2018

Bloom-forming macroalgae (*Ulva* spp.) inhibit the growth of co-occurring macroalgae and decrease eastern oyster larval survival

Lindsay A. Green-Gavrielidis
University of Rhode Island, lindsaygreen@uri.edu

Fiona MacKechnie
University of Rhode Island

Carol S. Thornber
University of Rhode Island, thornber@uri.edu

Marta Gomez-Chiarri
University of Rhode Island, gomezchi@uri.edu

Citation/Publisher Attribution

Green-Gavrielidis LA, MacKechnie F, Thornber CS, Gomez-Chiarri M (2018) Bloom-forming macroalgae (*Ulva* spp.) inhibit the growth of co-occurring macroalgae and decrease eastern oyster larval survival. Mar Ecol Prog Ser 595:27-37. https://doi.org/10.3354/meps12556
Available at: https://doi.org/10.3354/meps12556

Bloom-forming macroalgae (*Ulva* spp.) inhibit the growth of co-occurring macroalgae and decrease eastern oyster larval survival

Lindsay A. Green-Gavrielidis[1*], Fiona MacKechnie[1,2], Carol S. Thornber[1], and Marta Gomez-Chiarri[3]

[1]*Department of Natural Resources Science, University of Rhode Island, Kingston, RI 02881, U.S.A.*

[2]*Present address: Scottish Association for Marine Science, Scottish Marine Institute, Oban, Argyll, PA37 1QA, United Kingdom*

[3]*Department of Fisheries, Animal, and Veterinary Sciences, University of Rhode Island, Kingston, RI, 02881*

*Corresponding author lindsaygreen@uri.edu

Citation: Green-Gavrielidis LA, MacKechnie F, Thornber CS, Gomez-Chiarri M (2018) Bloom-forming macroalgae (*Ulva* spp.) inhibit the growth of co-occurring macroalgae and decrease eastern oyster larval survival. Mar Ecol Prog Ser 595:27-37. https://doi.org/10.3354/meps12556

Abstract

Macroalgal blooms have increased in frequency worldwide due to anthropogenic activities. Algal blooms can disrupt recreational activities, interfere with fisheries, and deplete oxygen during decomposition. Narragansett Bay has experienced macroalgal blooms dominated by blade-forming *Ulva* for over a century. Evidence from other systems has suggested that *Ulva* can negatively impact other organisms. The first objective of this study was to determine whether bloom-forming *Ulva compressa* and *U. rigida* inhibit the growth of co-occurring macroalgae, *Gracilaria vermiculophylla*, *Cystoclonium purpureum,* and *Chondrus crispus,* during co-culture via laboratory-based assays. We found that *U. compressa* and *U. rigida* significantly inhibited the growth of all three macroalgae. We were able to verify the negative effects of *Ulva compressa*, but not *U. rigida* on the growth of *G. vermiculophylla* in flow-through seawater tanks. Our second objective was to determine if *Ulva* exudate decreased the survival of eastern oyster larvae in laboratory challenge experiments. We documented a significant negative effect of *Ulva* exudate on oyster survival, which depended on both the *Ulva* species and the nutrient condition. The strongest effect on oyster larval survival was seen in larvae exposed to nutrient replete *Ulva compressa* exudate, which had less than 30% relative survival after one week. Our results indicate that bloom-forming *Ulva* has the potential to inhibit co-occurring macroalgae and cause oyster larval mortality.

KEY WORDS- *Ulva compressa*, *Ulva rigida*, macroalgal blooms, larval mortality.

Introduction

Macroalgal blooms, generally consisting of green ulvoid macroalgae (commonly referred to as “green tides”), have been increasing worldwide (Valiela et al. 1997, Nelson

et al. 2003, Teichberg et al. 2010, Liu et al. 2013, Smetacek & Zingone 2013) and are common occurrences on the northeastern coast of the United States (Bricker et al. 2008). Macroalgal blooms are typically driven by anthropogenic nutrient loading in shallow estuaries and can result in declines in seagrass (Valiela et al. 1997, McGlathery 2001), perennial algae, and overall community diversity (Worm & Lotze 2006). During bloom decomposition, macroinvertebrate abundance declines (Cummins et al. 2004) and dissolved organic nitrogen is released into the water column (Tyler et al. 2001) that can fuel further primary production (reviewed by Raffaelli et al. 1998). Macroalgal blooms are also costly to clean up (Atkins et al. 1993, Lapointe & Bedford 2007).

Narragansett Bay, Rhode Island, U.S.A is a 380 km^2 semi-diurnal, well mixed tidal estuary (Deacutis et al. 2006). The northern part of the bay is heavily populated and there are three major urban freshwater inflows that contribute anthropogenic nutrients to the system (Deacutis et al. 2006, Thornber et al. 2008). Greenwich Bay, a small sub-embayment on the western side of Narragansett Bay, has been plagued by persistent macroalgal blooms during the summer months for more than a century, dominated by *Ulva compressa* Linnaeus and *U. rigida* C. Agardh (Granger et al. 2000, Guidone et al. 2013, Thornber et al. 2017). For example, Granger et al. (2000) documented 100-400 g dry mass/m^2 (1015-4060 g wet mass/m^2 based on the conversion factors of Angell et al. 2012) of *Ulva* in Greenwich Bay in 1996, while Guidone & Thornber (2013) observed a maximum biomass of >1800 g wet mass/m^2 in 2010.

Although green macroalgal blooms can have significant deleterious impacts on coastal ecosystems (see Fletcher 1996), they have historically been considered non-toxic (Valiela & Cole 2002, Anderson 2009). Green macroalgae have been considered to be

less likely than red and brown macroalgae to inhibit or harm co-occurring organisms (Harlin 1987, Valiela et al. 1997), and therefore, competition between green macroalgae and co-occurring species has been investigated less than with red and brown macroalgae (Hurd et al. 2014). However, growing evidence has suggested that ulvoid species of green macroalgae (species in the Family Ulvophyceae) can inhibit the growth, germination, and/or development of co-occurring organisms (Nelson et al. 2003, Nan et al. 2008, Nelson & Gregg 2013, Van Alstyne et al. 2014, Van Alstyne et al. 2015; Table 1). Evidence of ulvoid species suppressing the growth of phytoplankton, especially species that cause harmful algal blooms, has been especially strong (*e.g.* Jin & Dong 2003, Nan et al. 2008, Wang et al. 2012, Accoroni et al. 2015).

While several researchers have reported positive and negative effects of ulvoid species on invertebrates (Nelson & Gregg 2013, Van Alstyne et al. 2014), to date there have been no reports of the potential effects of *Ulva* spp. on the economically important eastern oyster, *Crassostrea virginica*. Muñoz et al. (2012) showed that the presence of young *Ulva* thalli improved the post-larval growth rate of the commercially produced red abalone *Haliotis rufescens*, while Huggett et al. (2005) reported high settlement of the abalone *H. rubra* on two ulvoid species. Lamb (2015) noted that the presence of *Ulva* thalli in aquaculture bags resulted in slower growth of adult Pacific oysters, *Crassostrea gigas*. Currently there are 315 aquaculture farms that cultivate the eastern oyster *C. virginica* in the U.S. (USDA 2014), many of them in areas where *Ulva* is present. Therefore, it is important to understand the interactions between bloom-forming *Ulva* and the eastern oyster.

