive time-lagged correlation between the two organisms in each year when both were present, which indicates that presence of *Mesodinium* can be used as a leading indicator for a *Dinophysis* bloom. Analysis of images over the time series also revealed a wide range in the size of *Mesodinium* cells, which suggests that species other than *M. rubrum* may be present in the Gulf of Mexico. Finally, based on the occurrence of a *Dinophysis* bloom preceded by low abundances of *Mesodinium*, I believe that *Dinophysis* is able to utilize ciliates other than *M. rubrum* as prey. My observations indicate that these factors can affect initiation, presence or abundance of *Dinophysis* and thus may help in the prediction of future blooms.

# DEDICATION

This thesis is dedicated to my parents, Ron & Stephanie Harred, and my sister, Rachel Harred, for their love and support.

# ACKNOWLEDGMENTS

I would like to thank my committee chair, Dr. Lisa Campbell, and my committee members, Dr. George Jackson, Dr. Steve DiMarco, Dr. Heidi Sosik, and Dr. Masami Fujiwara, for their guidance and support throughout the course of this research. A special thanks to Dr. Campbell, for teaching the undergraduate oceanography course that led me to oceanography research. Thanks to Dr. Sosik for advice and help with Imaging FlowCytobot data processing, Dr. Jackson for help with data analysis, Dr. DiMarco for teaching the data methods course that taught me so much about data processing, and Dr. Fujiwara for the helpful advice and suggestions for this project. I also thank TAMUCC for providing the environmental data used in this project and all members of the Campbell laboratory for comments on the manuscript.

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# INTRODUCTION

Species of the genus *Dinophysis* are distributed worldwide in coastal and oceanic waters and are known to cause harmful algal blooms (Hallegraeff & Lucas, 1988). Recently, this toxic dinoflagellate has been observed blooming in the Gulf of Mexico (Campbell et al., 2010). Species of *Dinophysis* produce okadaic acid, dinophysis-toxins and pectenotoxins, which can cause diarrhetic shellfish poisoning (DSP) in humans (Yasumoto, 1985). Mixotrophic species of *Dinophysis* use a peduncle to consume the cell contents of their prey and can maintain photosynthetically active plastids for several generations, enabling growth in the absence of prey (Kim et al., 2008, 2012). Length of growth in the absence of prey varies among species and can range from one week to more than one month after feeding (Kim et al., 2008; Nielsen et al., 2012). Survival of *Dinophysis* in the absence of prey can be much longer; it has been reported that some species of *Dinophysis* can survive up to 3 months in the light, but maximum growth (0.4 – 0.9 divisions $day^{-1}$ at 15 – 20 ºC) will not be maintained (Hansen et al., 2013; Nielsen et al., 2012). *Mesodinium rubrum* (= *Myrionecta rubra*) has been identified as a prey item for *Dinophysis* when grown with the cryptophyte *Teleaulax sp.* in culture and is the only confirmed species of *Mesodinium* that *Dinophysis* utilizes as prey (Kim et al., 2008, 2012; Nagai et al., 2008; Nishitani et al., 2008, 2010; Park et al., 2006).

*M. rubrum* is a non-toxic, mixotrophic ciliate that is globally distributed (Crawford, 1989; Garcia-Cuetos et al., 2012; Johnson et al., 2013; Johnson and Stoecker, 2005). *M. rubrum* can maintain photosynthetic growth in the absence of prey for several

weeks and can survive without prey for several months (Hansen et al., 2013; Myung et al., 2013). It has been proposed that *Mesodinium* availability is one essential condition for a subsequent *Dinophysis* bloom (Diaz et al., 2013). Several culture experiments have reported an increased growth rate in *Dinophysis* with an increase in *M. rubrum* availability, showing the dependence of *Dinophysis* on *Mesodinium* (Kim et al., 2008; Riisgaard et al., 2009; Tong et al., 2010). It has also been reported that increased abundances of *M. rubrum* have preceded *Dinophysis* blooms in field studies in several locations (Campbell et al., 2010; Diaz et al., 2013; Minnhagen, 2010; Velo-Suarez et al., 2013).

In 2008, a large *Dinophysis ovum* bloom occurred in the Gulf of Mexico and early warning was provided using Imaging FlowCytobot (IFCB) images (Campbell et al., 2010; Swanson et al., 2010). This event led to the first closure of shellfish beds and recall of oysters in the United States due to high *D. ovum* abundance and okadaic acid contamination in shellfish. This shutdown of shellfish harvesting occurred shortly before a local annual oyster festival where up to 30,000 people might have been affected by DSP (Campbell et al., 2010; Deeds et al., 2010). Prior to this unexpected *D. ovum* bloom, *Mesodinium spp*. had a period of high abundance. Campbell and co-workers (Campbell et al., 2010) noted a wide range in size of the *Mesodinium* cells seen in IFCB images throughout the course of the bloom. Previously, differences in size of *Mesodinium* cells were attributed to variations in nutrients and prey availability (Montagnes et al., 2008). Recently, Garcia-Cuetos et al. (2012) compared 5 species of *Mesodinium* and reported a difference in size among the species.

The IFCB has provided image data of *Mesodinium* and *Dinophysis* abundance since the event in 2008. To investigate bloom dynamics of the two organisms, I examined IFCB cell abundance data for 2007 – 2012 to determine (i) if *Mesodinium*, as prey for *Dinophysis*, can be used as a predictor for a *Dinophysis* bloom, (ii) if environmental conditions have an influence on bloom onset or bloom formation of *Dinophysis* and *Mesodinium* and (iii) if differences in *Mesodinium* cell size is evidence of multiple species in the Gulf of Mexico. Results from this study will add to the understanding of bloom dynamics of the two organisms and may assist in predicting the occurrence of future *Dinophysis* blooms.

# METHODS

## Sampling Region and Data Acquisition

The IFCB has been deployed at the University of Texas Marine Science Institute (UTMSI) pier laboratory, located on the Port Aransas, TX, USA ship channel (27.84 ºN, 97.05 ºW) since September 2007. This relatively new imaging system collects real time, near-continuous observations of algal species abundance. Combining flow cytometry and video technology, the IFCB is designed to record images of phytoplankton cells within the size range ~10 – 100 μm (Olson & Sosik, 2007; Sosik & Olson, 2007). A 5mL sample is analyzed every 20 minutes and a file is produced containing images of the phytoplankton community. Many of these images can be identified to species (Campbell et al., 2010; Sosik & Olson, 2007). The Port Aransas ship channel is a well-mixed channel with strong tidal currents. Temperature ranges from 10 – 37 ºC (average ~23 ºC), salinity ranges from ~13 – 40 (average ~33), and tidal velocity ranges from -1.5 – 1.8 meters second$^{-1}$ where negative values indicate water movement into the channel.

## Data Classification

The IFCB data were processed and classified following the approach described in Sosik and Olson (2007) and Campbell et al. (2010) with the modification of replacing the support vector machine with the random forest approach described in Breiman (2001). Six automated classifiers were created with the intention to optimize accurate enumeration of the *Dinophysis* and *Mesodinium* categories. A different threshold of

classification probability scores was selected for each classifier from the random forest as implemented by the TreeBagger function in MATLAB. The different thresholds selected were the values that gave the least number of residuals between manual (see below) and classifier-estimated abundances.

Each classifier contains 53 categories that were chosen based on the community composition of phytoplankton seen in the sampling region. Training sets for each category except *Dinophysis* and *Mesodinium* were made up of images spanning the data set from 2007-2012. *Dinophysis* and *Mesodinium* training sets were modified to contain only images from one year of the data set for each year of the time series (six classifiers total). Each of the six classifiers was applied only to the year corresponding to *Dinophysis* and *Mesodinium* training set images (i.e., 2007 classifier applied only to 2007 data). The data were separated into 5 intervals, each ranging from September to August in order to cover the full blooms of *Mesodinium* and *Dinophysis* (e.g., September 2007 – August 2008).

To check the accuracy of each automated classifier, a large number of files (~300 – 2000) from each year of data were manually corrected. These files were visually inspected and images of *Dinophysis* and *Mesodinium* were manually sorted into their correct categories. A correlation between manual and automated results was computed for each of the 5 intervals (Table 1). By creating a different classifier for each year of data, the correlations of automated results to manual were higher than when one classifier was applied to the entire data set. A correction factor was applied to automated results of *Mesodinium* abundance from 2008 for the 2008/09 interval. By multiplying

*Mesodinium* abundance for September 1-December 31, 2008 by 4.5, the correlation of automated results to manual for the 2008/09 interval was improved. A correction was not required for any other year.

