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# LIFE CYCLE DYNAMICS OF THE HARMFUL BLOOM FORMING MACROALGAE *ULVA* SPP. IN NARRAGANSETT BAY, RI

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LIFE CYCLE DYNAMICS OF THE HARMFUL BLOOM FORMING

MACROALGAE *ULVA* SPP. IN NARRAGANSETT BAY, RI

BY

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**ABSTRACT**

Macroalgal blooms occur worldwide and have the potential to cause severe ecological and economic damage. Narragansett Bay, RI is a eutrophic system that experiences summer macroalgal blooms composed mostly of *Ulva compressa* and *Ulva rigida*. All *Ulva* species have isomorphic, biphasic life cycles, and the relative contribution of the haploid and diploid life history stages to bloom formation is poorly understood. In this study, we used flow cytometry to assess ploidy levels of *U. compressa* and *U. rigida* populations from five sites in Narragansett Bay, RI, USA. Both haploid gametophytes and diploid sporophytes were present for both species. Sites ranged from a relative overabundance of gametophytes to a relative overabundance of sporophytes, compared to the null model prediction of √2 gametophytes to 1 sporophyte. We also assessed growth rates and measured cell sizes to investigate potential differences between life history phases. We found no significant differences in growth rate between ploidy levels for either species. Sporophyte cells were significantly larger than gametophyte cells in *U. compressa*. Our results indicate the presence of both phases of each of the two dominant bloom forming species throughout the bloom season, and represent one of the first studies of *in situ Ulva* life cycle dynamics.

## ACKNOWLEDGMENTS

**DEDICATION**

I would like to dedicate this thesis to John Hall. He is one of the most caring and passionate teachers I have had the pleasure of knowing, and I am forever inspired by his unparalleled dedication to ecology and environmental conservation.

**PREFACE**

       This thesis is being submitted in manuscript format. It consists of one chapter which will be submitted for peer-reviewed publication in the journal Marine Ecology Progress Series.

**TABLE OF CONTENTS**

**LIST OF FIGURES**

**LIST OF TABLES**

CHAPTER 1

LIFE CYCLE DYNAMICS OF THE HARMFUL BLOOM FORMING

MACROALGAE *ULVA* SPP. IN NARRAGANSETT BAY, RI

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**INTRODUCTION**

Macroalgal blooms typically consist of large accumulations of ephemeral macroalgal biomass. These blooms occur worldwide, often in shallow areas with relatively low water mixing that are affected by coastal eutrophication, and they have the potential to cause severe ecological and economic damage (Rosenberg 1985, Thomsen & McGlathery 2006, Deacutis 2008). The largest documented bloom on record occurred four weeks before the 2008 Beijing Olympics, with a bloom of an estimated 20 million tons of *Ulva prolifera* in the Yellow Sea near Qingdao, China (Gao et al. 2010, Ye et al. 2011, Liu et al. 2013). The ecological effects of macroalgal blooms are often far reaching and indirect; algal blooms negatively affect seagrass, sessile invertebrates and perennial algae (Hauxwell et al. 1998, Hauxwell et al. 2003, Worm & Lotze 2006). Large blooms can create hypoxic environments that contribute to mass fish and invertebrate die-offs (Management 2003, Deacutis et al. 2006) and hydrogen sulfide from decaying algal mats can cause symptoms such as difficulty breathing and nausea in humans (Fulton et al. 2003). Blooms have increased worldwide over the years in frequency and intensity (Morgan et al. 2003, Smith et al. 2005, Lyons et al. 2009).

The green macroalgal genus *Ulva* forms large and dense sheets, a phenomenon known as green tides, and can proliferate by asexual (fragmentation) and sexual reproduction. Green tides include many genera of green algae, such as *Chaetomorpha*, and affect at least 37 countries worldwide (Morand & Merceron 2004, Merceron et al. 2007). *Ulva* is one of the most common macroalgal bloom-forming genera present in green tides and is the focus of this study.

Like many algae with complex life cycles, *Ulva* has a biphasic life cycle consisting of an alternation between two free-living forms, a haploid phase (1N, gametophyte) and a diploid phase (2N, sporophyte; Figure 1). These phases of *Ulva* are isomorphic, meaning that the gametophyte and sporophyte are morphologically similar and cannot be visually distinguished in the field. If the phases are ecologically equivalent, we expect a ratio √2 gametophytes to 1 sporophyte at equilibrium (Thornber & Gaines 2004). This predication is due to the reproductive success of the sporophyte phase, resulting in a relative overabundance of adult gametophytes. For isomorphic algal species, however, a wide range of distributions of ploidy ratio in have been documented in the field (for review see Thornber 2006).

There are two published studies on the *in situ* life cycle dynamics of *Ulva*; Hiraoka and Yoshida (2010) found a non-seasonal alternating dominance of the two phases for *U. pertusa*, and Alström-Rapaport and colleagues (2010) found a seasonal shift in the phases of *U. intestinalis*, although sporophytes were always more abundant. This lack of a broader understanding of *Ulva* life cycle dynamics may be due to the difficulty of discerning between isomorphic phases; however, ploidy can be rapidly determined using flow cytometry (Ulrich & Ulrich 1991, Le Gall et al. 1993, Doležel et al. 2007a). Flow cytometry quantitatively analyzes the DNA content of nuclei in a suspended solution and can allow for a more rapid and less expensive analysis than molecular techniques.

Although isomorphic sporophytes and gametophytes appear identical, they can occupy different ecological niches (Destombe et al. 1993, Dyck & De Wreede 1995). For example, one phase may be responsible for forming blooms, while the other may occur

during non-bloom forming months. In addition, the two phases may vary in growth rates, temperature optima, or susceptibility to herbivores (Thornber et al. 2006). Similarly, phases could vary in their response to environmental variables such as temperature and nutrients (Hannach & Santelices 1985, Destombe et al. 1993). If there are ecological differences between *Ulva* gametophytes and sporophytes, the distribution of life history phases will be partially dependent upon the physical and biological factors of the system.