Given the mounting evidence from other systems dominated by ulvoid macroalgae, we hypothesized that blade-forming species of *Ulva,* namely the bloom-forming *U. compressa* and *U. rigida,* inhibit the growth of co-occurring organisms in Narragansett Bay. The first objective of this study was to determine if *U. compressa* or *U. rigida* negatively affect the growth of co-occurring macroalgae. Our second objective was to determine if exudate from *U. compressa* or *U. rigida* affected the survival of eastern oyster larvae. Testing the impacts of *Ulva* exudate on oyster larvae was important for two reasons. First, *Ulva* blooms form in coastal ponds where eastern oyster populations co-occur and oyster cultivation is present (Thorne-Miller et al. 1983, Beutel 2017). Second, larvae should be included in assays because they are generally more sensitive to heavy metals and pollutants than adults (Connor 1972, His et al. 1999). We discuss our findings in light of increased coastal development and eutrophication, which will likely fuel increasing macroalgal blooms in the future.

Materials and Methods

Genetic identification of *Ulva*

The genus *Ulva* contains many blade-forming species that appear morphologically similar, however, the cell shape and numbers of pyrenoids can be used to distinguish between *U. compressa* and *U. rigida* (Guidone et al. 2013). We examined each blade of *Ulva* and determined its identity based on the morphological characteristics detailed by Guidone et al. (2013). *Ulva compressa* cells are polygonal with rounded corners and contain a single pyrenoid, while *U. rigida* cells are polygonal with angular corners and contain 2-4 pyrenoids. However, *Ulva* morphology can be highly variable

(Hofmann et al. 2010), so we also used DNA barcoding to verify the accuracy of our morphological identifications. We amplified a 678 bp segment from the *rbc*L gene of specimens used in these experiments following the methods of Guidone et al. (2013) except that we used a modified CTAB plant DNA extraction protocol based on Doyle and Doyle (1987). The raw sequence chromatograms were trimmed and proofread in 4Peaks (v.1.8, Nucleobytes) and sequences were aligned and assembled in Seq Man Pro (v.12, DNA Star Inc.).

Genetic identification of *Gracilaria*

Two species of *Gracilaria* occur in Narragansett Bay, the native *Gracilaria tikvahiae* McLachlan and the introduced *Gracilaria vermiculophylla* (Ohmi) Papenfuss (Nettleton et al. 2013). These two species have morphological characteristics that overlap, and therefore restricted fragment length polymorphism (RFLP) and selected DNA barcoding was performed to determine the species identification of material from the laboratory-based mesocosm trials.

DNA was extracted using the modified CTAB plant DNA extraction protocol based on Doyle and Doyle (1987). Polymerase chain reaction was performed in 50 µL volumes containing 10 µL of 5X GoTaq® Flexi DNA Polymerase (Promega Corporation), 7 µL of 25 mM Mg^{2+}, 1 µL of 2.5 mM dNTP, and 4 µL of extracted DNA template (10-50 ng). A 307 bp segment from the mitochondrial gene COX1 was used for species identification and was amplified with the forward primer CO1F328 and the reverse primer CO1R634 (Nettleton et al. 2013). The PCR profile consisted of an initial denaturation at 95°C for 2 minutes, followed by 30 cycles of 57°C for 1 minute, 73°C for

1 minute, and 95°C for 1 minute followed by a final minute at 57°C and a final extension at 73°C for 6 minutes. RFLP analysis was performed on the PCR samples after amplification following the protocol of Nettleton (2012). In addition to the RFLP analysis, three samples were chosen at random to be sequenced. PCR purification, sample preparation, sequencing, and sequence analysis were performed as described above (see "Genetic Identification of *Ulva*").

Effects of *Ulva* on co-occurring macroalgae

In order to determine whether *Ulva compressa* or *U. rigida* suppress the growth of other macroalgae, we performed a series of co-culture experiments. We co-cultured isolated tips of three species that are common in *Ulva* blooms in Narragansett Bay (Thornber, *unpub. data*), *Gracilaria vermiculophylla*, *Cystoclonium purpureum* (Hudson) Batters, and *Chondrus crispus* Stackhouse from adult thalli, in separate trials, with the bloom-forming *U. compressa* and *U. rigida.* We then conducted a series of semi-controlled trials with *U. compressa*, *U. rigida*, and *G. vermiculophylla* in outdoor flow-through seawater tanks.

All macroalgal material was collected in Narragansett Bay, Rhode Island during low tide in the intertidal or shallow subtidal zone and transported to the laboratory on ice for processing. Upon arrival at the laboratory, all material was cleaned with sterile seawater to remove epiphytes. Following epiphyte removal, tips of *G. vermiculophylla*, *C. purpureum*, and *C. crispus* were excised using sterile razor blades, rinsed three times with sterile seawater (30-32 psu), and placed in 250 mL flasks with sterile Von Stosch Enriched (VSE) natural seawater (Ott 1966) under acclimation conditions (20-23°C, 100

μmol photons m^{-2} s^{-1}, and a 16:8 Light: Dark photoperiod with constant aeration); total acclimation to laboratory conditions occurred for at least 3 days, with at least 24 hours allowed for wound healing following tip cutting. Natural seawater was obtained from the Marine Science Research Center (MSRC) at the University of Rhode Island's Narragansett Bay Campus, filtered to 0.2 μM, and autoclaved prior to use.

After the acclimation period, the blotted-dry wet mass of tips of *G. vermiculophylla* were taken and the tips were placed in individual 1 L mesocosms that were divided in half with mesh with 1 mm^2 openings and filled with 400 mL of sterile VSE seawater. On the other side of the mesh, 0.4 g of either *U. compressa* or *U. rigida* (=1 g/L) was added. Experimental culture conditions were equivalent to those provided during the acclimation period and light was supplied from the top to ensure no interspecific shading. In total, there were 21 mesocosms with seven replicates each of the *U. compressa* treatment, *U. rigida* treatment, and mesocosm control (*G. vermiculophylla* in mesh-divided mesocosm without *Ulva*) per trial. In order to prevent nutrient limitation, NO_3^- was measured daily as a proxy for nutrient concentrations, and all VSE nutrients ($NaNO_3$, $Na_2HPO_4 \cdot 12H_2O$, $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $Na_2EDTA \cdot 2H_2O$, Thiamine-HCl, Biotin, Vitamin B_{12}) were replenished based on nitrate depletion. Nitrate was measured using an API Nitrate Test Kit modified for a 1 mL sample. Nitrate was considered depleted if it was below 40 ppm (*i.e.* full VSE enrichment) and was replenished to this level daily. On average, we replenished nutrients in the *U. compressa* treatment every other day and the *U. rigida* treatment every 2.6 days.

On Days 2, 4, 6 and 8 of each trial, the blotted-dry wet mass of *G. vermiculophylla* tips was measured. Relative growth rate (RGR) was calculated using the

following equation: RGR (%) = 100 × [ln (L_2/L_1)/(t_2-t_1)], where L_2 and L_1 were the blade weight at times t_2 and t_1, respectively. A total of two *G. vermiculophylla* trials were performed. The same experimental design was used to conduct separate trials with *Cystoclonium purpureum* (2 trials) and *Chondrus crispus* (2 trials). Daily pH levels were determined for the first *Chondrus crispus* trial only using an EcoTestr™ pH meter (Oakton ®).

***Gracilaria vermiculophylla* control trials**

To confirm that the observed results were due to the presence of *Ulva* and not simply due to the presence of another macroalga, two *G. vermiculophylla* control trials with the same experimental design as the co-culture trials described above were performed. Seven replicates each of two treatments, *G. vermiculophylla* and mesocosm control, were included in each trial. The *G. vermiculophylla* treatment had 0.4 g (=1 g/L) of *G. vermiculophylla* on one side of the mesh and a tip of *G. vermiculophylla* on the other side.