Table 1. Correlation between automated classifier and manually corrected results

| | *Mesodinium* | *Dinophysis* |
|---|---|---|
| Time interval | Correlation coefficient | Correlation coefficient |
| 2007/08 | 0.96 | 0.94 |
| 2008/09 | 0.64 | 0.68 |
| 2009/10 | 0.83 | 0.97 |
| 2010/11 | 0.91 | 0.97 |
| 2011/12 | 0.79 | 0.98 |

All correlations are significant with $p < 0.01$

Manually corrected files span the data set from the onset of each bloom to termination in most cases; bloom termination for 2012 was not collected due to an instrument shutdown. Manual results were used to determine bloom initiation times for *Dinophysis* and *Mesodinium*. In this study, background cell abundance is defined as concentration < 2 cells $mL^{-1}$ and bloom initiation is defined as the first observation of concentration ≥ 2 cells $mL^{-1}$, both based on empirical observations of my time series. A bloom is defined as concentration ≥ 5 cells $mL^{-1}$, based on the legal limit of abundance necessary for the closure of shellfish harvesting for other HAB species as reported by the U.S. Food and Drug Administration (FDA, 2011).

Species identification of *Dinophysis* from the 2008 event was verified using molecular analysis and it was found that the bloom was primarily dominated by *D. ovum.* Images of *Dinophysis* from subsequent data were determined to be *D. ovum* based on visual comparisons to the *Dinophysis* images from 2008. Here, and in the remainder of this manuscript, *Dinophysis* refers to *D. ovum.*

**Size Analysis**

Cell size estimates were calculated from manually inspected IFCB images of *Mesodinium*. The estimated size of each cell was obtained using the cross sectional area of each image following the method described in Henrichs et al. (2011). The cross sectional area was used as a proxy for cell size and will be referred to as cell size throughout. Estimates of *Mesodinium* cell size were used to identify differences in size over the course of each bloom and among years. Approximated cross sectional area of *Mesodinium* species were calculated using the length and width ranges given by Garcia-Cuetos et al (2012) and the equation for the area of an ellipse, given the generalized geometric shape of *Mesodinium*.

**Environmental Data**

Environmental data were downloaded from two stations using the TAMU Corpus Christi Division of Nearshore Research website (http://lighthouse.tamucc.edu). Hourly water temperature and tidal velocity data were obtained from the Real-Time Navigation System Station (RTNS, Station 109) and hourly salinity data were obtained from the

Mission Aransas National Estuarine Research Reserve (MANERR #5, Station 149). Both stations are located on the UTMSI pier in Port Aransas. All data was linearly interpolated to replace missing values.

A portion of the 2008 salinity record is questionable with unexplained decreases on a two week frequency interval. This is not expected to interfere with results from this study; bloom initiation of *Dinophysis* and *Mesodinium* did not coincide with the questionable data.

**Statistical Analysis**

Statistical analysis was performed using MATLAB Statistics Toolbox (MATLAB R2011, The MathWorks Inc). All data were tested for normality; automated cell abundance data were not normally distributed and were log(x + 1) transformed prior to time series analysis, where x = cells $mL^{-1}$, in order to account for abundances with a value of zero throughout the time series. Cell abundance and environmental data were analyzed using one-way analysis of variance (ANOVA) and the Tukey-Kramer honestly significant difference (HSD) procedure to determine differences among years. ANOVA was used to determine whether variations among years of cell abundance data were related to variations in environmental variables. Time series of temperature, salinity and cell abundance were compared using time-lagged correlations to observe the interannual relationship between cell abundance and environmental variables. These time series were put into standard form prior to analysis (i.e., demeaned and divided by the standard deviation). A maximum lag of 2000 hours (~83 days) was chosen for the time-lagged

correlations in order to focus on the most influential time period surrounding the blooms of *Dinophysis* and *Mesodinium*. Because the time series of *Dinophysis* and *Mesodinium* abundance were non-stationary, significance for all computed correlations were obtained after degrees of freedom were calculated (Emery & Thomson, 2001). ANOVA and the Tukey-Kramer HSD procedure were used to determine differences among years of *Mesodinium* cell sizes. These data were found to be log normally distributed and were log transformed prior to the ANOVA.

# RESULTS

## Cell Abundance and Bloom Timing

Over the time series, *Dinophysis* blooms occurred in four of the five years: 2007/08, 2009/10, 2010/11 and 2011/12 (Figure 1). *Mesodinium* blooms also occurred in four of the five years: 2007/08, 2008/09, 2009/10 and 2010/11 (Figure 1). The 2007/08 blooms of *Dinophysis* and *Mesodinium* had the highest abundance reaching peaks of ~200 and ~300 cells $mL^{-1}$, respectively (Table 2). The highest abundance of *Mesodinium* occurred in late January and the highest abundance of *Dinophysis* occurred about one month later, in late February. In later years, cell abundance of *Dinophysis* and *Mesodinium* was lower and never reached concentrations comparable to the 2007/08 event. In 2008/09, although *Mesodinium* was present above bloom concentration, *Dinophysis* cell concentration remained below 1 cell $mL^{-1}$ for the entire year. In 2009/10, the peak in abundance of *Mesodinium* occurred in early February and cell concentration fluctuated above 10 cells $mL^{-1}$ until the end of April. The *Dinophysis* peak in abundance occurred in mid-April, which was ~2.5 months after the highest peak in abundance of *Mesodinium*. In 2010/11, the highest peak in abundance of *Mesodinium* occurred in mid-December, but cell counts remained above 10 cells $mL^{-1}$ through mid-January. The *Dinophysis* peak in abundance occurred 2 months later in mid-March. In 2011/12, although *Mesodinium* was present above background levels, it did not reach bloom concentrations prior to the *Dinophysis* bloom, which reached the highest peak in abundance in early February.

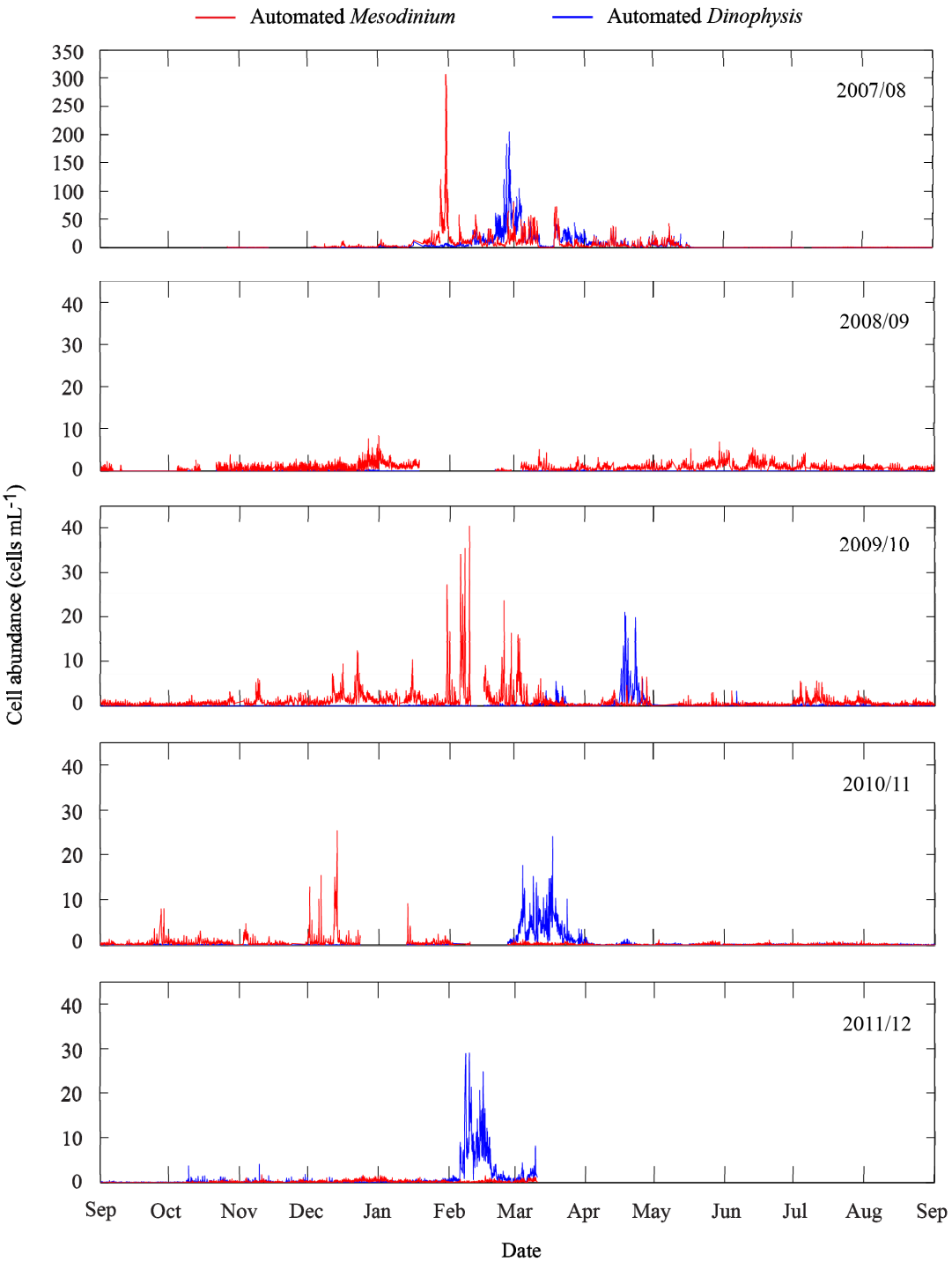


**Figure 1.** Time series of *Dinophysis* and *Mesodinium* at Port Aransas, TX, U.S.A (27.84 °N, 97.05 °W). Automated results for *Dinophysis* are in blue and *Mesodinium* in red. Note different scale in 2007/08.