We investigated life cycle dynamics in the bloom-forming macroalgae *Ulva compressa* L. and *Ulva rigida* C. Agardh, which are common in summer macroalgal blooms in the estuarine system of Narragansett Bay, Rhode Island (Guidone et al. 2010, Guidone et al. 2012). Macroalgal densities (comprised mostly of *Ulva*) peak in the summertime and vary significantly across sites, seasons, and years (Guidone 2012, Guidone & Thornber 2013, Guidone et al. 2013).

Our research focuses on four central questions regarding the life cycles and biology of *U. compressa* and *U. rigida*. Firstly, what is the relative abundance of sporophytes and gametophytes of both species? Secondly, how do these relative abundances correlate with physical and biological factors? Thirdly, do the phases have different growth rates, and lastly, do the phases have cells of different sizes? We interpret our data in the context of macroalgal bloom dynamics and the impacts of environmental variables in structuring bloom formation.

**METHODS**

**Collection of *Ulva***

4

We collected *Ulva* spp. monthly from June to October 2013 at several bloom-forming sites in Narragansett Bay, RI, including Chepiwanoxet, Sandy Point, Oakland Beach, Oakland Beach Cove, and Warwick City Park. We chose these sites to represent a range of typical *Ulva* spp. bloom intensity, with Oakland Beach Cove and Warwick City Park as high bloom sites, while Chepiwanoxet, Sandy Point and Oakland Beach as low bloom sites (Thornber, unpublished data). At each site, on each sampling date, we haphazardly collected individuals by hand from the shallow subtidal zone, put them in a plastic bag, and brought them back to the lab. We selected a minimum of 16 individuals and maximum of 40 individuals on each sampling date. Later, we identified *U. compressa* and *U. rigida* to the species level by microscopic examination and only used individuals with clear cellular characteristics (Guidone et al. 2013). Overall, we collected and analyzed 282 total *Ulva* individuals: 150 *U. compressa* and 132 *U. rigida*. Both species were collected at all sites, with a minimum of 10 individuals of each species at each site. Due to the nature of sampling and length of time necessary for preparing flow cytometry samples (which limited our ability to collect larger sample sizes), we present and analyze our data here in terms of the overall relative abundance of each *Ulva* species during the peak bloom-forming season at each site. However, we use collection date and month as covariates in building our logistic regression models for predicting the relative abundance of each phase (see Statistical Analysis section). We used sea surface temperature and sea surface salinity data for Greenwich Bay (Site F5) collected daily by the Rhode Island Department of Environmental Management Bay Assessment and Response Team (http://www.dem.ri.gov/bart/stations.htm).

We also determined *Ulva* biomass data from monthly subtidal surveys of the same sites, following the protocol in Guidone (2012). Briefly, at each site, we collected all algae in each of 0.16 m$^2$ subtidal quadrats placed 1 m apart along a transect line. All plots were < 2 m deep at mean lower low water.

Prior to thallus destruction for flow cytometry, we took a microscopic photograph at 400X of each individual that was analyzed for ploidy content. Using ImageJ (www.nih.gov), we created an overlying grid on the microscopic photograph, and measured the perimeter and area of the first ten cells at the intersections of the points of the grid to assess cell size differences between phases.

**Flow Cytometry**

We used flow cytometry to determine the relative abundance of gametophytes and sporophytes in *U. rigida* and *U. compressa*. Based on the C-values (haploid genome sizes) of *U. compressa* (0.13 pg, Le Gall et al. 1993) and *U. rigida* (0.16 pg, Le Gall et al. 1993), we used the freshwater unicellular alga *Chlamydomonas reinhardtii*, as an external flow cytometry control (C-value of 0.12 pg, Merchant et al. 2007). We specifically selected the cell wall-deficient mutant CC-400 cw15 mt+ as our control (University of Minnesota *Chlamydomonas* Center, chlamycollection.org).

We used an enzyme solution developed specifically for efficient production of *Ulva* protoplasts (Reddy et al. 2006), along with a modified version of the LB01 nuclear isolation buffer. Instead of the standard 0.1 % v/v concentration for Triton X-100, we modified the buffer to contain a 1% v/v concentration to ensure the nuclei were cleanly isolated (15 mM Tris, 2 mM EDTA, 0.5 mM Spermine tetrahydrochloride, 80 mM KCl,

20 mM NaCl, 1% vol/vol Triton X-100, 15 mM β-mercaptoethanol; Doležel et al. 2007a).

We weighed all *Ulva s*amples to 0.50 g wet weight, rinsed with them raw seawater to remove debris and epiphytes, and then thoroughly scrubbed them manually in 20 μm filtered seawater to remove smaller particles. *Ulva* samples were chopped with a razor blade in a large (85 mm x 25 mm) plastic Petri dish for one minute, and then the tissue was transferred into a small (55 mm x 15 mm) Petri dish that contained 5 mL of enzyme solution (Reddy et al. 2006).

Protoplasts were released by placing samples on a shaker at 50 rpm in the dark for two hours at room temperature (~21°C), then filtered with a 30 μm nylon mesh into a 5 mL polypropylene tube and spun for five minutes at 120 x g at 4°C. A total of 2 mL of supernatant was then removed and replaced with 2 mL of sterile filtered seawater. Centrifugation with subsequent replacement of fluid was repeated twice, and after the last round of centrifugation, all supernatant was removed and replaced with 1 mL of sterile filtered seawater. We observed successful protoplast isolation via microscopic examination at 400X. In preparation for the flow cytometer samples were spun for five minutes at 120 g at 4°C, the supernatant was removed, and samples were kept refrigerated or on ice.