Semi-controlled outdoor flow-through seawater tank trials

In order to determine whether *Ulva* suppressed the growth of co-occurring macroalgae, semi-controlled trials in outdoor flow-through seawater tanks were conducted at the MSRC during July 2015 (n=4). *G. vermiculophylla* (0.85 ±0.04 g) was co-cultured with 1.5 g of either *U. compressa, U. rigida,* or *G. vermiculophylla* (control) in separate flow-through tanks (n=3). *Ulva compressa, U. rigida,* or *G. vermiculophylla* (1 g/L) were placed in individual mesocosms (16 x 11.9 x 7.62 cm) covered with mesh on

all sides (mesh size=1.6 cm^2) that was connected with cable ties to a mesocosm containing *G. vermiculophylla*. The mesocosm pairs were arranged so that *Ulva* was upstream of *G. vermiculophylla*. The mass of *G. vermiculophylla* was measured on Day 0 and Day 7 and relative growth rate (RGR) was calculated. Due to space limitations, four individual trials were conducted with a single replicate from each treatment in each trial. Water temperature was measured using HOBO Tidbit v2 water temperature loggers (Onset Computer Corporation) and averaged 23.6°C (individual tanks ranged from 23.16°C ± 0.09 to 27.28°C ± 0.51; mean ± SE).

Effects of *Ulva* on oyster larvae

In order to determine whether exudate from *U. compressa* or *U. rigida* affected the survival of eastern oyster larvae, a series of challenge experiments were conducted. *Ulva compressa* and *U. rigida* (5 g/L) were cultured in nutrient replete (*i.e.* supplied full VSE nutrients) or nutrient deplete (*i.e.* no nutrients supplied) seawater for 2-3 days, under the same conditions outlined above, to produce *Ulva* exudate. This concentration of *Ulva* was chosen to reflect those present in *Ulva* blooms. Bloom biomass can exceed 8,000 g/m^3 in the subtidal and 3,000 g/m^2 in the intertidal (Thornber et al. 2017). In the nutrient replete cultures, NO_3^- was measured daily as a proxy for nutrient concentrations, and all VSE nutrients were replenished based on nitrate depletion. However, exudate was not collected for use in the challenge experiments until all NO_3^- was depleted in the nutrient replete cultures, since nitrate can be toxic to juvenile and adult shellfish (Epifanio & Srna 1975).

At the end of the culture period, *Ulva* material was removed from the seawater

and the pH of exudate was adjusted to 7.9-8.0. The exudate was then filter sterilized (0.2 μM). Oyster larvae were obtained from the Blount Shellfish Hatchery at Roger Williams University and acclimated to laboratory conditions in sterile natural seawater on a shaker plate (40 rpm). Larvae were fed 2 mL/L of Shellfish Diet 1800® (Reed Mariculture, Campbell, CA) every other day while in the laboratory. At the start of the experiments oyster larvae were between 3 and 9 days old.

Challenge experiments (3 trials) were conducted in 6-well culture plates following a slight modification of previously developed protocols (Karim et al. 2013, Sohn et al. 2016). Oyster larvae (~50-100) were collected onto 45 μM nylon mesh, washed with filtered sterile seawater and placed into each well with 5 mL of the assigned treatment water. Treatments included *U. compressa* + nutrients, *U. compressa* – nutrients, *U. rigida* + nutrients, and *U. rigida* – nutrients. Each well plate contained three wells of a treatment and three wells of control (sterile seawater). Larval survival was assessed on Days 3, 5, and 7 by counting dead larvae (*i.e.* empty shells) in each well using an inverted microscope. At the end of the experiment, larvae were fixed by adding 70% ethanol to each well to obtain a total count. Percent survival of oyster larvae was calculated for each day using the following equation: % survival = (total - dead) ÷ total × 100. In instances where % survival was less than 0, due to human error in counting, survival was adjusted to 0% (8 out of 141 observations). The relative percent survival (of control) was calculated by randomly pairing each treatment well with a control well from the same plate using the following equation: relative percent survival = (% survival of treatment ÷ % survival of control) × 100.

Statistical Analysis

We used separate split-plot analysis of variance (ANOVA) tests to determine the effect of co-culture with *U. compressa* and *U. rigida* on the growth rate of *G. vermiculophylla*, *C. purpureum*, and *C. crispus* with treatment as the main plot (3 levels) and time as the sub-plot (4 levels); Trial (n=2) was included as a blocking factor. We also used a split-plot ANOVA to test the effect of co-culture with *G. vermiculophylla* on the growth of *G. vermiculophylla* tips (*G. vermiculophylla* control trial). We used a one-way ANOVA to test the effect of treatment (3 levels) on the growth rate of *G. vermiculophylla* in semi-controlled outdoor flow-through seawater tank trials, with Trial (n=4) included as a blocking factor. We used a two-way split-split-plot ANOVA to determine the effect of *Ulva* species (main plot, 2 levels), nutrients (sub-plot, 2 levels), and day (sub-sub plot, 3 levels) on the percent survival (of control) of oyster larvae from the challenge experiment. Trial (n=3) was used as a blocking factor.

Prior to analyses, all data were examined for normality and homogeneity of variances and transformed where appropriate; *G. vermiculophylla* growth rate was log transformed to ensure homogeneity of variances. Our growth and percent survival data did not meet the assumption of normality, even after transformation; however ANOVA is robust to deviations from normality when experiments have balanced designs and reasonable sample sizes (Underwood 1997). Post-hoc comparisons were made using Tukey's Honestly Significant Differences tests. All statistical analyses were conducted using JMP (v.12.0.1, SAS Institute Inc.).

Results

Genetic identification of *Ulva*

All *U. compressa* specimens identified using morphological characteristics (n=14) in this study were verified by DNA barcoding using MegaAlign (v.12, DNA Star Inc.) to match *U. compressa* from the Northwest Atlantic (GenBank® Accession: KC582355.1). Although the holotype sequence for *U. compressa* is not currently available, our sequences agreed with the *U. compressa* concept identified by Guidone et al. (2013).

The topotype of *U. rigida* is labeled on GenBank® as *U. armorica* (Shimada et al. 2003; Guidone et al. 2013), which has since been synonymized with *U. rigida.* All *U. rigida* specimens identified using morphological characteristics in this study (n=14) were verified to match *U. rigida* from the Northwest Atlantic (GenBank® Accession: EU484395.1) and were 99% identical to the *U. rigida* topotype (GenBank® Accession: AB097630).

Genetic identification of *Gracilaria*

All *Gracilaria* specimens used in the laboratory co-culture experiments (n=6 for *Ulva* experiments and n=8 for *G. vermiculophylla* control) were identified through RFLP analysis as *G. vermiculophylla.* The three samples that were sequenced were identical to *G. vermiculophylla* from the Northwest Atlantic (GenBank® Accession: JQ675712.1) based on Nettleton et al. (2013).

Effects of *Ulva* on co-occurring macroalgae

The effect of treatment (*Ulva compressa, U. rigida,* and mesocosm control) on the relative growth rate of *Gracilaria vermiculophylla* was dependent on Day (Treatment x

Day: $F_{6,153} = 2.2$, $p = 0.048$; Figure 1a; Table S1). After eight days of co-culture, the RGR of *G. vermiculophylla* without *Ulva* (7.44 ± 1.35 % d^{-1}) was more than three times higher than *G. vermiculophylla* co-cultured with *U. rigida* (2.31 ± 0.69 % d^{-1}). *G. vermiculophylla* co-cultured with *U. compressa* had virtually no change in mass on Day 8 and grew significantly slower than *G. vermiculophylla* tips in the mesocosm control ($p=0.004$; Figure 1a).

Day significantly affected the relative growth rate of *Cystoclonium purpureum* ($F_{3,151} = 8.2$, $p < 0.001$) and was dependent on Treatment (Treatment x Day: $F_{6,151} = 3.9$, $p = 0.001$; Figure 1b; Table S2). There was no significant difference between the RGR of *C. purpureum* tips co-cultured with *U. rigida* (0.94 ± 1.28 % d^{-1}) and the mesocosm control (6.37 ± 0.96 % d^{-1}), after 8 days of co-culture. However, after 8 days of co-culture tips co-cultured with *U. compressa* grew significantly slower (-5.39 ± 1.22 % d-1) than tips co-cultured with *U. rigida* ($p=0.024$) or alone ($p<0.001$; Figure 1b; Table 2).