Table 2. Bloom peak abundance and timing of automated time series

| Time interval | *Mesodinium* | | *Dinophysis* | |
|---|---|---|---|---|
| | Peak time | Peak abundance [a] | Peak time | Peak abundance [a] |
| 2007/08 | 01/31/2008 | 306 | 02/27/2008 | 205 |
| 2008/09 | 01/01/2009 | 8 | N/A [b] | N/A [b] |
| 2009/10 | 02/09/2010 | 40 | 04/18/2010 | 21 |
| 2010/11 | 12/13/2010 | 25 | 03/17/2011 | 24 |
| 2011/12 | N/A [b] | N/A [b] | 02/09/2012 | 29 |

[a] abundance in cells $mL^{-1}$

[b] No bloom occurred

*Mesodinium* blooms occurred between mid-September and May. Correlations between *Mesodinium* abundance of bloom years with temperature and salinity were not significant. Most blooms of *Mesodinium* corresponded to temperature and salinity values that were below the inter-quartile range ($25^{th}$ – $75^{th}$ percentiles) of their distribution (Figure 2A). The bloom initiation of *Mesodinium* during bloom years ranged from mid-September through the end of October (Table 3). Temperature and salinity during bloom initiation of bloom years ranged from ~25 – 29 ºC and ~30 – 34 respectively. Bloom initiation occurred during an incoming tide in each year except 2009/10. A bloom initiation date for *Mesodinium* could not be identified for 2008/09 because cell concentrations continued to fluctuate above the 2 cells $mL^{-1}$ threshold after the large 2007/08 bloom until the end of the 2008/09 bloom.

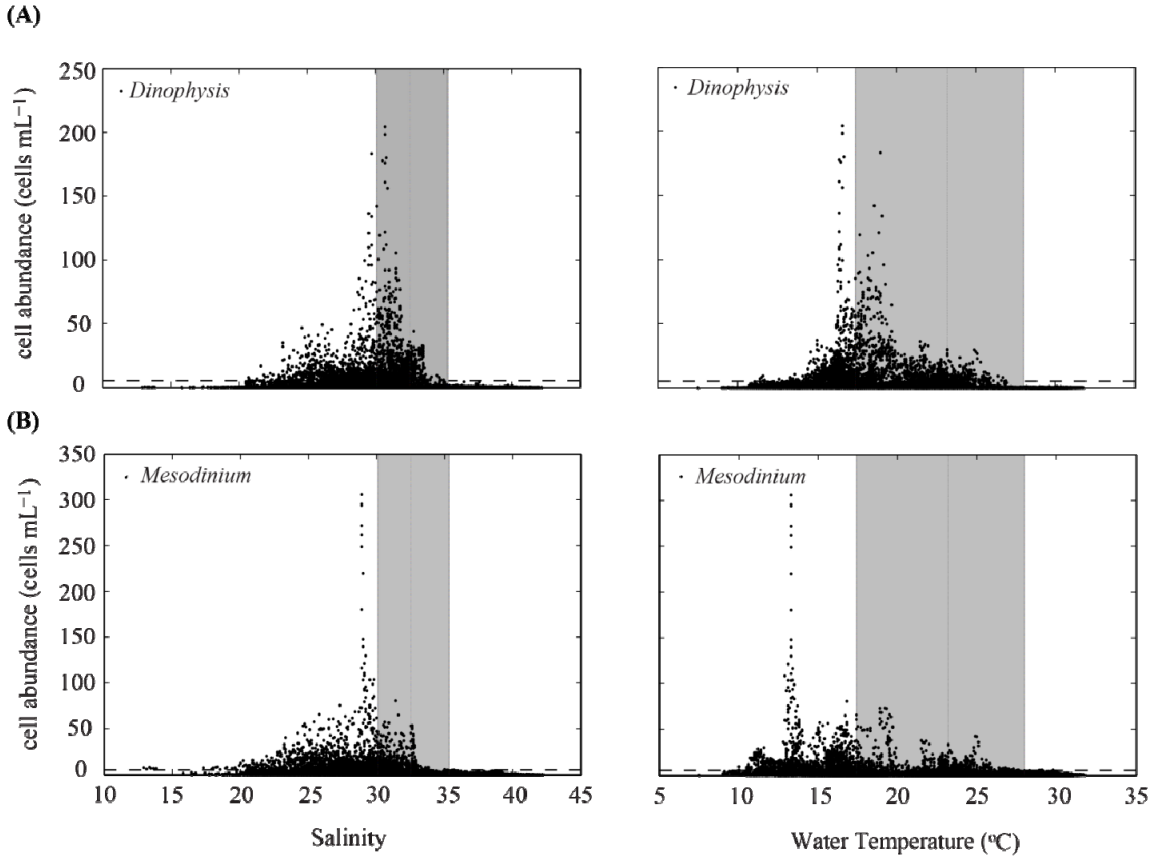


**Figure 2.** Temperature and salinity values plotted with **(A)** *Dinophysis* and **(B)** *Mesodinium* abundance. The solid block represents the 25th – 75th percentile range of temperature and salinity. Note the difference in scale for *Dinophysis* and *Mesodinium* abundance.

*Dinophysis* blooms occurred between the end of January and the end of May. Correlations of *Dinophysis* abundance of bloom years with temperature and salinity were not significant. Most blooms of *Dinophysis* corresponded to temperature and salinity values that were within or slightly below the inter-quartile range of their distribution (Figure 2B). Bloom initiation of *Dinophysis* for the time series ranged from the end of January to mid-March (Table 3). Temperature and salinity during bloom initiation ranged from ~12 – 18 ºC and ~29 – 33, respectively. Bloom initiation occurred on, or

just after, an incoming tide each year, with the exception of 2009/10, when velocity = 0 after the incoming tide.

Table 3. Environmental conditions during bloom initiation of *Dinophysis* and *Mesodinium*

| | *Mesodinium* | | | | *Dinophysis* | | | |
|---|---|---|---|---|---|---|---|---|
| Time interval | Bloom initiation date | T (°C) | Salinity | Tidal velocity | Bloom initiation date | T (°C) | Salinity | Tidal velocity |
| 2007/08 | 10/27/2007 | 24.6 | 33.4 | -0.66 | 01/20/2008 | 12.0 | 30.4 | -0.91 |
| 2008/09 | N/A[a] | N/A[a] | N/A[a] | N/A[a] | No Bloom[b] | No Bloom[b] | No Bloom[b] | No Bloom[b] |
| 2009/10 | 10/13/2009 | 26.6 | 33.9 | -0.67 | 03/14/2010 | 15.2 | 28.6 | 0 |
| 2010/11 | 09/19/2010 | 29.4 | 29.5 | 0.27 | 03/01/2011 | 17.7 | 31.9 | -0.73 |
| 2011/12 | 11/10/2011[c] | 19.9[c] | 34.7[c] | -0.56[c] | 02/06/2012 | 17.2 | 32.5 | -0.55 |

[a] No bloom initiation of *Mesodinium* for 2008/09
[b] No bloom of *Dinophysis* occurred in 2008/2009
[c] Bloom initiation of *Mesodinium* reported for 2011/12, but no bloom occurred

To determine if variations among years of cell abundance were related to variations among years of temperature, salinity and tidal velocity, ANOVA was used. Results showed that all years of automated cell counts of *Dinophysis* and *Mesodinium* were significantly different (Figure A1, Figure A2). All years of salinity were significantly different, and only temperature values from 2007/08 and 2010/11 were found to have no significant difference from each other (Figure A3, Figure A4).

**Time Series Analysis**

Time-lagged cross correlations are used to help determine if one variable can be used as a leading indicator of another. In this study, I found that there was a positive trend in correlations between *Dinophysis* and *Mesodinium* abundance each year except

in 2008/09 when *Dinophysis* was not present (Table 4; Figure 3). The time lag for the highest positive correlation values ranged from 46 – 62 days, and the correlation coefficients ranged from r = 0.38 – 0.50 (P < 0.01).

There was a negative pattern of correlations between *Dinophysis* abundance and temperature at zero lag each year except in 2009/10, but the correlations were not significant. The correlations between *Dinophysis* abundance and salinity were negative at zero lag each year, but were not significant. There was a negative pattern of correlation for *Mesodinium* abundance with temperature and salinity at zero lag each year except 2008/09, but the correlation was only significant in 2009/10 (P < 0.05). Results showed a positive trend correlation between temperature and salinity for most lag phases every year, but the correlations were not significant.