To liberate the nuclei, we added 1 mL of modified LB01 nuclear buffer kept on ice to each sample, vortexed and tapped the tube occasionally for eight minutes, and then added 0.5 mL of PI/RNase Staining Buffer (BD Science). After five minutes the samples were run on a BD Influx flow cytometer at the RI EPSCoR Marine Life Sciences Facility on the University of Rhode Island's Narragansett Bay Campus. This machine was

optimized for marine applications and is equipped with three lasers (355 nm, 488 nm, and 561 nm). We used a green (532 nm) or a blue (488 nm) laser and quantified fluorescence at 610 nm (20 nm bandwith) on a linear scale. Since sporophytes have twice the amount of genetic material and gametophytes, sporophytes have twice the amount of fluorescence as gametophytes (Figure 2). To measure the spread of the distribution of the data we used the coefficient of variation (CV), which is the standard deviation expressed as a percentage of the population mean. Our CV values ranged from 3-8%. This range is due to the small genome size and the preponderance of PI to bind to remaining cell wall polysaccharides from the extraction of *Ulva* protoplasts, which makes obtaining CV values less than 3% challenging (Kagami et al. 2005, Doležel et al. 2007b).

**Growth Experiments**

We assessed growth rates of gametophytes and sporophytes of *U. rigida* and *U. compressa* in outdoor flow-through ambient temperature seawater tanks on the University of Rhode Island's Narragansett Bay campus. We collected healthy *Ulva* individuals from the shallow subtidal zone in Greenwich Bay in the summer of 2013. In total, we used 90 *U. compressa* individuals (62 sporophyte and 28 gametophyte) and 61 *U. rigida* individuals (38 sporophyte and 23 gametophyte) for this analysis. We conducted growth experiments in June, July, and August to assess differences in growth over the peak bloom-forming months.

In the lab, we determined the species identity of each specimen via microscopic examination. We placed one 1.0g *Ulva* individual in each 2.5 L bucket with mesh sides; after 14 days, all growth experiments concluded and the *Ulva* was re-weighed. For each month, we had a sample size of at least five (up to a maximum of 36) individuals of each

phase of each species, except for *U. rigida* sporophytes in August, when we only had three individuals. All *Ulva* were spun 20 times in a salad spinner prior to each weighing to ensure consistent mass, and all individuals were analyzed using flow cytometry for ploidy content (see above).

**Statistical Analyses**

To assess ploidy ratios in field populations of *U. compressa* and *U. rigida*, we used a $\chi^2$ analysis to determine if the relative abundances of each species, at each site, were significantly different from the null model hypothesis. We then assessed the relationship of several variables (site, species, salinity, temperature, month of collection, date of collection, total *Ulva* biomass, total algal biomass, total *Ulva* biomass) to the ploidy ratio, using a logistic regression model with a binomial response variable (gametophyte vs. sporophyte). We selected the model with the highest AIC as it best explained the distribution of gametophytes and sporophytes in Greenwich Bay.

The AIC measures the relative quality of a statistical model, taking into consideration the number of parameters and the information lost with the model. Model coefficient estimate values predict the odds ratio of gametophytes and sporophytes in the population. The model has a binomial response variable with sporophytes chosen as success and gametophytes as failure. Therefore, negative estimate values are associated with higher proportions of gametophytes while positive estimate values are associated with higher proportions of sporophytes.

Based on the results for the logistic regression model described above, we then selected the three significant continuous variables (salinity, salinity two weeks prior to specimen collection, and total *Ulva* biomass) and analyzed each individually in separate

models for representation in graphical models. Data analyses were conducted in R

(Wickham 2009, Team 2013) and JMP (JMP®, Version 10. SAS Institute Inc., Cary, NC,

1989-2013).

Growth data were analyzed with a two way fixed factor ANOVA to measure

differences across ploidy levels and months. Cell sizes were compared between

gametophytes and sporophytes for each species using t-tests with unequal variances in

JMP.


**RESULTS**

**Ploidy**

We found both gametophytes and sporophytes of each species present at each of

the sampling location sites. There were significant differences among the relative ploidy

levels at each site (Figure 3), compared to the null model prediction of $\sqrt{2}$ gametophytes

to 1 sporophyte ($\chi^2$ likelihood test, Table 1). *U. compressa* in Oakland Beach Cove

(OBC) and Sandy Point (SP) differed from this null prediction with a relative

overabundance of sporophytes. *U. rigida* in Warwick City Park (WCP) and Sandy Point

(SP) differed from the null prediction with a relative overabundance of sporophytes in

WCP and dominance of gametophytes in SP.

Based on AIC values, the strongest predictive model for ploidy relative

abundance included the variables species, site, salinity at time of sampling, and total *Ulva*

biomass (Table 2) and not temperature, month of sampling, date of sampling, or total

algal biomass. While salinity measurements with a time lag of two weeks prior were

significant, they were not included in the model with the strongest AIC.

10

When we analyzed the significant continuous variables individually for their correlation to ploidy ratios, we found that the relative abundance of sporophytes was positively correlated with higher *Ulva* biomass at the time of collection (Figure 4A; $\chi^2_3$= 16.10, p<0.01). We found increasing proportions of *Ulva* sporophytes at higher salinities at the date of sampling for both species (Figure 4B; $\chi^2_3$= 13.36, p<0.01). Interestingly, salinity measurements with a time lag of two weeks prior yielded significantly increasing proportions of *Ulva* gametophytes at higher salinities (Figure 4C; $\chi^2_3$= 10.54, p=0.01) for both species.