The relative growth rate of *Chondrus crispus* was significantly affected by Treatment (*U. compressa, U. rigida*, and mesocosm control; $F_{2,152} = 39.3$, $p = 0.025$) with the effect of Treatment dependent on Day (Day: $F_{3,151} = 8.7$, $p < 0.001$; Treatment x Day: $F_{6,151} = 2.6$, $p = 0.018$; Figure 1c; Table S3). *C. crispus* thalli grown without *Ulva* grew significantly faster than thalli grown with *U. rigida* ($p=0.025$) or *U. compressa* ($p=0.019$) after six days of co-culture (Figure 1c; Table 3). On Day 8, *C. crispus* thalli in both *Ulva* treatments were losing mass while *C. crispus* cultured without *Ulva* was growing at a RGR of 5.7 ± 0.5 % d^{-1} (Figure 1c, Table 3). In the first *C. crispus* trial, the pH levels were 8.1 ±0.03, 9.2±0.2, and 8.9±0.2 in the mesocosm control, *U. compressa* treatment, and *U. rigida* treatment, respectively.

There was no effect of co-culture with *G. vermiculophylla* on the relative growth rate of tips of *G. vermiculophylla* in the control trials (Treatment: $F_{1,101} = 3.2$, $p = 0.157$; data not shown). The average RGR of *G. vermiculophylla* cultured alone was 6.29 ± 0.52 % d^{-1}, while tips co-cultured with *G. vermiculophylla* had an average RGR of 3.79 ± 0.90 % d^{-1}.

The overall relative growth rate of *G. vermiculophylla* in outdoor flow-through seawater tank trials was significantly different among treatments ($F_{2,6} = 5.8$, $p = 0.0393$). There was no significant difference in the RGR of *G. vermiculophylla* between the mesocosm control (8.5 ± 1.7 % d^{-1}) and *U. rigida* (7.7 ± 3.0 % d^{-1}) treatments or between *U. rigida* and *U. compressa* (3.9 ± 0.9 % d^{-1}) treatments. However, *G. vermiculophylla* in the mesocosm control grew significantly faster than *G. vermiculophylla* co-cultured with *U. compressa* ($p=0.043$).

Effects of *Ulva* on oyster larvae

Survival in the control oyster larvae wells was good throughout the 7-day challenge experiment. Mean survival in the controls was 97.8% ±1.3% on Day 3, 89.4% ±2.5% on Day 5 and 71.9% ±3.8% on Day 7 (mean ± SE, n=48).

Relative percent survival of oyster larvae was significantly lower when larvae were cultured in exudate from *U. compressa* (79.5 ± 1.9 %) than from *U. rigida* (98.1 ± 1.9 %; $F_{1,2} = 47.4$, $p = 0.008$; Figure 2; Table S4). The effect of *Ulva* species on oyster larval survival was dependent on nutrients and time (*Ulva* species × nutrients × day= $F_{2,122} = 6.7$, $p = 0.002$; Table S4). Post hoc analysis revealed no difference between the treatments after 3 days of culture. However, oyster survivorship was significantly lower

when cultured in *U. compressa* + nutrients than *U. compressa* – nutrients ($p<0.001$) and *U. rigida* + nutrients ($p<0.001$) after 5 days of culture (Figure 2). This pattern was consistent after 7 days, when oyster survival in the *U. compressa* + nutrients treatment was less than 30% (Figure 2).

Discussion

While green macroalgae have been traditionally thought of as non-toxic, increasing evidence has shown that species of ulvoid macroalgae can inhibit co-occurring phytoplankton (Nan et al. 2008; Tang & Gobler 2011), macroalgae (Gao et al. 2014), and invertebrates (Nelson & Gregg 2013, Van Alstyne et al. 2014, Peckol & Putnam 2017). Here, we found that two dominant bloom-forming ulvoid species, *Ulva compressa* and *U. rigida*, inhibit the growth of the co-occurring red macroalgae, *Gracilaria vermiculophylla*, *Cystoclonium purpureum*, and *Chondrus crispus* at *Ulva* concentrations that are observed during blooms (Thornber et al. 2017). Thornber et al. (2017) documented blooms dominated by *Ulva* that reached a biomass of >3000g/m^2 in the intertidal and >8000g/m^3 in the subtidal zone; blooms with over 12,000 g/m^3 were recently documented in a coastal salt pond (Green-Gavrielidis et al. 2017). We were able to validate the negative effect of *U. compressa* on the growth rate of *G. vermiculophylla* through trials in outdoor flow-through seawater tanks.

Previous studies have reported that species of *Ulva* (e.g. *Ulva linza;* Gao et al. 2014) inhibited the growth and photosynthesis of *G. lemaneiformis* in co-culture experiments, through a combination of chemical and nutrient competition. In our study, we attempted to eliminate the effects of nutrient competition by replenishing nutrients

daily. It should be noted, however, that nitrate was used as a proxy for all nutrients in the seawater media and concentrations of other essential nutrients (e.g. phosphorus, trace minerals) were not measured. Despite this, we believe that nutrient limitation was unlikely since the uptake rate of nitrogen is generally several times higher than the uptake of other nutrients in macroalgae (Wallentinus 1984). Additionally, although previous studies have indicated that *Ulva* and *Gracilaria* have similar nitrogen uptake rates (Wallentinus 1984, Naldi & Wheeler 2002), we saw no negative effect on the growth rate of *G. vermiculophylla* in our control trials, which suggests that nitrogen limitation did not occur. However, we cannot completely eliminate the possibility that nutrient competition played a role in our study. Future studies should test the concentrations of all nutrients to eliminate nutrient competition as a mechanism.

We found that nutrient replete *U. compressa* caused significant mortality in oyster larvae, while nutrient deplete *Ulva* extract had no significant effect on larval mortality. Other studies have shown that bryozoan and hydroid larvae can be negatively impacted by brown algae (Schmitt et al. 1998), red algae can cause necrosis in soft corals (de Nys et al. 1991), and green algae can negatively affect the development of Pacific oyster larvae (Nelson & Gregg 2013), growth rate of adult Pacific oysters (Nelson et al. 2003, Nelson & Gregg 2013, Van Alstyne et al. 2014), and metamorphosis of crab larvae (Van Alstyne et al. 2014). Interestingly, several studies have reported that the toxicity of phytoplankton increased under nutrient limitation. For example, the haptophyte *Prymesium parvum* causes significant mortality in other phytoplankton species and the toxicity of *P. parvum* was enhanced under nutrient limited conditions (Granéli & Johansson 2003, Uronen et al. 2005, reviewed by Granéli et al. 2008). Ribalet et al.

(2007) reported that production of toxic polyunsaturated aldehydes (PUAs) by marine diatoms increased under nutrient limitation. Our results indicate that *U. rigida* grown under nutrient deplete conditions had a stronger negative effect on oyster larval survival, although this trend was not statistically significant. Contrastingly, Nan et al. (2008) showed that *Ulva lactuca* caused mortality in microalgae under nutrient replete conditions, similar to our findings for *U. compressa*.

Previous researchers have also demonstrated that the effect of macroalgae on co-occurring species is dependent on species-specific characteristics. For example, Accoroni et al. (2015) showed that co-culture with fresh thalli of the brown alga *Dictyota dichotoma* had a stronger negative effect on the growth of the benthic diatom *Ostreopsis* cf. *ovata* than co-culture with *U. rigida.* There are also species-specific effects within the Ulvales. Nelson et al. (2003) showed that extract from *U. obscura* more strongly inhibited the germination of *Fucus gardneri* than extract from *U. fenestrata*.