Table 4. Strongest correlations with lag from time series analysis

| Time interval | D-M[a] | D-T[b] | D-S[c] | M-T[d] | M-S[e] | T-S[f] |
|---|---|---|---|---|---|---|
| 2007/08 | 0.78 (0) | -0.71 (31) | -0.45 (0) | -0.69 (20) | -0.55 (0) | 0.67 (-23) |
| 2008/09 | 0.10 (-17)* | -0.33 (24) | -0.26 (-56) | 0.22 (-61)* | 0.21 (-60)** | 0.56 (-38) |
| 2009/10 | 0.38 (62)** | 0.31 (-77)** | -0.25 (50)* | -0.45 (-4) | -0.51 (0)* | 0.66 (0) |
| 2010/11 | 0.38 (51)** | -0.60 (27)* | 0.26 (78) | -0.43 (-32)* | -0.33 (0) | 0.46 (0) |
| 2011/12 | 0.50 (46)** | -0.47 (47) | -0.56 (0) | -0.55 (0) | -0.44 (3) | 0.77 (-1) |

Lag (in days) of correlation in parenthesis, * $p < 0.05$, ** $p < 0.01$

[a] *Dinophysis* - *Mesodinium*

[b] *Dinophysis* - Temperature

[c] *Dinophysis* - Salinity

[d] *Mesodinium* - Temperature

[e] *Mesodinium* - Salinity

[f] Temperature - Salinity

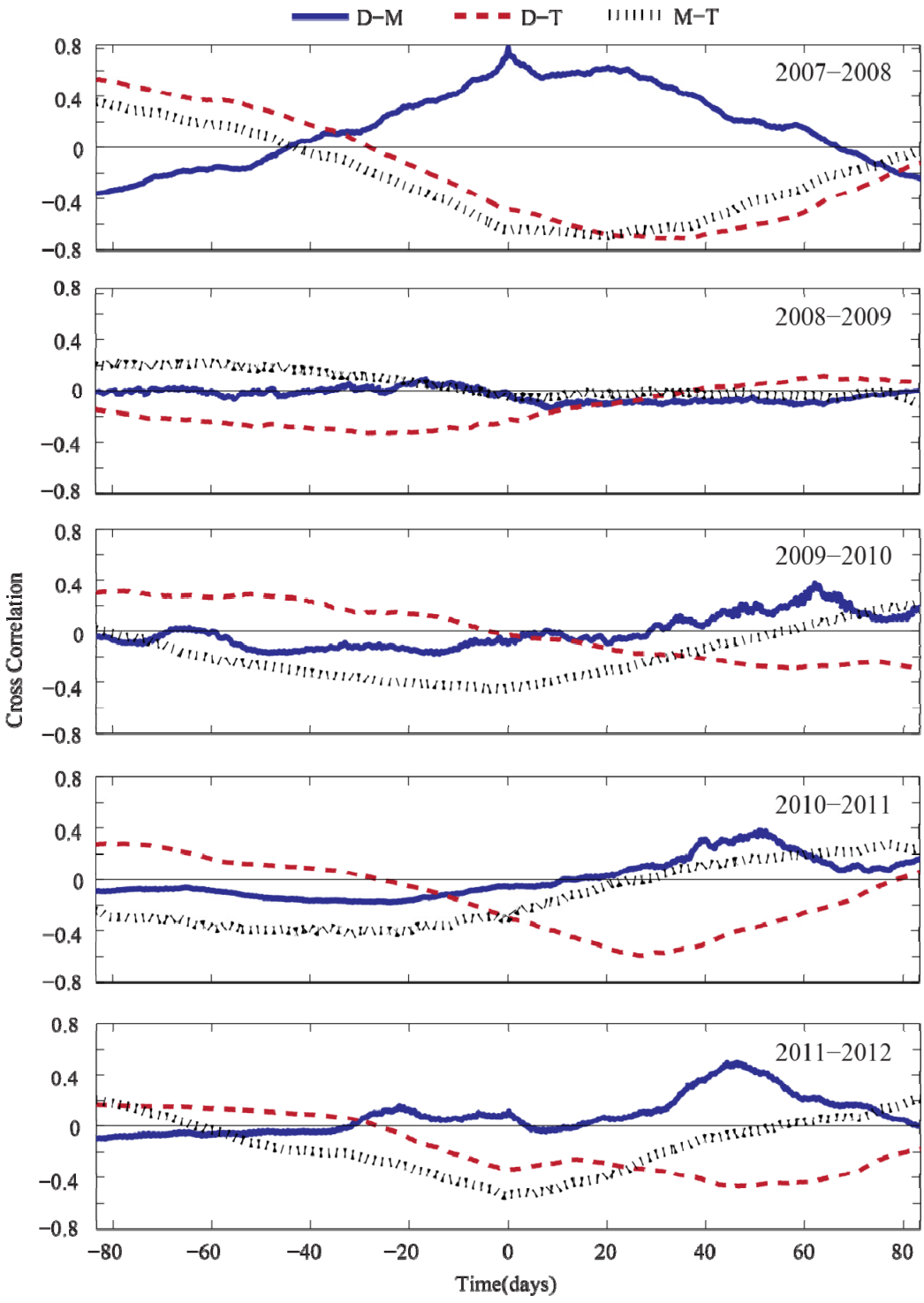


**Figure 3.** Cross correlations of automated cell abundance of *Dinophysis* and *Mesodinium* with temperature and cross correlation of *Dinophysis* with *Mesodinium*. D - *Dinophysis*, M - *Mesodinium*, T - Temperature.

**Size Analysis**

The cross sectional area of *Mesodinium* cells ranged from 224 $\mu m^2$ – 4415 $\mu m^2$ (Figure 4). Using cross sectional area as a proxy for cell size, *Mesodinium* cell size was greatest in 2007/08 and lowest in 2008/09 with average values 2094 $\mu m^2$ and 731 $\mu m^2$, respectively. There was a wide range in *Mesodinium* cell size throughout the course of each bloom and among years (Figure 5). The widest range in sizes occurred in 2007/08 (~283 $\mu m^2$ – 4415 $\mu m^2$) and the smallest range occurred in 2011/12 (~ 224 $\mu m^2$ – 2433 $\mu m^2$). The results from the ANOVA showed that *Mesodinium* average cell sizes were significantly different in each year of the time series (Figure A5).

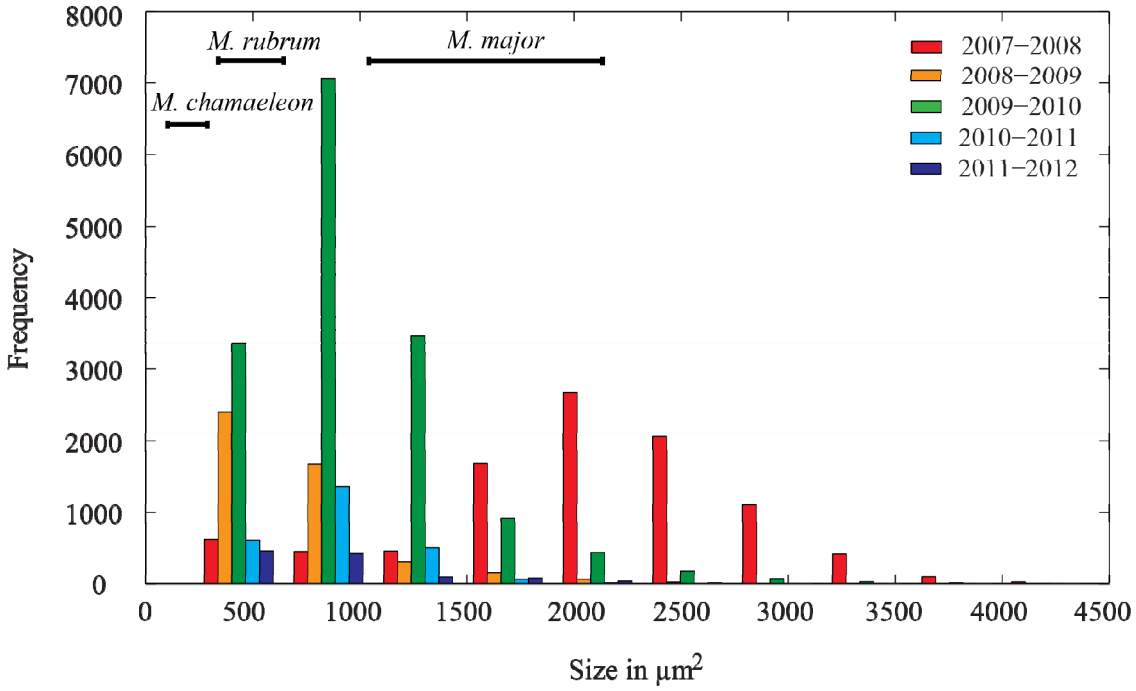


**Figure 4.** Histogram of *Mesodinium* cell sizes for each bloom interval. Black bars represent area estimates using size ranges from Garcia – Cuetos (2013) for *M. rubrum*, *M. major* and *M. chamaeleon*.