**Growth**

We found no significant differences in growth rate between phases for either species, (Figure 5; *U. compressa*, $F_{1,84}$=0.88, p=0.35; *U. rigida*, $F_{1,54}$=0.19, p=0.66) but we did find differences in growth rate across months (*U. compressa*, $F_{2,84}$=6.61, p<0.01; *U. rigida*, $F_{2,54}$=2.25, p=0.02). There was no significant interaction between month and ploidy for either species (*U. compressa*, $F_{2,84}$=0.37, p=0.69; *U. rigida*, $F_{2,54}$=2.00, p=0.15). *U. compressa* had a significantly higher growth rate in July than in June or August (post-hoc Tukey-Kramer, $F_{2,87}$=8.96, p<0.01), with a mean growth rate (g/day in June=0.11, July=0.21, and August=0.15). *U. rigida* also had significantly higher growth rates in July and August vs. June (post-hoc Tukey-Kramer, $F_{2,57}$=5.87, p<0.01), with a mean growth rate (g/day in June=0.05, July=0.07, and August=0.11).

**Cell Area**

*U. compressa* sporophytes (mean area = 87.13±2.76 $\mu m^2$) had a larger cell area than gametophytes (mean area = 75.24±3.88 $\mu m^2$; $t_{58}$=-2.49, p=0.01). *U. rigida* had a trend toward larger sporophyte (mean area = 152.38±12.23 $\mu m^2$) over gametophyte

11

(mean area = 134.41±6.11 $\mu m^2$) cell area sizes, although it was not significant ($t_{41}$=-1.31,

p=0.09; Figure 6).
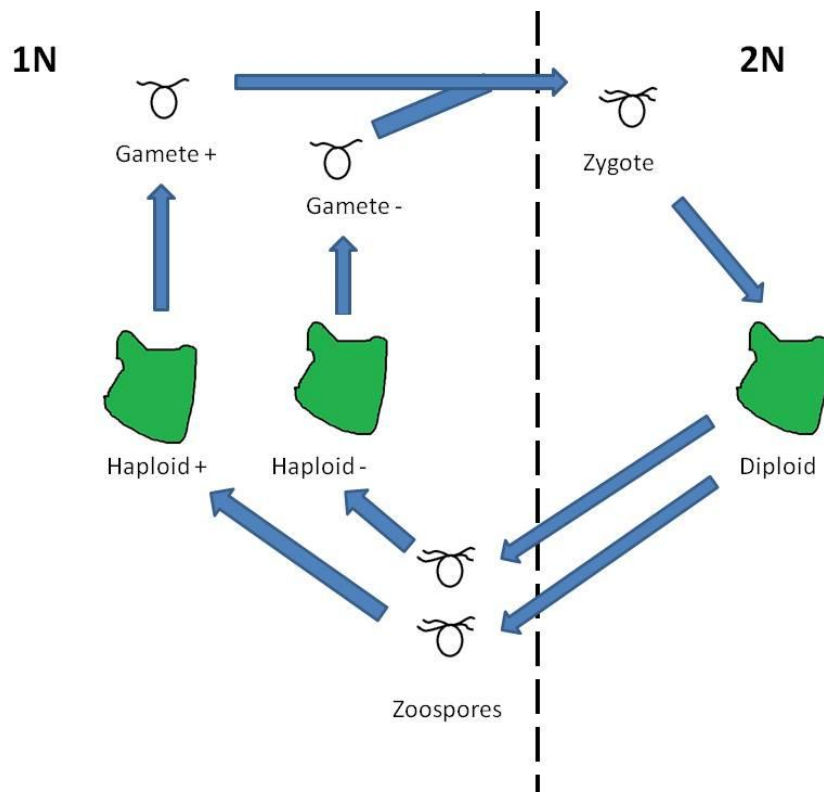
Figure 1. Isomorphic biphasic life cycle of *Ulva*. *Ulva* cycles between two morphologically similar multicellular adult phases, a haploid gametophyte and a diploid sporophyte. Diploid sporophytes produce haploid zoospores that develop into gametophytes. Haploid gametophytes produce haploid gametes. When a "+" and "–" gamete fuse they form a zygote, which develops into a diploid sporophyte.
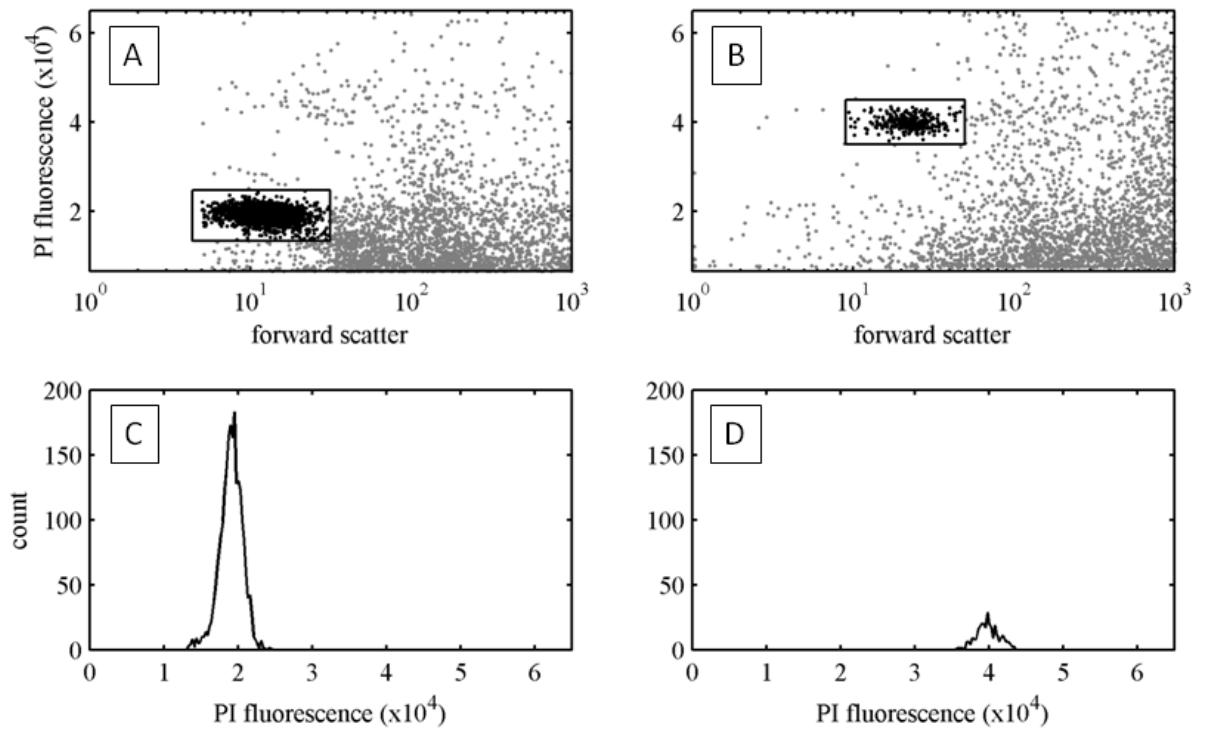
Figure 2. Flow cytometry graphs. The graphs on the left (A, C) represent an *U. compressa* gametophyte, while the two graphs on the right (B, D) represent an *U. compressa* sporophyte. Figures 2A and 2B show the forward scatter by fluorescence, while figures 2C and 2D represent the count of nuclei from 20,000 events. The sporophyte (B, D) has twice the fluorescence as the gametophyte (A,C), with the gametophyte mean fluorescence near 19,000 and the sporophyte mean fluorescence near 38,000.

