A Survey of the Tidal Cycle Influence on the Differential Phytoplankton Diversity of the Duplin River at Marsh Landing on Sapelo Island, Georgia

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Abstract:

This study aimed to analyze the fluctuations in diversity of phytoplankton during tidal cycle. The purpose of this experiment was to observe changes in phytoplankton diversity in the Duplin River. The hypothesis was that Mid Tide would have the highest diversity because of the increased movement of water that accompanies the ebb from high to low tide. Plankton tows were conducted during high tide, mid ebb (mid tide), and low tide from Marsh Landing on Sapelo Island, Georgia. The samples were then fixed with Lugol's Iodine to help differentiate between detritus and phytoplankton cells. The resulting solution was plated on depression slides. The cells on the slides were then counted by type of algae. The hypothesis was rejected as high tide had the highest diversity followed by low tide ($p < 0.0001$). Mid tide had the lowest diversity due to the high dominance of the dinoflagellates and little brown balls in the samples.

Objective:

The study aimed to analyze the differences in diversity of phytoplankton as a result of tidal cycles. The purpose of this experiment is to help identify changes in phytoplankton diversity over a tidal cycle.

Introduction:

Phytoplankton are photosynthetic protists that are estimated to produce 73-84% of the net global production of oxygen (Barnard 2014). Phytoplankton are the primary source of net primary production and use chlorophyll to convert PAR light and dissolved inorganic carbon into sugars (Weithoff et al. 2015). The phytoplankton – in addition to bacteria – are also a base of the aquatic food chains (Chou et al. 2012).

When conditions are right, phytoplankton populations erupt into a bloom. The climate plays a major role in determining when blooms occur. In temperate regions, like the one containing the study site, blooms are most common in the spring months (Hahnke et al. 2014). Some common causes of blooms include idealized water temperatures, nitrogen and phosphorus additions through water column mixing caused by a turnover effect, and an increased in the dissolved organic matter concentrations (Dammrich et al. 2014).

It is important to understand the taxonomic differences between the phytoplankton groups. All phytoplankton are in the domain Eukarya (Taujale and Yin 2015) . Phytoplankton used to be in kingdom Protista, but that kingdom has since dissolved into other kingdoms due to genomics (Pérez et al. 2015). Diatoms are in kingdom Chromista, Phylum Ocrophyta, and subphylum Bacilliariophytina (Boudouresque 2015). Dinoflagellates are in kingdom Aveolata and phylum Dinoflagellata (Klueter et al. 2015). Green Algae are in kingdom Vidriplantae with the unicellular algae in phylum Chlorophyta and multicellular algae in phylum Charophyta (Pérez et al. 2015). “Little brown balls” and “little

green balls" are operational terms that will be used here for picoplankton. These classifications are for algal or phytoplankton cells that are too small to identify using a light microscope. These terms are very vague and nondescript as they are only an informal classification (Potter et al. 1997).

The impact of the tidal cycle was an important aspect of the experiment. The tidal cycle is driven by the lunar cycle with about 12.5 hour cycles (Porter et al. 2014). Every new and full moon, there is a spring tide and halfway in between the two, there is a neap tide. The magnitude also depends on the earth's orbit position around the sun (Boersma and Terwindt 1981).

The primary focus of the experiment was to examine diversity changes of phytoplankton over the course of the tidal cycle. Kwon et al. (2014) indicated that several marine phytoplankton groups have tidal rhythms, meaning that they have a vertical migration based on the tidal cycle. The tidal cycle also fluctuates the residual flow, which can increase the amount of phytoplankton movement during the tidal changes (Macdonald et al. 2014).

The null hypothesis for the experiment was that tides would have no effect on phytoplankton diversity because the phytoplankton move towards the surface at all tides to be closer to PAR from the sun. The alternative hypothesis was that mid tide would have the highest diversity because of the increased movement of water that accompanies the ebb from high to low tide, causing turbulence that mixes benthic macroalgae in the water column.