In our laboratory-based mesocosms studies, both *U. compressa* and *U. rigida* inhibited the growth of *G. vermiculophylla* and there was no significant difference in the growth of *G. vermiculophylla* between the *Ulva* treatments. However, our results from outdoor flow-through seawater tank trials showed that only *U. compressa* significantly suppressed the growth of *G. vermiculophylla.* Although there was a trend of reduced *G. vermiculophylla* growth in the *U. rigida* treatment, there was no significant effect of co-culture with *U. rigida*, likely due to low replication. Furthermore, we documented consistent, contrasting responses of oyster larvae to exudate of *U. compressa* and *U. rigida.* Therefore, we hypothesize that the mechanisms responsible for the negative effects of *U. compressa* and *U. rigida* on co-occurring organisms are species specific.

The three co-occurring macroalgae tested here also responded differently to *U. compressa* and *U. rigida.* For example, the native *C. purpureum* began to lose mass or show negligible growth in the presence of *U. compressa* and *U. rigida* after 4 and 6 days of co-culture, respectively. *Chondrus crispus* grown in the presence of both *Ulva* species had lower growth rates, but only began to lose mass after 8 days of co-culture with *U. compressa*. Interestingly, the non-native *G. vermiculophylla* appeared to be the least affected of the three macroalgae tested; *G. vermiculophylla* did not experience a significant reduction in growth rate in either *Ulva* treatment until 8 days of co-culture had passed and never began to lose mass. Differences in the response of species to ulvoids have also been documented in phytoplankton (Tang & Gobler 2011) and could have ecological consequences for species presence and abundance in or near macroalgal blooms. In Narragansett Bay, *Gracilaria* spp. is very common in blooms (Thornber et al. 2017), perhaps owing to its ability to coexist with *U. compressa* and *U. rigida*. Species interactions shape ecological communities and the impacts of *U. compressa* and *U. rigida* on community composition require further research.

The responses documented here could be the result of allelopathy (*i.e*. chemical inhibition) by *U. compressa* and *U. rigida*. However, identifying chemically mediated interactions depends on detection of chemicals at or near the alga surface (Steinberg and de Nys 2002). Therefore, this hypothesis cannot be validated until allelochemicals are detected, isolated, and identified from *U. compressa* and *U. rigida*, and the effect of those isolated allelochemicals on target species is tested. Furthermore, it is important to note that *Ulva* can also compete with co-occurring macroalgae through other mechanisms such as nutrient competition (discussed above) or through pH alteration. For example,

Ulva intestinalis has been shown to raise the pH of rockpools to a level (>10) where seaweeds cannot utilize external carbonic anhydrase (CA) to covert HCO_3- to CO_2 for use in photosynthesis, and therefore become carbon limited (Bjork et al. 2004). *Chondrus crispus* utilizes HCO_3- only through external CA and becomes bleached when growing in rockpools dominated by *U. intestinalis* due to high pH (Bjork et al. 2004). Although pH was only measured in the first *Chondrus crispus* trial, we did document pH levels that were potentially high enough to interrupt external CA activity. Alterations in pH, however, cannot explain all of the results documented here. In particular, research has shown that species of *Gracilaria* and closely related *Gracilariopsis* use both external CA (sensitive to high pH) and a direct HCO_3- transporter (not sensitive to pH) simultaneously to take up inorganic carbon (Andría et al. 1999, Pérez-Lloréns et al. 2004), yet we documented a negative effect of *U. compressa* on the relative growth rate of *Gracilaria vermiculophylla* in closed mesocosms. Additionally, in the oyster larval survival assays, we adjusted the pH of the *Ulva* exudate to match control seawater (7.9-8.0) prior to use. If pH were responsible for the negative effects of *Ulva* on oyster larvae, we should have seen no difference in the survival between treatments.

We have demonstrated for the first time that *U. compressa* has a significant negative effect on the survival of eastern oyster larvae, an important aquaculture crop in the U.S. (USDA 2014), when cultured under eutrophic conditions. Approximately two-thirds of U.S. coastal waterways, including Narragansett Bay, are considered degraded by an excess of nitrogen (N) from anthropogenic influences (Howarth & Marino 2006). Excess nutrients are known to cause blooms of ulvoid macroalgae (Teichberg et al. 2010) and our results suggest that *U. compressa* can cause mortality in oyster larvae in these

systems especially when oyster spawning coincides with the occurrence of *Ulva* blooms. Interestingly, *U. rigida* did not cause significant mortality of oyster larvae, although it did inhibit the growth of co-occurring macroalgae. One important caveat of this study is the lack of validation of these effects *in situ.* While we did use ecologically relevant concentrations of *Ulva* in our study, these results are likely to change as a result of hydrodynamics (Steinberg et al. 2002). Further research is required to examine the effects of *Ulva* on other economically important bivalves (e.g. clams and scallops) and on post-larval eastern oysters and to verify these effects *in situ*.

Acknowledgements

We would like to thank I. Burns, K. Carpentier, L. Dansereau, and C. Anderson for laboratory and/or logistical help. Funding was provided by the Rhode Island Science and Technology Council and the National Science Foundation EPSCoR Cooperative Agreement #EPS-1004057. This research is based in part upon work conducted using the Rhode Island Genomics and Sequencing Center and the Marine Life Sciences Facility. We are thankful to three anonymous reviewers for their insightful feedback that greatly improved this manuscript.

References

Accoroni S, Percopo I, Cerino F, Romagnoli T, Pichierri, S, Perrone C, Totti C (2015) Allelopathic interactions between the HAB dinoflagellate *Ostreopsis* cf. *ovata* and macroalgae. Harmful Algae 49:147-155

Angell AR, Pirozzi I, de Nys R, Paul NA (2012) Feeding preferences and the nutritional value of tropical algae for the abalone *Haliotis asinina*. PLoS ONE 7: e38857.

1 Anderson DM (2009) Approaches to monitoring, control and management of harmful
2 algal blooms (HABs). Ocean Coast Manage 52:342–347
3 Andría JR, Pérez-Lloréns L, Vergara JJ (1999) Mechanisms of inorganic carbon
4 acquisition in *Gracilaria gaditana* nom. prov. (Rhodophyta). Planta 208:564-573
5 Atkins RP, Deeley DM, Alpine KW (1993) Managing the aquatic environment. Fert Res
6 36:171–175
7 Beutel D (2017) Aquaculture in Rhode Island: 2016 Annual Status Report. Coastal
8 Resources Management Council. Wakefield, RI.
9 Bjork M, Axelsson L, Beer S (2004) Why is *Ulva intestinalis* the only macroalga
10 inhabiting isolated rockpools along the Swedish Atlantic coast? Mar Ecol Prog Ser
11 284:109-116
12 Borowsky R, Borowsky B (1990) Feeding inhibition of the salt marsh amphipod
13 *Gammarus palustris* Bousfield, 1969 by heat-labile substances in *Ulva lactuca* L.
14 Crustaceana 59: 299-301
15 Bricker SB, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J (2008)
16 Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful
17 Algae 8:21–32
18 Connor PM (1972) Acute toxicity of heavy metals to some marine larvae. Mar Pollut
19 Bull 3(12):190-192.
20 Cummins SP, Roberts DE, Zimmerman KD (2004) Effects of the green macroalga
21 *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow
22 coastal estuary. Mar Ecol Prog Ser 266:77–87
23 de Nys R, Coll JC, Price IR (1991) Chemically mediated interactions between the red

alga *Plocamium hamatum* (Rhodophyta) and the octocoral *Sinularia cruciata* (Alcyonacea). Mar Biol 108:315-320

Deacutis CF, Murray D, Prell W, Saarman E (2006) Hypoxia in the upper half of Narragansett Bay, RI, during August 2001 and 2002. Northeast Nat 13:173–198

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11-15

Epifanio CE, Srna RF (1975) Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. Mar Biol 33:241–246

Fletcher RL (1996) The British Isles. In: Schramm W, Nienhuis PH, editors. Marine benthic vegetation: recent changes, the effects of eutrophication. Berlin: Springer. pp. 7-223

Gao, Z, Xu D, Meng C, Zhang X, Wang Y, Li D, Zou J (2014) The green tide-forming macroalga *Ulva linza* outcompetes the red macroalga *Gracilaria lemaneiformis* via allelopathy and fast nutrients uptake. Aquat Ecol 48:53-62

Granéli E, Johansson N (2003) Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. Harmful Algae 2:135-145

Granéli E, Weberg M, Salomon PS (2008) Harmful algal blooms of allelopathic microalgal species: The role of eutrophication. Harmful Algae 8:94-102

Granger S, Brush MJ, Buckley BA, Schwartz ML (2000) An assessment of eutrophication in Greenwich Bay. In: Schwartz M (ed) Restoring Water Quality in Greenwich Bay A Whitepaper Series. Narragansett, R.I.