14
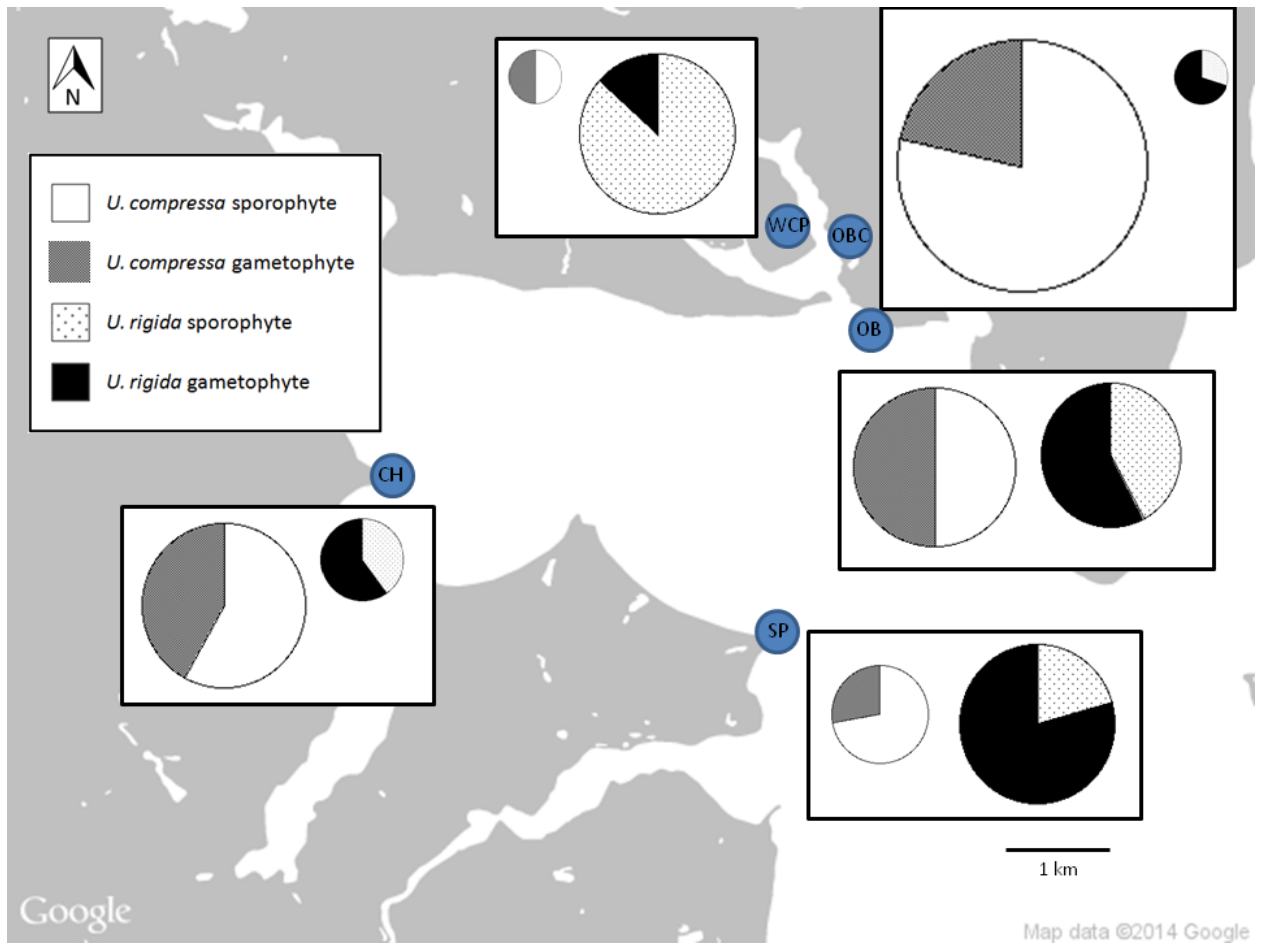
Figure 3. Map of Greenwich Bay (a subset of Narragansett Bay, Rhode Island). This figure shows the relative proportion of gametophytes and sporophytes present at five sites in Greenwich Bay during the 2013 bloom forming season. The sites are Warwick City Park (WCP), Oakland Beach Cove (OBC), Oakland Beach (OB), Sandy Point (SP), and Chepiwanoxet (CH). Pie chart sizes represent the relative number of individuals sampled.