Materials and Methods:

i. Sampling

Sampling was conducted at Marsh Landing on Sapelo Island, GA (31º25’3.306” N, 81º17’45.390” W), the site of a National Oceanic and Atmospheric Administration (NOAA) weather station and National Science Foundation (NSF) Long-term Ecological Research (LTER) study (Di Iorio et al. 2011). The weather conditions were around 30ºC and high relative humidity. Samples were collected by conducting a surface plankton tow by dragging a 0.5 m plankton net with 100 micron mesh along the surface of the water adjacent to the floating dock farthest from the ferry entrance four full lengths of the floating dock as detailed by a field manual (Goswami 2004). Once collected, the entire volume of water collected was poured into a 50 mL Falcon Tube. The sampling was conducted during the afternoon high tide, mid tide, and low tide on three consecutive days.

ii. Sample Processing

5.0 mL of the sample was pipetted into three 25 mL Falcon Tubes. 50.0 µL of Lugol’s Iodine was pipetted into each of the subsamples. The Lugol’s Iodine was the stain applied to fix the plankton tow solution as detailed in a methods paper regarding algal staining (Owen Jr et al. 1978). Then, 50.0 µL of the fixed sample from each subsample was pipetted onto a depression slide. The slide was covered and sealed with nail polish. The nail polish was allowed to dry before sample analysis.

iii. Sample Analysis

The fixed slides were viewed under a compound microscope to identify the general group of phytoplankton. Those groups were diatoms, dinoflagellates, single-celled green algae, multicellular green algae, “little brown balls,” and “little green balls.” The groups were identified using the classification detailed by Bellinger and Sigee (Bellinger and Sigee 2015). The slides were analyzed with the 10x lens as a stratified random sample using random numbers to determine size and area to be analyzed, and a multiplication factor was used to approximate the census numbers. A multiple tally counter was used to count the individuals in each group.

iv. Data Analysis

Analysis included diversity indices, Morista cladograms, and a diversity profile using PAST (Hammer et al. 2001). A Whittaker plot was also constructed to evaluate evenness. For statistics, a chi-squared analysis was conducted in R (Team 2015).

Results:

Table 1: Abundance of Phytoplankton Types by Tide per 450 µL of Sample

Phytoplankton Type	Low Tide	Midtide	High Tide
Diatoms	2853	13354	2847
Dinoflagellates	1977	39587	1420
Unicellular Green	1392	1054	908
Multicellular Green	457	287	545
Little brown Balls	4135	40600	2640
Little Green Balls	4327	22870	2422

The total abundance by group (Table 1) was calculated by summing all replications (different days) and pseudo replications (subsamples) to obtain the total population. A chi-squared analysis was conducted on the table to determine if the variations in the phytoplankton groups were due to the tidal cycle or due to chance sampling. The chi-squared statistic is 15310 ($df = 10$, $p < 0.0001$).

A Whittaker Plot (Figure 1) was constructed to show the evenness of proportions of the groups of algae by tide. Number of species is the number of preselected groups present. Species are in order of proportional abundance, not by tabular values. The proportion of the first species is called the Berger-Parker Index.

Figure 1: Whittaker plot of the abundances of different algal types based on tide.

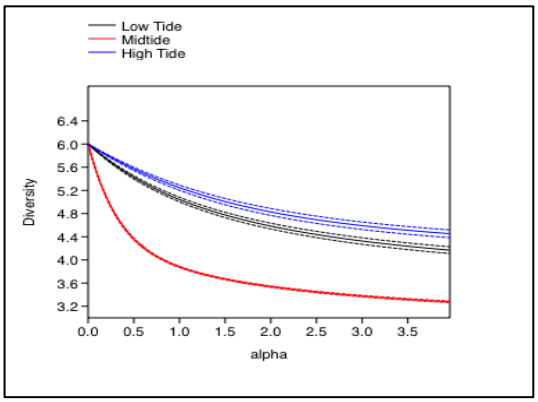

Figure 2: Diversity Profile Based on Tide with 95% Confidence Intervals

Diversity is a measure of richness and evenness. There are multiple diversity indices (Table 2) to represent the different factors that make up diversity. Taxa Richness is the number of groups present in the treatments. Individuals are sum of all dependent variables. Dominance is a measure of the evenness of the independent variables. Simpson is one of the indices used to model diversity in a diversity profile. HShannon (Shannon Entropy or Shannon Diversity Index) is a comparative measure of diversity of an independent variable. Evenness is a measure of diversity in terms of the second criteria of the diversity definition. Berger-Parker is the highest proportion of individual dependent variables.