Green-Gavrielidis L, Ernst E, Valentin Guttandin J, Thornber C (2017) Monitoring seaweed abundance and species composition at Napatree lagoon. In: Stassi J, editor, The State of Napatree Report: 2017, 191 pp.

Guidone M, Thornber CS (2013) Examination of *Ulva* bloom species richness and relative abundance reveals two cryptically co-occurring bloom species in Narragansett Bay, Rhode Island. Harmful Algae 24:1–9

Guidone M, Thornber C, Wysor B, O'Kelly CJ (2013) Molecular and morphological diversity of Narragansett Bay (RI, USA) *Ulva* (Ulvales, Chlorophyta) populations. J Phycol 49:979–995

Harlin MM (1987) Allelochemistry in marine macroalgae. Crit Rev Plant Sci 5:237-249.

His E, Beiras R, Searman MNL (1999) The assessment of marine pollution: bioassays with bivalve embryos and larvae. In: AI Southward, PA Tyler, and CM Young (Eds) Advances in Marine Biology, Vol. 37. London: Academic Press

Hofmann LC, Nettleton JC, Neefus CD, Mathieson AC (2010) Cryptic diversity of *Ulva* (Ulvales, Chlorophyta) in the Great Bay Estuarine System (Atlantic USA): introduced and indigenous distromatic species. Eur J Phycol 45:230–239

Howarth RW, Marino R (2006) Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnol Oceanogr 51:364–376

Huggett MJ, de Nys R, Williamson JE, Heasman M, Steinberg PD (2005) Settlement of larval blacklip abalone, *Haliotis rubra*, in response to green and red macroalgae. Mar Biol 147:1155–1163

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology:

second edition. Cambridge University Press, 551 pp.

Jin Q, Dong S (2003) Comparative studies on the allelopathic effects of two different strains of *Ulva pertusa* on *Heterosigma akashiwo* and *Alexandrium tamarense*. J Exp Mar Biol Ecol 293:41-55

Johnson DA, Welsh BL (1985) Detrimental effect of *Ulva lactuca* (L.) exudates and low oxygen on estuarine crab larvae. J Exp Mar Biol Ecol 86:73-83

Karim M, Zhao W, Rowley D, Nelson D, Gomez-Chiarri M (2013) Probiotic strains for shellfish aquaculture: protection of Eastern oyster, *Crassostrea virginica*, larvae and juveniles against bacterial challenge. J Shellfish Res 32:401-408

Lamb A (2015) Understanding the impact of sea lettuce (*Ulva* spp.) density on Pacific oyster (*Crassostrea gigas*) growth in Puget Sound, Washington. Master's Thesis, The Evergreen State College

Lapointe BE, Bedford BJ (2007) Drift rhodophyte blooms emerge in Lee County, Florida, USA: Evidence of escalating coastal eutrophication. Harmful Algae 6:421–437

Liu D, Keesing JK, He P, Wang Z, Shi Y, Wang Y (2013) The world's largest macroalgal bloom in the Yellow Sea, China: Formation and implications. Estuar Coast Shelf S 129:2–10

Magre EJ (1974) *Ulva lactuca* L. negatively affects *Balanus balanoides* (L.)(Cirripedia Thoracica) in tidepools. Crustaceana 27:231-234

McGlathery KJ (2001) Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. J Phycol 37:453–456

Muñoz P, Ambler R, Bulboa C (2012) Settlement, survival, and post-larval growth of red

abalone, *Haliotis rufescens*, on polycarbonate plates treated with germlings of *Ulva* sp. J World Aquacult Soc 43:890–895

Naldi M, Wheeler PA (2002) ^{15}N measurements of ammonium and nitrate uptake by *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta): comparison of net nutrient disappearance, release of ammonium and nitrate, and ^{15}N accumulation in algal tissue. J Phycol 38:135-144

Nan C, Zhang H, Lin S, Zhao G, Liu X (2008) Allelopathic effects of *Ulva lactuca* on selected species of harmful bloom-forming microalgae in laboratory cultures. Aquat Bot 89:9-15

Nelson TA, Gregg BC (2013) Determination of EC50 for normal oyster larval development in extracts from bloom-forming green seaweeds. Nautilus 127:156–159

Nelson TA, Lee DJ, Smith BC (2003) Are "green tides" harmful algal blooms? Toxic properties of water-soluble extracts from two bloom-forming macroalgae, *Ulva fenestrata* and *Ulvaria obscura* (Ulvophyceae). J Phycol 39:874–879

Nettleton JC (2012) Tracking environmental trends in the Great Bay Estuarine System: An examination of water quality and nuisance macroalgal blooms. Dissertation, University of New Hampshire

Nettleton JC, Mathieson AC, Thornber C, Neefus CD, Yarish C (2013) Introduction of *Gracilaria vermiculophylla* (Rhodophyta, Gracilariales) to New England, USA: Estimated arrival times and current distribution. Rhodora 115(961): 28-41

Ott FD (1966) A selected listing of xenic cultures. *Systematics-Ecology Program* No. 72 (Mar. Biol. Lab., Woods Hole, MA), pp 1-45

Peckol P, Putnam AB (2017) Differential toxic effects of *Ulva lactuca* (Chlorophyta) on

the herbivorous gastropods, *Littorina littorea* and *L. obtusata* (Mollusca) J Phycol 53:361-367

Pérez-Lloréns JL, Brun FG, Andría J, Vergara JJ (2004) Seasonal and tidal variability of environmental carbon related physico-chemical variables and inorganic C acquisition in *Gracilariopsis longissima* and *Enteromorpha intestinalis* from Los Toruños salt marsh (Cádiz Bay, Spain). J Exp Mar Biol Ecol 304:183-201

Rafaelli D, Raven JA, Poole LJ (1998) Ecological impact of mass blooms of benthic algae. Mar Biol Oceanogr Ann Rev 36: 97-125

Ribalet F, Wichard T, Pohnert G, Ianora A, Miralto A, Casotti R (2007) Age and nutrient limitation enhance polysaturated aldehyde production in marine diatoms. Phytochemistry 68:2059-2067

Schmitt TM, Lindquist N, Hay ME (1998) Seaweed secondary metabolites as antifoulants: effects of *Dictyota* spp. diterpenes on survivorship, settlement, and development of marine invertebrate larvae. Chemoecology 8:125-131

Shimada S, Hiraoka M, Nabata S, Iima M, Masuda M (2003) Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae), with special reference to the free-floating *Ulva.* Phycol Res 51:99-108

Smetacek V, Zingone A (2013) Green and golden seaweed tides on the rise. Nature 504:84–88

Sohn S, Lundgren KM, Tammi K, Karim M, Smolowitz R, Nelson DR, Rowley DC, Gómez-Chiarri M (2016) Probiotic strains for disease management in hatchery larviculture of the Eastern oyster *Crassostrea virginina*. J Shellfish Res 35:307-317

Steinberg PD, de Nys R (2002) Chemical mediation of colonization of seaweed surfaces.