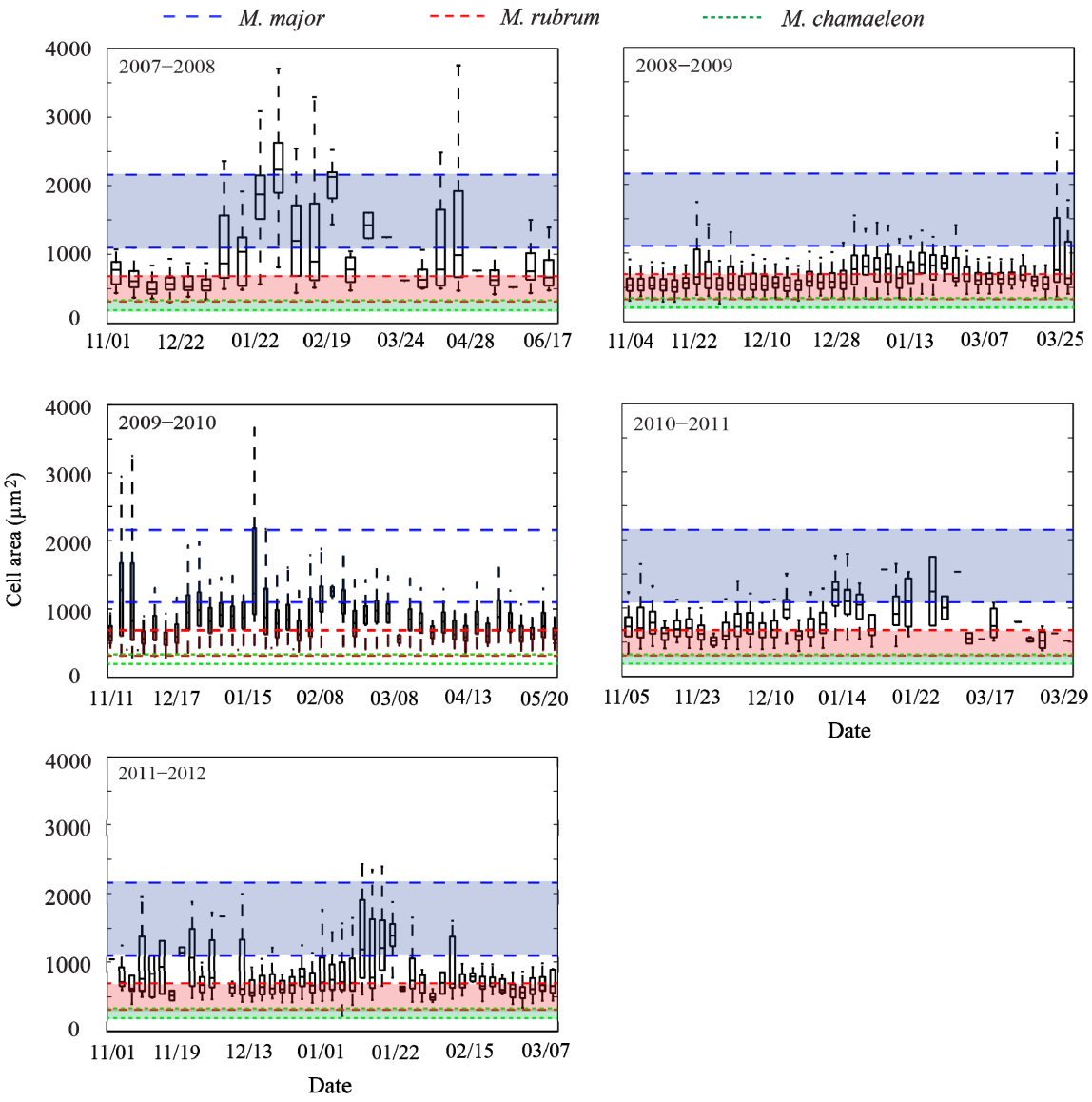


**Figure 5.** Size of *Mesodinium* cells over the course of the bloom period. Boxplots show median and the inter – quartile range (25th - 75th percentiles). The dotted horizontal lines represent the size ranges as described by Garcia - Cuetos (2012) of *M. rubrum*, *M. major* and *M. chamaeleon*. There is no *Mesodinium* species described by Garcia-Cuetos (2012) in the size range between estimates for *M. rubrum* and *M. major.*

# DISCUSSION

## Time Series of Cell Abundance

Results from this study have shown that *Mesodinium* bloomed prior to *Dinophysis* blooms each year except 2011/12, when *Mesodinium* was present but did not exceed the defined bloom threshold concentration. These observations provide evidence that *Mesodinium* availability may be necessary for the formation of a *Dinophysis* bloom, as suggested by recent studies (Diaz et al., 2013), and that presence of *Mesodinium* can be used as a predictor for *Dinophysis* blooms. However, the ratio of prey to predator necessary for a bloom is not yet known. I suggest that bloom concentrations of *Mesodinium* each year were related to the bloom concentration of *Dinophysis*, except in 2011/12. The *Mesodinium* bloom in 2007/08 was the largest of the time series and was followed by the largest *Dinophysis* bloom of the time series. This leads to the hypothesis that the high abundance of *Dinophysis* in this year was directly linked to the high abundance of *Mesodinium*.

I observed a wide range in the timing of bloom initiation for both *Mesodinium* (09/19 – 11/10) and *Dinophysis* (01/20 – 03/14). The temperature and salinity values during bloom initiation periods for *Dinophysis* were narrow (~12 – 18 ºC and ~29 – 33 respectively), and I believe that these conditions are favorable for the formation of a bloom. Similarly, there was a narrow range of temperature and salinity during bloom initiation periods for *Mesodinium* bloom years (~25 – 29 ºC and ~30 – 34 respectively). In 2011/12, *Mesodinium* was present above 2 cells $mL^{-1}$, and bloom initiation for this

year was observed, but a bloom (≥ 5 cells $mL^{-1}$) did not occur. The temperature during bloom initiation for 2011/12 was much lower than any other year (20 ºC) and salinity was higher than any other year (35). I propose that a temperature range of 25 – 29 ºC and a salinity range of 30 – 34 are favorable to *Mesodinium* for bloom formation and given that the temperature and salinity values were outside of this range in 2011/12, a bloom did not occur. Nevertheless, additional years of data will be needed to confirm this explanation for the absence of a *Mesodinium* bloom in 2011/12. The temperature ranges observed during blooms of *Dinophysis* and *Mesodinium* are comparable to previous field and culture studies (Hansen et al., 2013; Johnson et al., 2013). The salinity ranges observed are similar to many culture studies, but are higher than many field observations (Johnson et al., 2013; Kim et al., 2012; Park et al., 2008; Yih et al., 2013).

I found that in most cases (except 2010/11) *Mesodinium* bloom initiation occurred during or just after an incoming tide. I also observed that cell concentrations increased during incoming tide in many cases (Figure 6) as was shown in Campbell et al. (2010). This leads to the conclusion that the blooms are originating offshore before they are seen in the Port Aransas ship channel. In a recent study, it was proposed that wind speed and direction along the Texas coast affects the occurrence of blooms in my sampling region (Ogle, 2012). More specifically, the along-shore wind component (used as an indicator for upwelling/downwelling strength of the coastal circulation) for September was related to bloom presence for *Karenia brevis*, a harmful algal bloom species that typically initiated in late September-mid October.

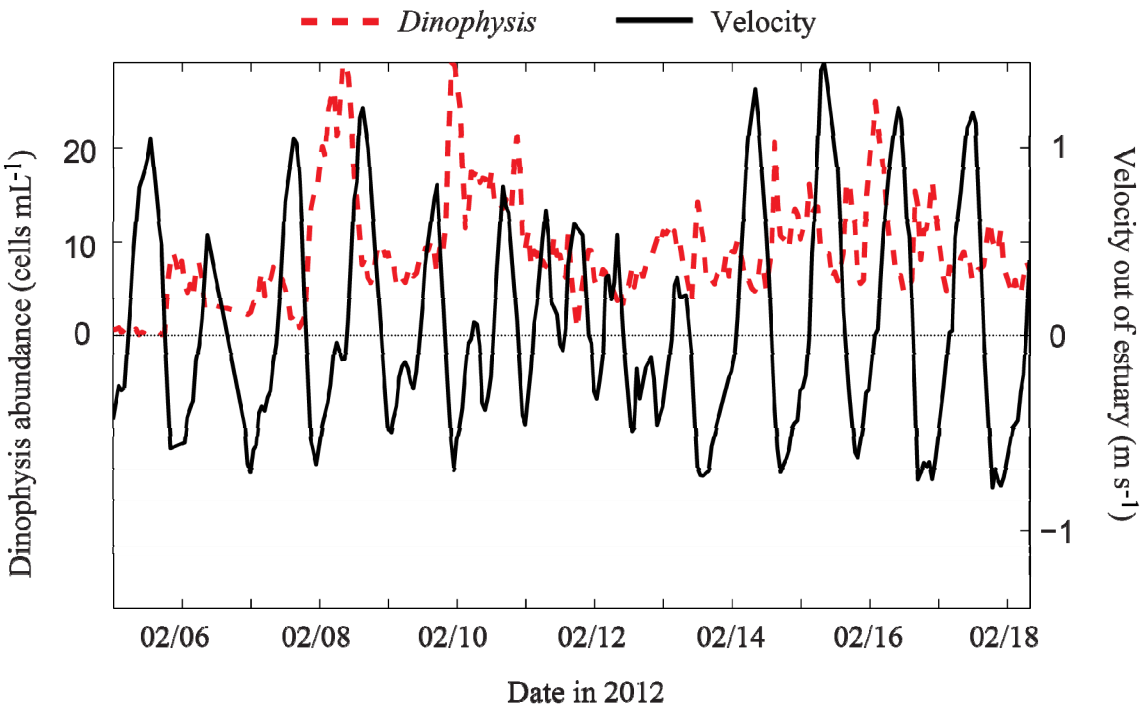


**Figure 6.** Dinophysis abundance plotted with tidal velocity for a 2 week period in February 2012. Negative values of velocity indicate water movement into the estuary.