| Site | Species | $\chi^2$ | Sample Size | Overabundant Phase | Prob> $\chi^2$ |
|------|---------|----------|-------------|--------------------|----------------|
| Chepiwanoxet | *U. compressa* | 3.651 | 31 | Expected | 0.056 |
| Oakland Beach | *U. compressa* | 0.988 | 30 | Expected | 0.320 |
| Oakland Beach Cove | *U. compressa* | 27.877 | 47 | Sporophyte | **<0.001** |
| Sandy Point | *U. compressa* | 7.188 | 18 | Sporophyte | **0.007** |
| Warwick City Park | *U. compressa* | 0.329 | 10 | Expected | 0.566 |
| Chepiwanoxet | *U. rigida* | 0.006 | 15 | Expected | 0.937 |
| Oakland Beach | *U. rigida* | 0.018 | 26 | Expected | 0.892 |
| Oakland Beach Cove | *U. rigida* | 0.519 | 10 | Expected | 0.471 |
| Sandy Point | *U. rigida* | 5.401 | 29 | Gametophyte | **0.020** |
| Warwick City Park | *U. rigida* | 27.024 | 30 | Sporophyte | **<0.001** |

Table 1. Testing against the null model prediction of √2 gametophytes to 1 sporophyte by site and species. The overabundant phase column indicates which phase was more abundant than predicted by the null model. Numbers in bold indicate significant (<0.05 values).

| Coefficient | Estimate | Std. Error | z value | Pr(>|z|) |
|---|---|---|---|---|
| Intercept | -2.480 | 0.953 | -2.603 | **0.009** |
| *U. rigida* | -1.130 | 0.629 | -1.798 | 0.072 |
| Oakland Beach | -0.956 | 0.579 | -1.650 | 0.099 |
| Oakland Beach Cove | 0.684 | 0.523 | 1.309 | 0.191 |
| Sandy Point | -0.547 | 0.683 | -0.801 | 0.423 |
| Warwick City Park | -0.688 | 0.766 | -0.898 | 0.369 |
| Salinity | 0.075 | 0.019 | 3.923 | **<0.001** |
| *Ulva* biomass | 0.088 | 0.033 | 2.667 | **0.008** |
| Oakland Beach*U. rigida* | 0.503 | 0.860 | 0.585 | 0.559 |
| Oakland Beach Cove*U. rigida* | -0.118 | 0.940 | -0.125 | 0.900 |
| Sandy Point*U. rigida* | -0.794 | 0.946 | -0.839 | 0.402 |
| Warwick City Park*U. rigida* | 2.891 | 1.051 | 2.750 | **0.006** |