J Phycol 38:621-629

Steinberg PD, de Nys R, Kjelleberg S (2002) Chemical cues for surface colonization. J Chem Ecol 28:1935-1951

Tang YZ, Gobler CJ (2011) The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. Harmful Algae 10: 480-488

Teichberg M, Fox S, Olsen Y, Valiela I, Martinetto P, Iribarne O, Muto EY, Petti M, Corbisier T, Soto-Jimenez M, Paez-Osuna F, Castro P, Freitas H, Zitelli A, Cardinaletti M, Tagliapietra D (2010) Eutrophication and macroalgal blooms in temperate and tropical coastal waters: nutrient enrichment experiments with *Ulva* spp. Glob Change Biol 16:2624–2637

Thornber CS, Dimilla P, Nixon SW, McKinney RA (2008) Natural and anthropogenic nitrogen uptake by bloom-forming macroalgae. Mar Pollut Bull 56:261–269

Thornber CS, Guidone M, Deaucutis C, Green L, Ramsay CN, Palmisciano M (2017) Spatial and temporal variability in macroalgal blooms in a eutrophied coastal estuary. Harmful Algae 68:82-96.

Thorne-Miller B, Harlin M, Thursby G, Brady-Campbell M, Dworetzky B (1983) Variations in the distribution and biomass of submerged macrophytes in five coastal lagoons in Rhode Island, U.S.A. Botanica Marina 26, 231-242

Tyler AC, McGlathery KJ, Anderson IC (2001) Macroalgae mediation of dissolved organic nitrogen fluxes in a temperate coastal lagoon. Estuar Coast Shelf S 53:155–168

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation

using analysis of variance. Cambridge University Press. 508 pp.

Uronen P, Lehtinen S, Legrand C, Kuuppo P, Tamminen T (2005) Haemolytic activity and allelopathy of the haptophyte *Prymnesium parvum* in nutrient-limited and balanced growth conditions. Mar. Ecol. Prog. Ser. 299:137-148.

USDA (United States Department of Agriculture) (2014) Census of Aquaculture 2013.

Valiela I, Cole ML (2002) Comparative evidence that salt marshes and mangroves may protect seagrass meadows from land-derived nitrogen loads. Ecosystems 5:92–102

Valiela I, McClelland J, Hauxwell J, Behr PJ, Herh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol Oceanogr 42:1105–1118.

Van Alstyne KL, Harvey EL, Cataldo M (2014) Effects of dopamine, a compound released by the green-tide macroalga *Ulvaria obscura* (Chlorophyta), on marine algae and invertebrate larvae and juveniles. Phycologia 53:195–202

Van Alstyne KL, Nelson TA, Ridgway RL (2015) Environmental chemistry and chemical ecology of "green tide" seaweed blooms. Integr Comp Biol 55:518-532

Wallentinus I (1984) Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar Biol 80:215-225

Walters LJ, Hadfield MG, Smith CM (1996) Waterborne chemical compounds in tropical macroalgae: positive and negative cues for larval settlement. Mar Biol 126:383-393.

Wang R, Feng W, Tang X, Wang J, Dong S (2012) Allelopathic growth inhibition of *Heterosigma akashiwo* by the three *Ulva* species (*Ulva pertusa, Ulva linza, Enteromorpha intestinalis*) under laboratory conditions. Acta Oceanol Sin 31:138–144

1 Worm B, Lotze HK (2006) Effects of eutrophication, grazing, and algal blooms on rocky
2 shores. Limnol Oceanogr 51:569–579
3

Figure Legend

Figure 1. Mean relative growth rate (% d^{-1}) of a) *Gracilaria vermiculophylla* b) *Cystoclonium purpureum* and c) *Chondrus crispus* co-cultured with *U. compressa, U. rigida*, or alone (control). Error bars represent ±1SE. Asterisks (*) denote a statistically significant difference based on Tukey's HSD post hoc comparisons. The results of posthoc comparisons for *C. purpureum* and *C. crispus* are available in Table 2 and 3, respectively.

Figure 2. Relative percent survival of oyster larvae exposed to exudate from *U. compressa* and *U. rigida* grown under nutrient replete (+ Nutrients) or nutrient deplete (- Nutrients) conditions. Bars with a letter in common are not statistically different based on Tukey's HSD post hoc comparisons. Error bars represent ±1SE.

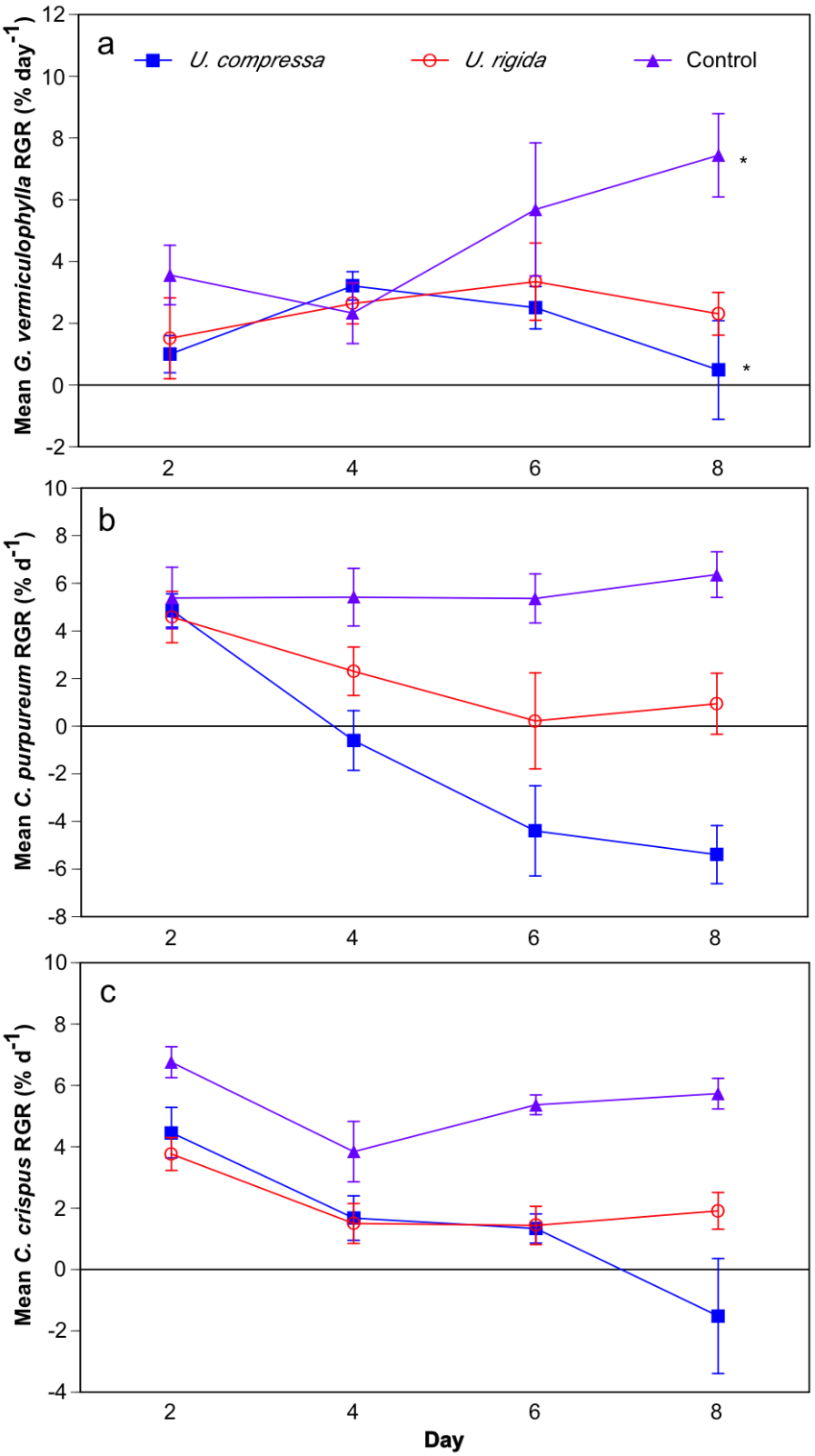
a
U. compressa
U. rigida
Control
Mean G. vermiculophylla RGR (% day-1)
b
Mean C. purpureum RGR (% d-1)
c
Mean C. crispus RGR (% d-1)
Day
*
*

Figure 1.