The diversity profile (Figure 2) uses several of these values to calculate diversity. Alpha = 0.0 represents Taxa Richness. Alpha = 1.0 represents HShannon. Alpha = 2.0 represents the Simpson Index. The rest of the alpha values are extrapolated from those three values using a preset diversity algorithm.

Table 2: Diversity Indices

Index	Low Tide	Mid Tide	High Tide
Taxa Richness	6	6	6
Individuals	15141	117752	10782
Dominance (D)	0.2182	0.2826	0.2071
Simpson (1-D)	0.7818	0.7174	0.7929
HShannon	1.618	1.356	1.658
Evenness (e^H/S)	0.8403	0.6465	0.8747
Berger-Parker	0.2858	0.3448	0.2641

A Morista cladogram is a cladogram that groups similar data sets together. The Morista cladograms for phytoplankton groups (Figure 3) and for tides (Figure 4) show relationships between the groups in the data set. The similarity index on the y-axis represents the proportion of similarity of groups in a data set. For example, the High Tide and Low Tide groups are 97% similar while those groups are only 85% similar to Mid Tide. Cladogram abbreviations are as follows: LGB is "Little Green Balls," LBB is "Little Brown Balls," UCG is Unicelllular Green Algae, and MCG is Multicellular Green Algae.

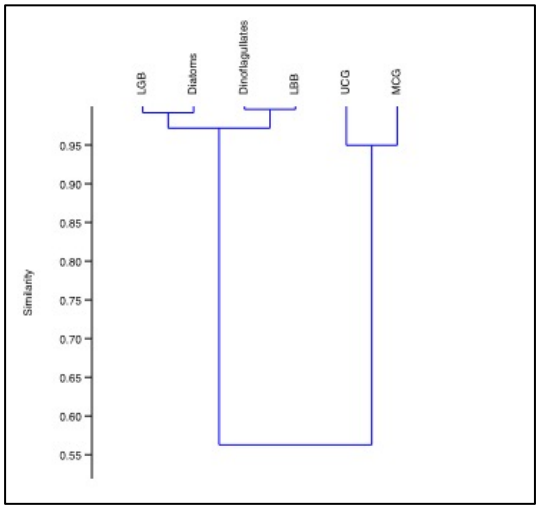

Figure 3: Morista Cladogram of Phytoplankton Groups

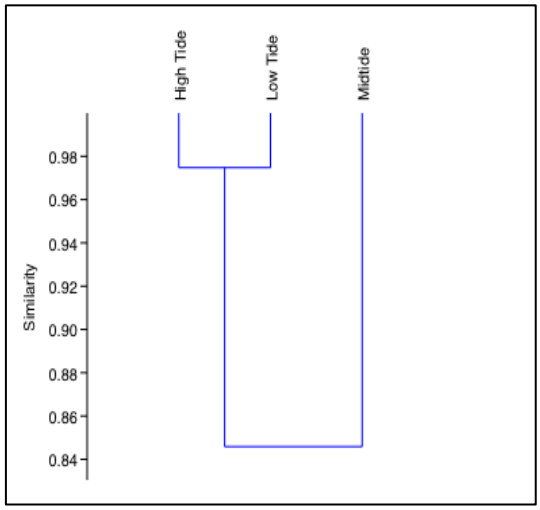

Figure 4: Morista Cladogram of Tides

Discussion:

The null hypothesis for the experiment was that tides would have no effect on phytoplankton diversity. The alternative hypothesis was that mid tide would have the highest diversity. Both the null hypothesis and the alternative hypothesis were rejected as a result of experimentation. The chi-square had a p-value less than 0.0001, which means that $p < \alpha = 0.001$. Furthermore, the 95% confidence intervals on the diversity profile (Figure 2) do not cross.