As this time period is very similar to the bloom initiation of *Mesodinium* in this study, I compared the presented data for bloom and non-bloom years. It appears that strong downwelling (i.e., Ekman transport toward the shore) occurred in September of bloom years and weak downwelling occurred during the non-bloom year (Table 5). This observation adds to the understanding that blooms of *Mesodinium* are originating offshore. Similar analysis should be done for the *Dinophysis* bloom period.

When comparing the cell abundance data to environmental variables in the ANOVA, although there were significant differences among most variables, few consistent patterns were seen. I observed that the two years with the highest mean

salinities correspond to the years with the lowest *Mesodinium* peak size and abundance. Similarly, I found that the year of temperature with the highest mean corresponds to the only year with no *Mesodinium* bloom (data not shown). More observations are needed to determine whether significantly higher values of salinity and temperature over the course of a bloom can be factors for decreased *Mesodinium* abundance.

Table 5. September mean along-shore wind speed for bloom and non-bloom years of *Mesodinium*

| Bloom years | Velocity (kts)[a] |
|---|---|
| 2007/08 | -2.4 |
| 2008/09 | -3.1 |
| 2009/10 | -1.3 |
| 2010/11 | -1.8 |
| Non-bloom year | |
| 2011/12 | -0.3 |

[a] negative values indicate down-welling

**Time Series Analysis**

I suggest that a short lag between peaks and overlap of the two organisms are key factors for the formation of a large bloom of *Dinophysis*. There was a positive correlation between *Dinophysis* and *Mesodinium* at different time lags in every year

except 2008/09 when a *Dinophysis* bloom did not occur. Although *Mesodinium* remained below bloom concentration in 2011/12, a significant positive correlation between *Dinophysis* and *Mesodinium* was still present. The time lag for highest correlation corresponded to lag between peaks of the blooms of *Dinophysis* and *Mesodinium* and typically ranges from ~1 – 2 months. This is relevant to culture studies that show the ability of some *Dinophysis* species to continue photosynthetic growth without food for periods longer than one month (Nielsen et al., 2012; Park et al., 2008). The longest lag (62 days) occurred in 2009/10 and is associated with the highest peaks in abundance of *Mesodinium* and *Dinophysis* for this interval. A two month lag between blooms is quite long, but *Mesodinium* abundance remained well above background levels after its highest peak and increased above 15 cells $mL^{-1}$ several times before *Dinophysis* bloomed. The shortest lag and highest correlation occurred in 2007/08 and is associated with the largest blooms of the time series. The lag for highest correlation in this year is zero due to the overlap of the two organisms. A second peak in correlation occurs at ~20 days and corresponds to the lag between the highest peak of *Dinophysis* and *Mesodinium*. It is important to note that *Mesodinium* abundance remained well above background levels and reached abundances > 20 cells $mL^{-1}$ throughout the course of the *Dinophysis* bloom in 2007/08, which I believe to be significant. Although an overlap of the two organisms was present in other years, the abundance of both species was much lower than in 2007/08.

Time series analysis of salinity and temperature with cell abundance data were used to investigate why a *Dinophysis* bloom did not occur in 2008/09. The cross

correlation patterns for salinity and temperature were similar in each year except 2008/09. The correlations of environmental variables with *Dinophysis* were different in 2008/09 because no bloom occurred, but this does not explain why the correlation patterns of environmental variables and *Mesodinium* were different in this year. In every year apart from 2008/09, there was a negative trend in correlation between *Mesodinium* abundance with salinity and temperature at zero lag. In 2008/09, there was no correlation for either pair at zero lag. Because I found that every year of salinity and *Mesodinium* abundance, and most years of temperature were significantly different in the ANOVA, it is difficult to determine the cause for the difference in correlation patterns in this year. Although the bloom in 2008/09 was the smallest bloom of *Mesodinium*, low abundance does not seem to be a factor since the negative correlation was seen in 2011/12, the year with the lowest *Mesodinium* abundance. More data are needed to determine whether this anomaly in 2008/09 is significant.

**Size Analysis**

The cross sectional areas of *Mesodinium* cells seen in the IFCB images ranged from ~225 $\mu m^2$ to ~4400 $\mu m^2$. According to Garcia-Cuetos et al. (2012), length and width of *M. rubrum* range from 25 – 35 μm and 16 – 25 μm respectively, giving an approximated cross sectional area range ~315 – 685 $\mu m^2$. Although many of the cross sectional areas obtained in this study fall within the size range of *M. rubrum*, smaller and larger areas were seen in every year (Figure 4). The largest species reported, *M. major*, ranges in length 40 – 55 μm and in width 35 – 50 μm giving an approximated cross

sectional area ranging ~1100 – 2160 μm$^2$. The smallest species reported, *M. chamaeleon*, ranges 19 – 25 μm in length and 13 – 17 μm in width giving an approximated cross sectional area ranging ~195 – 335 μm$^2$ (Garcia-Cuetos et al., 2012). These three size classes account for a large majority of my results. Variations in cell area were previously attributed to prey and nutrient availability and all cells were assumed to be *M. rubrum* regardless of size (Montagnes et al., 2008). The wide range in sizes of the different *Mesodinium* species presented by Garcia-Cuetos et al. (2012) and my observations suggest that the variation in cell area could be associated with multiple species of *Mesodinium* in the Gulf of Mexico.

In laboratory studies, the only confirmed species of *Mesodinium* that *Dinophysis* utilizes as prey is *M. rubrum* (Hansen et al., 2013; Kim et al 2008; Minnhagen et al., 2011; Nishitani et al., 2008, 2010; Park et al., 2006). The presence of multiple species of *Mesodinium* in the Gulf of Mexico could be a cause for varying bloom abundance of *Dinophysis* in my time series. As it is not certain which species or size range is preferable to *D. ovum*, the species at my study site, it is possible that a portion of the *Mesodinium* cells in a bloom are not utilized by *Dinophysis* as prey.

In 2007/08, the majority of *Mesodinium* cells were larger than the *M. rubrum* size range (90% of cells were larger). As this was the year of the largest *Dinophysis* bloom, it is possible that *D. ovum* favors other, larger species of *Mesodinium*. This is one explanation for why *Dinophysis* did not bloom in 2008/09 though *Mesodinium* was present. Average cross sectional area of *Mesodinium* cells in 2008/09 was much smaller

than in 2007/08 and although the majority were within the *M. rubrum* size range (78% compared to 10% in 2008), there were very few larger cells.

In 2011/12, the majority of cells were in the *M. rubrum* size range (72%) but abundance was low. A *Dinophysis* bloom still occurred in this year, meaning that the *Dinophysis* must have obtained enough prey to grow to bloom concentrations. One possible explanation is that *Dinophysis* ingested most of the *Mesodinium* offshore and thus no bloom was seen in my samples, but this did not occur in any other year. It has been suggested that *Dinophysis spp*. may feed on other marine ciliates such as *Laboea*, *Tontonia* and *Strombidinium* due to their ability to acquire plastids from many different algal groups including the cryptophyte genus *Teleaulax*. Evidence of *Dinophysis* feeding on other ciliates has not been found, but it has been reported that some species contain plastids of several different microalgal origins, implying that *Dinophysis* can utilize other ciliates as prey (Kim et al., 2012; Nishitani et al., 2012). I propose that this may be the case for 2011/12, when the *Dinophysis* bloom was not preceded by a *Mesodinium* bloom. Abundance of ciliate groups other than *Mesodinium* were not analyzed in this study but should be considered in future studies.

# CONCLUSION

Results from this study suggest that the presence of *Mesodinium* can be used as a predictor for subsequent *Dinophysis* blooms. I suggest that the temperature and salinity ranges observed during *Dinophysis* and *Mesodinium* bloom initiation during bloom years are ideal conditions for the formation of a bloom. I propose that differences in the *Mesodinium* cross sectional areas observed across years of the time series are different *Mesodinium* species, but molecular analysis for species identification is needed for confirmation. Finally, based on the occurrence of a *Dinophysis* bloom preceded by very low abundances of *Mesodinium*, I propose that *Dinophysis* is able to utilize ciliates other than *M. rubrum* as prey. Direct evidence of this has not yet been reported, but future studies should include analysis of other ciliate groups prior to *Dinophysis* bloom events.