Table 2. Table for the best-fit logistic regression with a binomial distribution and ploidy as the independent variable. Model follows the form logit($\hat{y}$) = $\beta_0$ + $\beta_1 x_1$+ $\beta_2 x_2$ +… (e.g. -2.480 – 1.130*U. rigida* – 0.946*Oakland Beach +…) Numbers in bold indicate significant (<0.05 values).
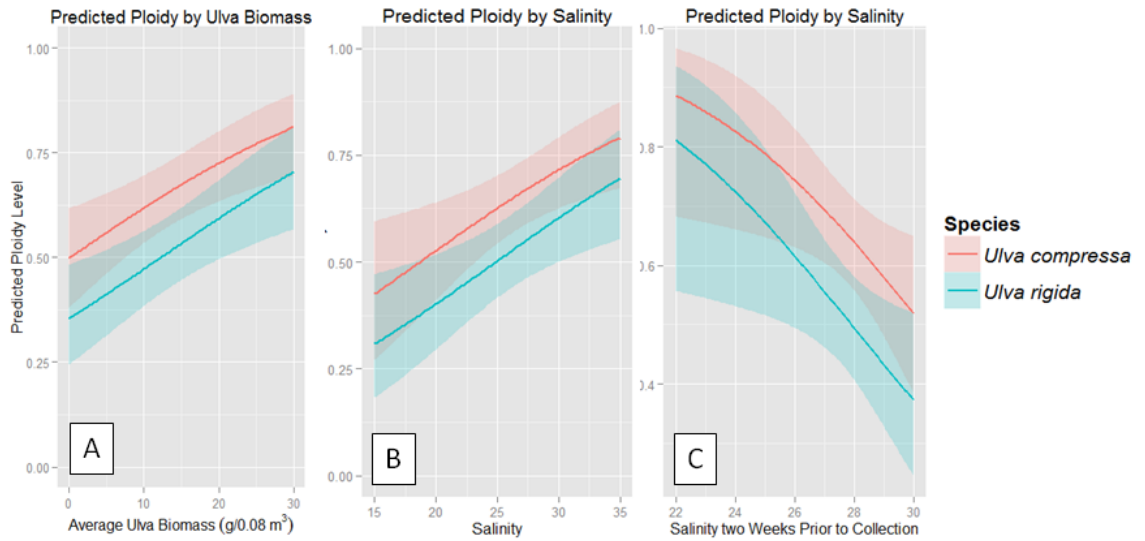
Figure 4. This figure describes the predicted probability of an individual being a gametophyte or sporophyte under different environmental conditions based on the model estimate. Sporophytes are represented by one, while gametophytes are represented by zero. A value of one indicates 100 percent sporophyte abundance. Variables measured include *Ulva* biomass (g/0.08 m$^3$) present at time of collection (4A), surface salinity values from the date collection (4B) and from two weeks prior to collection (4C), The upper line is *U. compressa* and the lower line is *U. rigida*. The shading indicates 95% confidence intervals.
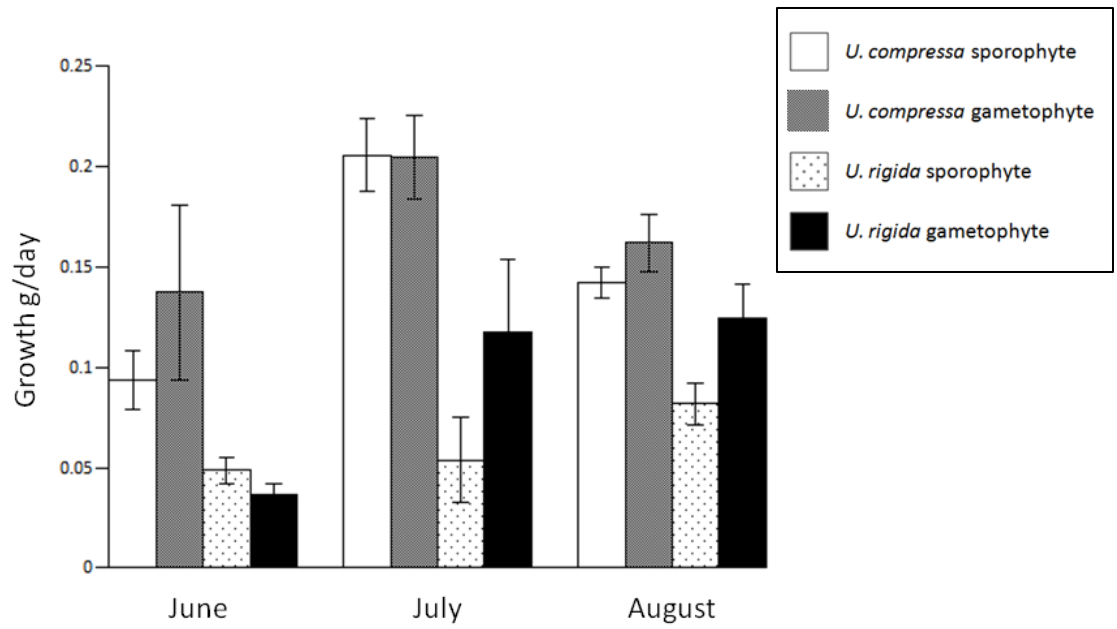
Figure 5. Growth of *U. rigida* and *U. compressa* gametophytes and sporophytes. There are no significant differences in growth rate between ploidy levels in either species. Data are means ± one standard error. Numbers represent the sample size for each bar.
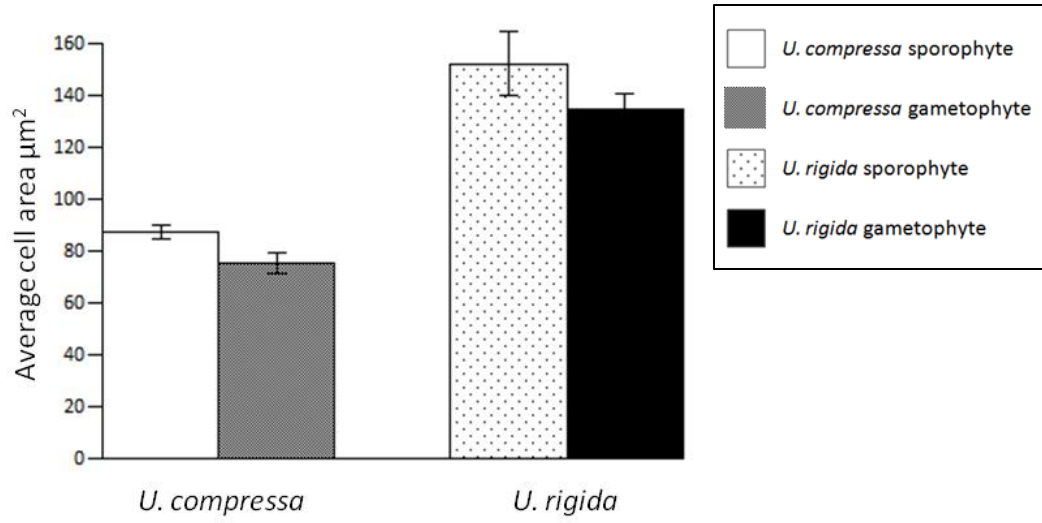
Figure 6. Cell size by species and ploidy for *U. compressa* and *U. rigida*. *U. compressa* has significantly larger sporophyte cell sizes then gametophyte cell sizes. Data are means $\pm$ one standard error.

## DISCUSSION

## Ploidy Distribution

Macroalgal blooms are a problem worldwide and cause severe ecological and economic damage (Liu et al. 2013). Prior to this study, only two publications Hiraoka and Yoshida (2010) and Alström-Rapaport et al (2010) had assessed abundance of *Ulva* gametophytes and sporophytes, and found an alternating abundance of gametophytes and sporophytes that was seasonal only in the latter study. Here, our data indicate that both phases are present for each species throughout the peak bloom forming season, and that relative phase abundance is correlated with both abiotic and biotic factors. We found a high variability among sites in ploidy ratio among sites, with some sites matching the null model prediction of relative abundance, while others exhibited an overabundance of gametophytes or sporophytes. These deviations could be due to ecological differences among phases and/or environmental differences among sites. Sandy Point, which differed from the null hypothesis for both species, is a more exposed site and experiences more water mixing than the other sites (Sankaranarayanan & Ward 2006). However, as *U. compressa* had an overabundance of sporophytes and *U. rigida* had an overabundance of gametophytes at this site, the relative impacts of environmental factors are challenging to assess and may represent specific environmental factors unique to each species. Warwick City Park and Oakland Beach Cove, which differed from the null hypothesis in *U. compressa* and *U. rigida* respectively, are more sheltered sites and experience less water mixing (Sankaranarayanan & Ward 2006).