Diversity is measured in a combination of richness and in evenness. According to Table 1, mid tide had the most individual richness of any of the tidal groups. This agrees with a Brazilian phytoplankton diversity study (Silva et al. 2004). However, according to Figure 2, mid tide was the least diverse. High tide was the most diverse, closely followed by low tide. This is likely due to the high dominance factor exhibited by the mid tide samples (Table 1). This contradicts a recent study evaluating the physiochemical effects of tide changes on algae that found mid tide to be the most diverse (Juneau et al. 2015).

The results were analyzed using diversity indices (Table 2) detailed in two phytoplankton diversity studies (Washington 1984, Sun and Liu 2003). All groups have the same taxa richness since all three had individuals in all six groups of phytoplankton. Mid tide had a dominance index that was 30% higher than the other tides. In terms of HShannon, high tide and low tide were 20% higher than mid tide. Furthermore, the evenness of high and low tides was 30% higher than mid tide. In terms of the Berger-Parker Index, mid tide was 21% higher than low

tide and 31% higher than mid tide. These factors were used in the computation of total diversity (Figure 2).

The Morista Cladogram for comparing phytoplankton groups (Figure 3) closely matches taxonomic differences between the groups. The clades split at 56% similarity. One clade are the unicellular and multicellular green algae groups which split at 95% similarity. This group is primarily freshwater algae, which would distinguish it from the other groups taxonomically, which matches the diversity data from the experiment. The other groups, which are the most abundant groups in water with non-zero salinity, split into two clades at 96% similarity. Those two resulting clades are little green balls and diatoms, which split at 97% similarity, and dinoflagellates and little brown balls, which also split at 97% similarity. Both clades are composed of taxonomically similar groups.

The Morista Cladogram for comparing tides (Figure 4) closely matched the diversity profile (Figure 2). The three tides split into two clades at 85% similarity with mid tide on one clade and high and low tides branching off the other clade at 95% similarity. This indicates that high and low tides are more similar to each other in terms of the experimental diversity data than they are to mid tide.

There were several sources of error in the experiment. First was that low tide was always after dark, which may have affected the readings. The second was that the assumption was made that the phytoplankton were evenly distributed along the slide since a random sampling was conducted and multiplied by a factor to simulate a census count. Phytoplankton tend to sink to the bottom of the sampling tube, so it is possible that a representative sampling

was not achieved due to the subsampling from the surface to avoid spillage when fixing the samples. Another bias was that the counting was conducted with the 10x lens, meaning that some of the little brown balls and little green balls may have been identifiable under 40x or 100x. The taxonomic identifications were very broad groups; species are generally used for diversity studies. Overall, these errors likely had no affect on the differences in diversity between tides.

Improvements on the experimental design could include using current data as well as more replications to create a mathematical model to predict phytoplankton diversity of an estuarine system. One possible future experiment is to conduct a diurnal sample to understand how time of day and incoming versus ebbing tides impact the phytoplankton diversity. Furthermore, the experiment would identify the phytoplankton to genus or species for a more accurate representation of the diversity.

In conclusion, Mid Tide had the highest abundance of phytoplankton of any testing group. However, due to high dominance, the group exhibited the lowest diversity of any group. It appears that the mixing of benthic macroalgae increases abundance, but reduces the overall diversity of the water column. It can be concluded that the tidal changes foster the greatest diversity of phytoplankton while the mid ebb fosters the highest phytoplankton abundance due to mixing of benthic and surface water in the estuarial system of the Duplin River.

References:

Barnard, M. A. 2014. Algal system for improving water quality.*in* I. P. Organization, editor. Google Patents. Google Patents, International.

Bellinger, E. G., and D. C. Sigee. 2015. Freshwater algae: identification and use as bioindicators. John Wiley & Sons.

Boersma, J., and J. Terwindt. 1981. Neap–spring tide sequences of intertidal shoal deposits in a mesotidal estuary. Sedimentology **28**:151-170.

Boudouresque, C.-F. 2015. Taxonomy and Phylogeny of Unicellular Eukaryotes. Pages 191-257 *in* J.-C. Bertrand, P. Caumette, P. Lebaron, R. Matheron, P. Normand, and T. Sime-Ngando, editors. Environmental Microbiology: Fundamentals and Applications. Springer Netherlands.