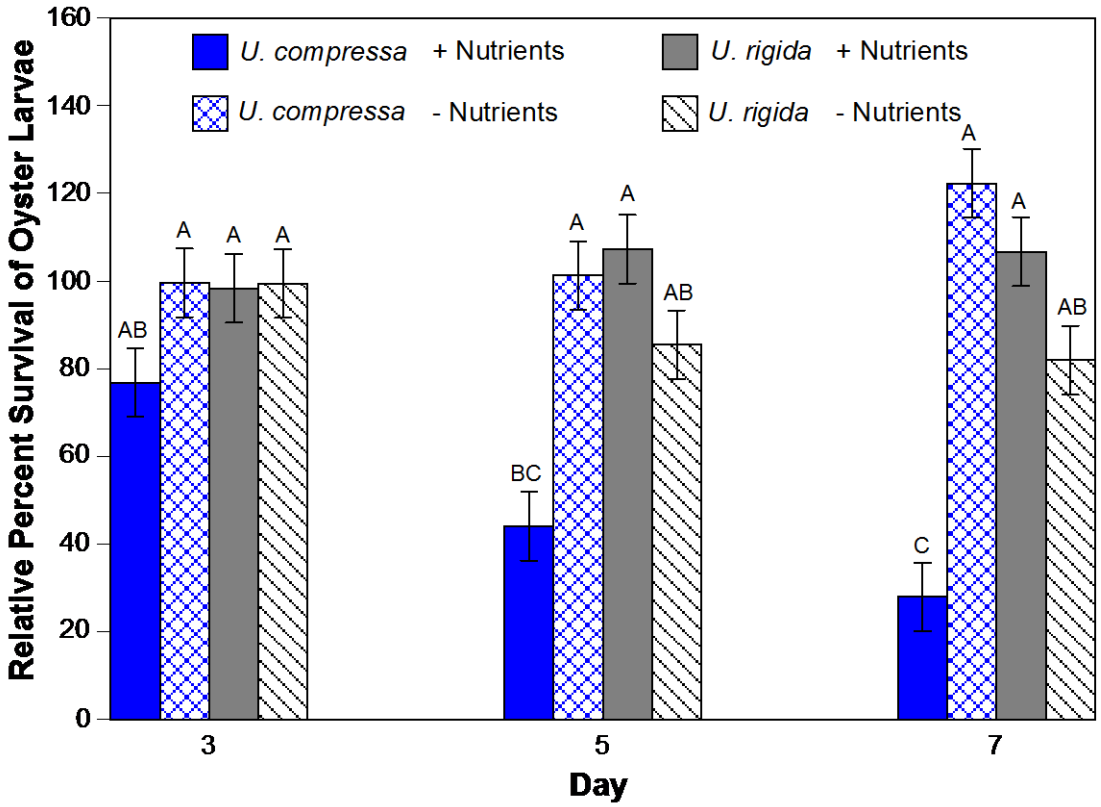
U. compressa + Nutrients
U. compressa - Nutrients
U. rigida + Nutrients
U. rigida - Nutrients
Relative Percent Survival of Oyster Larvae
160
140
120
100
80
60
40
20
0
AB
A
A
A
BC
A
A
AB
C
A
A
AB
3
5
7
Day

Figure 2.

Table 1: Selected examples of studies documenting the effects of ulvoid macroalgae (Family Ulvophyceae) on co-occurring organisms.

Location	Macroalgal Taxa	Documented Effects	Reference(s)
Washington, U.S.A	*U. obscura*	Inhibited development of *Fucus* zygotes and crab larvae, growth of *Ulva lactuca*	Van Alstyne et al. 2014
Washington, U.S.A	*U. lactuca, U. obscura*, and/or *U. fenestrata*	Inhibited development of *Fucus* zygotes, growth of *Ulva*, *Ulvaria*, and epiphytic macroalgae, inhibited/killed oyster larvae	Nelson et al. 2003, Nelson & Gregg 2013
Hawaii, U.S.A.	*U. reticulata*	Inhibited/killed fouling invertebrates	Walters et al. 1996
New York, U.S.A.	*U. lactuca*	Inhibited feeding of amphipod; inhibited growth of multiple harmful microalgal species	Borowsky & Borowsky 1990, Tang & Gobler 2011
Connecticut, U.S.A.	*U. lactuca*	Killed barnacles; killed zoeae crab larvae	Magre 1974, Johnson & Welsh 1985
Sirolo, Italy	*U. rigida*	Inhibited growth of toxic benthic dinoflagellate	Accoroni et al. 2015
Qingdao, China	*U. pertusa, U. linza, U. intestinalis*, and/or *U. lactuca*	Inhibited growth of red tide microalgae	Jin & Dong 2003, Nan et al. 2008, Wang et al. 2012

Table 2. Mean relative growth rate (RGR; % d^{-1}) of *Cystoclonium purpureum* tips cultured with *U. compressa, U. rigida,* or alone (mesocosm control). Means without a common superscript letter differ significantly ($p<0.05$) based on Tukey's HSD post hoc comparisons.

Days of Co-culture	Treatment	RGR (% d^{-1}) ±1SE
2	*U. compressa*	4.89 ± 0.70^{abc}
	U. rigida	4.58 ± 1.08^{abc}
	Mesocosm control	5.38 ± 1.29^{ab}
4	*U. compressa*	-0.60 ± 1.25^{cde}
	U. rigida	2.31 ± 1.02^{abc}
	Mesocosm control	5.42 ± 1.21^{ab}
6	*U. compressa*	-4.40 ± 1.89^{de}
	U. rigida	0.22 ± 2.01^{bcde}
	Mesocosm control	5.36 ± 1.03^{ab}
8	*U. compressa*	-5.39 ± 1.22^{e}
	U. rigida	0.94 ± 1.28^{abcd}
	Mesocosm control	6.37 ± 0.96^{a}

Table 3. Mean relative growth rate (RGR; % day^{-1}) of *Chondrus crispus* tips cultured with *U. compressa, U. rigida,* or alone (mesocosm control). Means without a common superscript letter differ significantly ($p<0.05$) based on Tukey's HSD post hoc comparisons.

Days of Co-culture	Treatment	RGR (mg d^{-1}) ±1SE
2	*U. compressa*	4.46 ± 0.82^{abc}
	U. rigida	3.76 ± 0.54^{abc}
	Mesocosm control	6.76 ± 0.50^{a}
4	*U. compressa*	1.68 ± 0.72^{cd}
	U. rigida	1.50 ± 0.65^{cd}
	Mesocosm control	3.84 ± 0.98^{abc}
6	*U. compressa*	1.34 ± 0.47^{cd}
	U. rigida	1.44 ± 0.62^{cd}
	Mesocosm control	5.37 ± 0.32^{ab}
8	*U. compressa*	-1.52 ± 1.87^{d}
	U. rigida	1.91 ± 0.60^{bcd}
	Mesocosm control	5.73 ± 0.50^{a}