REFERENCES

Breiman, L. (2001) Random forests. *Mach. Learn.,* 45, 5-32.

Campbell, L., Olson, R. J., Sosik, H. M. *et al.* (2010). First harmful *Dinophysis* (*Dinophyceae, Dinophysiales*) bloom in the U.S. is revealed by automated imaging flow cytometry. *J. Phycol.,* 46, 66-75.

Crawford, D. W. (1989) *Mesodinium rubrum* – The phytoplankter that wasn't. *Mar. Ecol. - Prog. Ser.,* 58, 161-174.

Deeds, J. R., Wiles, K., Heideman VI, G. B. *et al*. (2010) First U.S. report of shellfish harvesting closures due to confirmed okadaic acid in Texas Gulf coast oysters. *Toxicon,* 55, 1138-1146.

Diaz, P. A., Reguera, B., Ruiz-Villarreal, M. *et al*. (2013) Climate variability and oceanographic settings associated with interannual variability in the initiation of *Dinophysis acuminata* blooms. *Mar. Drugs,* 11, 2964-2981.

Emery, W. J., and Thomson, R. E. (2001). *Data analysis methods in physical oceanography*. Elsevier.

Food and Drug Administration (FDA) (2011) National shellfish sanitation program(NSSP): Guide for the control of molluscan shellfish. U.S. Department of Health and Human Services, 2011 revision.

Garcia-Cuetos, L., Moestrup, Ø., Hansen, P. J. (2012) Studies on the Genus Mesodinium II. Ultrastructural and Molecular Investigations of Five Marine Species Help Clarifying the Taxonomy. *J Eukaryot Microbiol,* 59, 374-400.

Hallegraeff, G. M. and Lucas, I. A. N. (1988) The marine dinoflagellate genus *Dinophysis* (*Dinophyceae*) – photosynthetic, neritic and non-photosynthetic, oceanic species. *Phycologia,* 27, 25-42.

Hansen, P. J., Nielsen, L. T., Johnson, M. *et al*. (2013) Acquired phototrophy in *Mesodinium* and *Dinophysis* - A review of cellular organization, prey selectivity, nutrient uptake and bioenergetics. *Harmful Algae,* 28, 126-139.

Henrichs, D. W., Sosik, H. M., Olson, R. J. *et al*. (2011) Phylogenetic analysis of *Brachidinium capitatum* (*Dinophyceae*) from the Gulf of Mexico indicates membership in the *Kareniacae*. *J. Phycol.,* 47, 366-374.

Johnson, M. D. and Stoecker, D. K. (2005) Role of feeding in growth and photophysiology of *Myrionecta rubra*. *Aquat. Microb. Ecol.,* 39, 303-312.

Johnson, M. D., Stoecker, D. K. and Marshall, H. G. (2013) Seasonal dynamics of *Mesodinium rubrum* in Chesapeake Bay. *J. Plankton Res.,* 35, 877-893.

Kim, M., Nam, S., Shin, W. *et al*. (2012) *Dinophysis caudata* (*Dinophyceae*) sequesters and retains plastids from the mixotrophic ciliate prey *Mesodinium rubrum*. *J. Phycol.,* 48, 569-579.

Kim, S., Kang, Y., Kim, H. *et al.* (2008) Growth and grazing responses of the mixotrophic dinoflagellate *Dinophysis acuminata* as functions of light intensity and prey concentration. *Aquat. Microb. Ecol.,* 51, 301-310.

Minnhagen, S. (2010) Kleptoplasty in *Dinophysis spp:* Ecological role and evolutionary implications. Linnaeus University Dissertations No 19/2010.

Minnhagen, S., Kim, M., Salomon, P. *et al*. (2011) Active uptake of kleptoplastids by *Dinophysis caudata* from its ciliate prey Myrionecta rubra. *Aquat. Microb. Ecol.,* 62, 99-108.

Montagnes, D. J. S., Allen, J., Brown, L. *et al*. (2008) Factors controlling the abundance and size distribution of the phototrophic ciliate *Myrionecta rubra* in open waters of the North Atlantic. *J. Eukaryot. Microbiol.,* 55, 457-465.

Nagai, S., Nitshitani, G., Tomaru, Y. *et al*. (2008) Predation by the toxic dinoflagellate *Dinophysis fortii* on the ciliate *Myrionecta rubra* and observation of sequestration of ciliate chloroplasts. *J. Phycol.,* 44, 909-922.

Nielsen, L. T., *et al.* (2012) Effects of light and food availability on toxin production, growth and photosynthesis in Dinophysis acuminata. *Mar. Ecol-Prog. Ser.* 471, 37-50.

Nishitani, G., Nagai, S., Baba, K. *et al.* (2010) High-level congruence of *Myrionecta rubra* prey and *Dinophysis* species plastid identities as revealed by genetic analyses of isolates from Japanese coastal waters. *Appl. Environ. Microb.,* 76, 2791-2798.

Nishitani, G., Nagai, S., Hayakawa, S. *et al*. (2012) Multiple plastids collected by the dinoflagellate *Dinophysis mitra* through kleptoplastidy. *Appl. Environ. Microb.,* 78, 813-821.

Nishitani, G., Nagai, S., Sakiyama, S. *et al*. (2008) Successful cultivation of the toxic dinoflagellate *Dinophysis caudata* (*Dinophyceae*). *Plankton and Benthos Research,* 3, 78-85.

Ogle, M. (2012) Physical mechanisms driving harmful algal blooms along the Texas coast. MS thesis, Texas A&M University, College Station, TX.

Olson, R. J., and Sosik, H. M. (2007) A submersible imaging-in-flow instrument to analyze nano-and microplankton: Imaging FlowCytobot. *Limnol. Oceanogr.,* methods 5, 195-203.

Park, M., Kim, S., Kim, H. *et al*. (2006) First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquat. Microb. Ecol.,* 45, 101-106.

Park, M. G., Park, J. S., Kim, M. *et al.* (2008) Plastid dynamics during survival of *Dinophysis caudata* without its ciliate prey. *J. Phycol.,* 44, 1154-1163.

Riisgaard, K., and Hansen, P. J. (2009) Role of food uptake for photosynthesis, growth and survival of the mixotrophic dinoflagellate *Dinophysis acuminata*. *Mar. Ecol. - Prog. Ser.,* 381, 51-62.

Sosik, H. M., and Olson, R. J. (2007) Automated taxonomic classification of phytoplankton sampled with imaging-in-flow cytometry. *Limnol. Oceanogr.,* 5, 204-216.

Swanson, K., Flewelling, L., Byrd, M. *et al*. (2010) The 2008 Texas *Dinophysis ovum* bloom: Distribution and toxicity. *Harmful algae,* 9, 190-199.

Tong, M. M., Zhou, Q. X., Kulis, M. D. *et al*. (2010) Culture techniques and growth characteristics of *Dinophysis acuminata* and its prey. *Chin. J. Oceanol. Limn.,* 28**,** 1230-1239.

Velo Suarez, L., Gonzalez-Gil, S., Pazos, Y. *et al*. (2013) The growth season of *Dinophysis acuminata* in an upwelling system embayment: A conceptual model based on is situ measurements. . *Deep-Sea Res.,* II.

Yasumoto, T., Murata, M., Oshima, Y. *et al*. (1985) Diarrhetic shellfish toxins. *Tetrahedron,* 41, 1019-1025.

APPENDIX

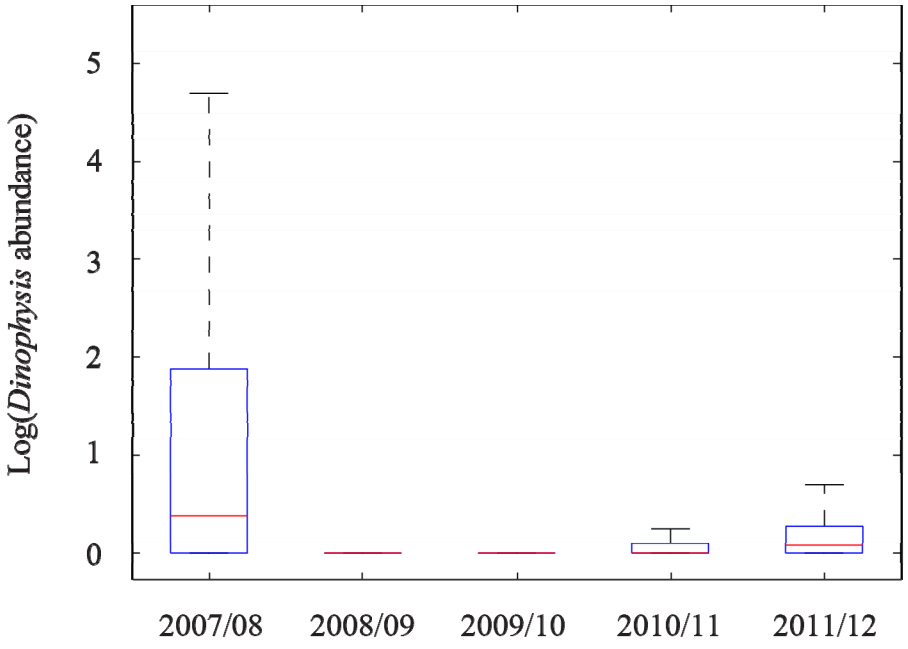


**Figure A1.** ANOVA of *Dinophysis* cell abundance. Data were log transformed before analysis. All years were found to be statistically different.