Chou, W.-R., L.-S. Fang, W.-H. Wang, and K. Tew. 2012. Environmental influence on coastal phytoplankton and zooplankton diversity: a multivariate statistical model analysis. Environmental Monitoring & Assessment **184**:5679-5688.

Dammrich, T., J. E. van Beusekom, and A. Engel. 2014. Characteristics of marine aggregates during the phytoplankton spring bloom in a temperate tidal basin.

Di Iorio, D., D. Hurley, P. Hagan, and W. Sheldon. 2011. Climate data from the SINERR/GCE/UGAMI weather station at Marsh Landing on Sapelo Island, Georgia, from 01-Jan-2011 to 31-Dec-2011. Georgia Coastal Ecosystems LTER.

Goswami, S. 2004. Zooplankton Methodology, Collection & identyification-A field manual.

Hahnke, R. L., C. M. Bennke, B. M. Fuchs, A. J. Mann, E. Rhiel, H. Teeling, R. Amann, and J. Harder. 2014. Dilution cultivation of marine heterotrophic bacteria abundant after a spring phytoplankton bloom in the North Sea. Environmental microbiology.

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica.

Juneau, P., A. Barnett, V. Méléder, C. Dupuy, and J. Lavaud. 2015. Combined effect of high light and high salinity on the regulation of photosynthesis in three diatom species belonging to the main growth forms of intertidal flat inhabiting microphytobenthos. Journal of Experimental Marine Biology and Ecology **463**:95-104.

Klueter, A., J. B. Crandall, F. I. Archer, M. A. Teece, and M. A. Coffroth. 2015. Taxonomic and Environmental Variation of Metabolite Profiles in Marine Dinoflagellates of the Genus Symbiodinium. Metabolites **5**:74-99.

Kwon, B.-O., C.-H. Koh, J. S. Khim, J. Park, S.-G. Kang, and J. H. Hwang. 2014. The Relationship between Primary Production of Microphytobenthos and Tidal Cycle on the Hwaseong Mudflat, West Coast of Korea. Journal of Coastal Research **30**:1188-1196.

Macdonald, R., D. Bowers, D. McKee, G. Graham, and W. Nimmo-Smith. 2014. Vertical migration maintains phytoplankton position in a tidal channel with residual flow. MARINE ECOLOGY PROGRESS SERIES **509**:113-126.

Owen Jr, B. B., M. Afzal, and W. R. Cody. 1978. Staining preparations for phytoplankton and periphyton. British Phycological Journal **13**:155-160.

Pérez, W., J. D. Hall, R. M. McCourt, and K. G. Karol. 2015. Oospore dimensions and morphology in North American Tolypella (Charophyceae, Charophyta). Journal of Phycology **51**:310-320.

Porter, K., R. Simons, and J. Harris. 2014. Laboratory investigation of scour development through a spring-neap tidal cycle. Scour and Erosion:667-677.

Potter, D., T. Lajeunesse, G. Saunders, and R. Anderson. 1997. Convergent evolution masks extensive biodiversity among marine coccoid picoplankton. Biodiversity & Conservation **6**:99-107.

Silva, A. P., S. Neumann-Leitão, R. Schwamborn, and L. M. d. O. Gusmão. 2004. Mesozooplankton of an impacted bay in North Eastern Brazil. Brazilian Archives of Biology and Technology **47**:485-493.

Sun, J., and D. Liu. 2003. The application of diversity indices in marine phytoplankton studies. Acta Oceanologica Sinica **26**:62-75.

Taujale, R., and Y. Yin. 2015. Glycosyltransferase Family 43 Is Also Found in Early Eukaryotes and Has Three Subfamilies in Charophycean Green Algae. PLoS ONE **10**:e0128409.

Team, R. C. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Washington, H. 1984. Diversity, biotic and similarity indices: a review with special relevance to aquatic ecosystems. Water research **18**:653-694.

Weithoff, G., M. R. Rocha, and U. Gaedke. 2015. Comparing seasonal dynamics of functional and taxonomic diversity reveals the driving forces underlying phytoplankton community structure. Freshwater Biology **60**:758-767.