We found a significant correlation of physical and biological factors on the relative abundance of gametophytes and sporophytes in our study system (Table 2, Figure

21

4). In this study system, low salinities are typically a result of increased freshwater flow from rivers caused by storms. In Narragansett Bay, increased flow in rivers yields higher concentrations of dissolved inorganic nitrogen and phosphorus (Nixon et al. 1995). Therefore, although nutrient data are not available for our sampling period, low salinities can be used as a proxy for increased nutrients. Lower salinities from the date of sample collection were correlated with higher relative levels of gametophytes, while lower salinities from two weeks prior to specimen collection were correlated with more sporophytes (Figure 4). This shift in ploidy ratios may be due to several factors, such as salinity tolerance, positive response to nutrient availability from one phase over the other, or shift to asexual reproduction (Bliding 1963). While it is unlikely that a reproductive event would result in the presence of new adults after only two weeks (Leletkin et al. 2004), lower salinities may trigger more rapid growth of one phase from a microscopic to a macroscopic size (Hannach & Santelices 1985). Due to the biphasic life cycle, increased nutrients may either impact mortality and/or fecundity rates of either phase (Adams & Hansche 1974, Lewis 1985), with differential effects on the relative balance of phases. In addition, vegetative fragmentation of mature blades, germination of unfused gametes, and/or asexual production of diploid spores by sporophytes may impact the ploidy ratio (Van Den Hoek et al. 1995).

We also found a positive correlation between the relative abundance of sporophytes for total *Ulva* biomass for both species. One potential explanation is that gametophytes could be more successful when there is less competition for resources, while sporophytes are better competitors than gametophytes under higher competitive pressure for resources. While other environmental factors, such as temperature (Deacutis

et al. 2006), have an impact on bloom abundance (Rivers & Peckol 1995, Kim et al. 2011) or growth rates (Guidone 2012), we found no impact of temperature on the relative abundance of gametophytes and sporophytes.

Previous studies have found a seasonal dominance of one ploidy phase (Dyck & De Wreede 1995) or a long term (11-20 month) non-seasonal cyclic dominance (Hiraoka & Yoshida 2010), or no seasonal trend (Thornber & Gaines 2003). As our sampling was limited to the bloom forming season, a cycling trend in ploidy for *U. compressa* and/or *U. rigida* could exist. However, due to the scarcity of *Ulva* specimens during non bloom forming periods (Guidone & Thornber 2013), this would be challenging to assess.

**Growth and Cell Size**

We did not find any significant differences in growth rates of adult gametophytes and sporophytes of either species, but this does not preclude the possibility of differences at the germling stage (Hannach & Santelices 1985). In addition, growth rates can vary based on nutrient levels (Peckol et al. 1994); as nutrient levels shift in Narragansett Bay over seasonal cycles (Oviatt et al. 2002, Nixon et al. 2008), differences in *Ulva* growth rates between phases may emerge.

Based on our *U. compressa* cell size data, future studies of *U. compressa* life cycle dynamics may be much more rapid and less expensive. Individuals can be predicted as gametophytes or sporophytes based on their cell size, with a subset confirmed using ploidy analysis. This would increase the ability to have larger sample sizes and more rapid assessment. Similarly, *U. rigida* trended toward larger sporophytes over gametophytes (significant at the 90 percent confidence level).

Differences in *U. compressa* cell sizes between phases can impact the surface area to volume ratio, allowing for faster uptake of nutrients in smaller cells (Lewis 1985). This is especially relevant in single-celled spores, gametes, and small juveniles, and may impact *Ulva* individuals in their early growth stages. *U. rigida* zoospores are 9-15 µm x 5-10 µm while gametes are 7-11 µm x 4-6 µm (Clayton 1992). Since gametes are much smaller than zoospores, they may have a survival advantage in their enhanced ability for nutrient uptake. There may also be other ecological differences between either phases across their lifespan, such as susceptibility to herbivores, light tolerance, salinity tolerances, and temperature optima (Destombe et al. 1993, Thornber 2006, Thornber et al. 2006, Guillemin et al. 2013), that may explain differences in ploidy ratios. Gametophytes do not have heterozygosity, so they do not have the genetic capacity to deal with environmental changes as efficiently as sporophytes. However, both beneficial and deleterious mutations will immediately be expressed in gametophytes, so deleterious mutations will be eliminated faster than in sporophytes, and if a beneficial mutation should arise in a gametophyte population, it will reach fixation faster than in sporophyte populations (Otto & Gerstein 2008).

**Flow Cytometry Method**

We designed our flow cytometry ploidy analysis methods from similar analyses in higher plants (Doležel et al. 1989, Doležel & Bartos 2005, Doležel et al. 2007a, b), which has been successful for other macroalgal studies (e.g. Le Gall et al. 1993). We first attempted chopping *Ulva* tissues with a razor blade in the presence of a nuclear isolation buffer to obtain isolated nuclei (essentially removing our protoplast isolation step). This method, which is successful in higher plants for flow cytometric analysis (Galbraith et al.

24

1983), was unsuccessful for *Ulva*. The amount of nuclei obtained was small and contaminated with other materials, likely organelle genomes and bacteria (Nakanishi et al. 1996). In addition, *Ulva* has high concentrations of anionic polysaccharides in its cell walls (Abdel-Fattah & Edrees 1972) which can interfere with obtaining a sufficient number of nuclei by binding to the  positively charged nuleus, inhibiting the propidium iodide from attaching. Given these constraints, protoplast isolation was necessary to obtain sufficient numbers of nuclei for flow cytometry analyses (Kagami et al. 2005), which is successful yet time consuming and expensive (Reddy et al. 2006), thus limiting our abilities to obtain larger sample sizes.

Given the abundance of *Ulva* blooms worldwide (Morand & Merceron 2004, Merceron et al. 2007), it is crucial that we understand the basic ecology of *Ulva*, including the life cycle, to make more informed management decisions for bloom mitigation. This research presents an innovative step into further understanding *Ulva* life cycle dynamics and their relationships to associated abiotic and biotic factors, through the use of flow cytometry techniques.

## ACKNOWLEDGEMENTS

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