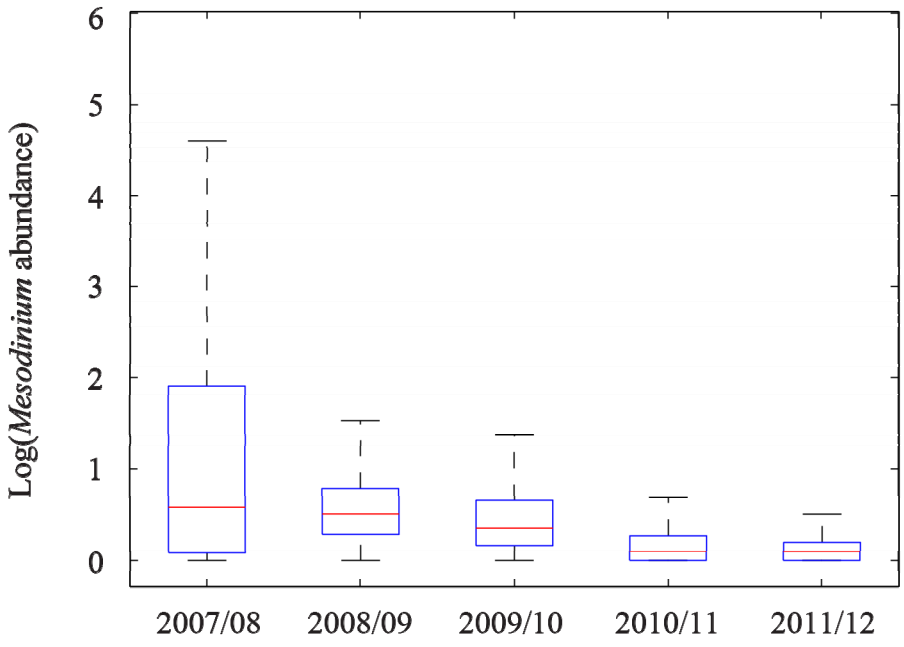


**Figure A2.** ANOVA of *Mesodinium* cell abundance. Data were log transformed before analysis. All years were found to be statistically different.

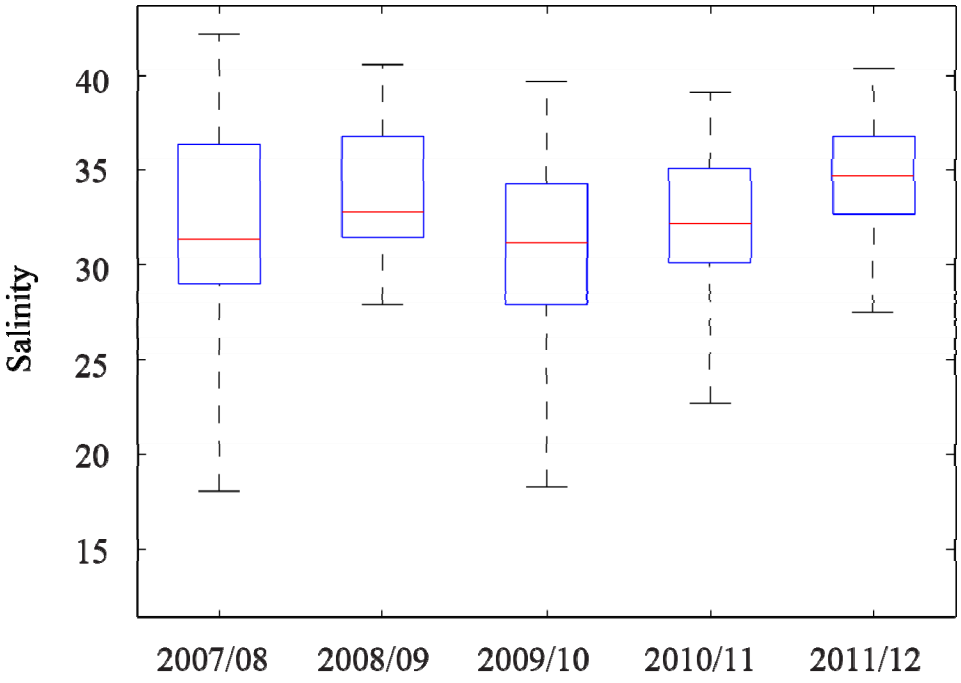


**Figure A3.** ANOVA of Salinity data. All years were found to be statistically different.

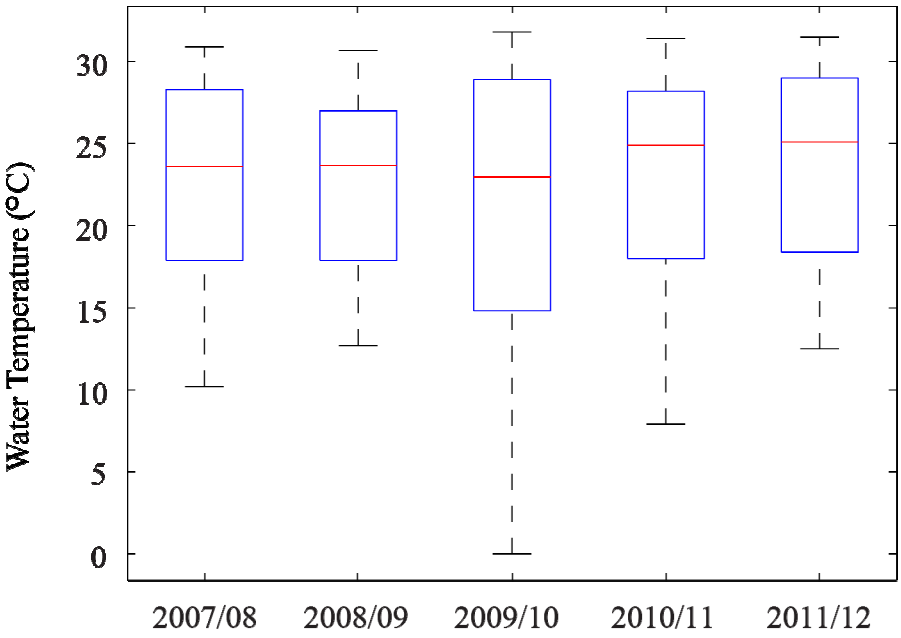


**Figure A4.** ANOVA of water temperature data. All years except 2007/08 and 2010/11 were found to have no significant difference from each other

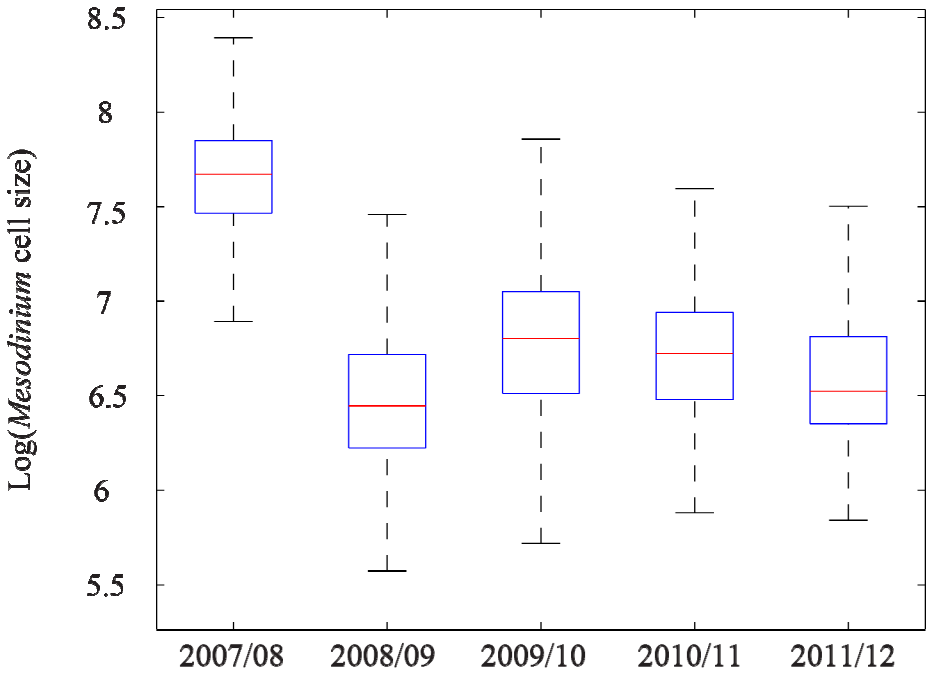


**Figure A5.** ANOVA of *Mesodinium* cell size. Data were log transformed before analysis. All years were found to be statistically different