Vol. 595: 27–37, 2018
https://doi.org/10.3354/meps12556

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published May 14

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

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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 macroalgae of the genus *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 *U. 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 3 macroalgae. We were able to verify the negative effect of *U. 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 *U. compressa* exudate, which had <30% relative survival after 1 wk. 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

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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), which 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, USA, 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 3 major urban freshwater inflows

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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 to 400 g dry mass m^{-2} (1015 to 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, 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 2 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 United States (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 is important for 2 reasons. First, *Ulva*

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

Location	Macroalgal taxa	Documented effects	Reference
Washington, USA	*U. obscura*	Inhibited development of *Fucus* zygotes and crab larvae, growth of *Ulva lactuca*	Van Alstyne et al. (2014)
Washington, USA	*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, USA	*U. reticulata*	Inhibited/killed fouling invertebrates	Walters et al. (1996)
New York, USA	*U. lactuca*	Inhibited feeding of amphipod; inhibited growth of multiple harmful microalgal species	Borowsky & Borowsky (1990), Tang & Gobler (2011)
Connecticut, USA	*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)

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). *U. compressa* cells are polygonal with rounded corners and contain a single pyrenoid, while *U. rigida* cells are polygonal with angular corners and contain 2 to 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 & 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).

Genetic identification of *Gracilaria*

Two species of *Gracilaria* occur in Narragansett Bay, the native *G. tikvahiae* McLachlan and the introduced *G. vermiculophylla* (Ohmi) Papenfuss (Nettleton et al. 2013). These 2 species have morphological characteristics that overlap, and therefore restricted fragment length polymorphism (RFLP) and selected DNA barcoding were 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 & Doyle (1987). Polymerase chain reaction (PCR) was performed in 50 µl volumes containing 10 µl of 5× GoTaq® Flexi DNA Polymerase (Promega Corporation), 7 µl of 25 mM Mg^{2+}, 1 µl of 2.5 mM deoxynucleotides (dNTP), and 4 µl of extracted DNA template (10 to 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 min, followed by 30 cycles of 57°C for 1 min, 73°C for 1 min, and 95°C for 1 min followed by a final 1 min at 57°C and a final extension at 73°C for 6 min. RFLP analysis was performed on the PCR samples after amplification following the protocol of Nettleton (2012). In addition to the RFLP analysis, 3 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 *U. compressa* or *U. rigida* suppress the growth of other macroalgae, we performed a series of co-culture experiments. We co-cultured isolated tips of 3 species that are common in *Ulva* blooms in Narragansett Bay (C. S. Thornber unpubl. 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, 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 3 times with sterile seawater (30 to 32 psu), and placed in 250 ml flasks with sterile Von Stosch enriched (VSE) natural seawater (Ott 1966) under acclimation conditions (20 to 23°C, 100 µmol photons m^{-2} s^{-1}, and a 16 h light:8 h dark photoperiod with constant aeration); total acclimation to laboratory conditions occurred for at least 3 d, with at least 24 h allowed for wound healing following tip cutting. Natural seawater was obtained from the Marine Science Re-

search 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 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^{-1}) 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 7 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 d.

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:

$$\mathrm{RGR}\ (\%) = 100 \times [\ln (L_2 / L_1) / (t_2 - t_1)] \quad (1)$$

where L_2 and L_1 are the blade weight at times t_2 and t_1, respectively. A total of 2 *G. vermiculophylla* trials were performed. The same experimental design was used to conduct separate trials with *C. purpureum* (2 trials) and *C. crispus* (2 trials). Daily pH levels were determined for the first *C. crispus* trial only using an EcoTestr™ pH meter (Oakton®).

G. 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, 2 *G. vermiculophylla* control trials with the same experimental design as the co-culture trials described above were performed. A total of 7 replicates each of 2 treatments, *G. vermiculophylla* and mesocosm control, were included in each trial. The *G. vermiculophylla* treatment had 0.4 g (=1 g l^{-1}) 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; mean ± SE) was co-cultured with 1.5 g of either *U. compressa*, *U. rigida*, or *G. vermiculophylla* (control) in separate flow-through tanks (n = 3). *U. compressa*, *U. rigida*, or *G. vermiculophylla* (1 g l^{-1}) were placed in individual mesocosms (16 × 11.9 × 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 RGR was calculated. Due to space limitations, 4 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 ± 0.09°C to 27.28 ± 0.51°C).

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. *U. compressa* and *U. rigida* (5 g l^{-1}) were cultured in nutrient-replete (i.e. supplied full VSE nutrients) or nutrient-deplete (i.e. no nutrients supplied) seawater for 2 to 3 d, 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 8000 g m^{-3} in the subtidal and 3000 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 between 7.9 and 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^{-1} of Shellfish Diet 1800® (Reed Mariculture) every other day while in the laboratory. At the start of the experiments oyster larvae were between 3 and 9 d 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 to 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 3 wells of a treatment and 3 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:

$$\% \text{ survival} = (\text{total} - \text{dead}) / \text{total} \times 100 \quad (2)$$

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 % survival (of control) was calculated by randomly pairing each treatment well with a control well from the same plate using:

$$\text{Relative \% survival} = (\text{\% survival of treatment} / \text{\% survival of control}) \times 100 \quad (3)$$

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 1-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 2-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 % 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 % 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).

RESULTS

Genetic identification of *Ulva*

All *Ulva compressa* specimens identified using morphological characteristics (n = 14) in this study were verified by DNA barcoding using MegaAlign v.12 (DNA Star) 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 *Gracilaria vermiculophylla* control) were identified through RFLP analysis as *G. vermiculo-*

phylla. The 3 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 (*U. compressa*, *U. rigida*, and mesocosm control) on the RGR of *G. vermiculophylla* was dependent on day (treatment × day: $F_{6,153}$ = 2.2, p = 0.048; Fig. 1a, Table S1 in the Supplement at www.int-res.com/articles/suppl/m595p027_supp.pdf). After 8 d of co-culture, the RGR of *G. vermiculophylla* without *Ulva* (7.44 ± 1.35% d^{-1}; mean ± SE) was more than 3 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; Fig. 1a).

Day significantly affected the RGR of *Cystoclonium purpureum* ($F_{3,151}$ = 8.2, p < 0.001) and was dependent on treatment (treatment × day: $F_{6,151}$ = 3.9, p = 0.001; Fig. 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 d of co-culture. However, after 8 d 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; Fig. 1b, Table 2).

The RGR 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 × day: $F_{6,151}$ = 2.6, p = 0.018; Fig. 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 6 d of co-culture (Fig. 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} (Fig. 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 RGR 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

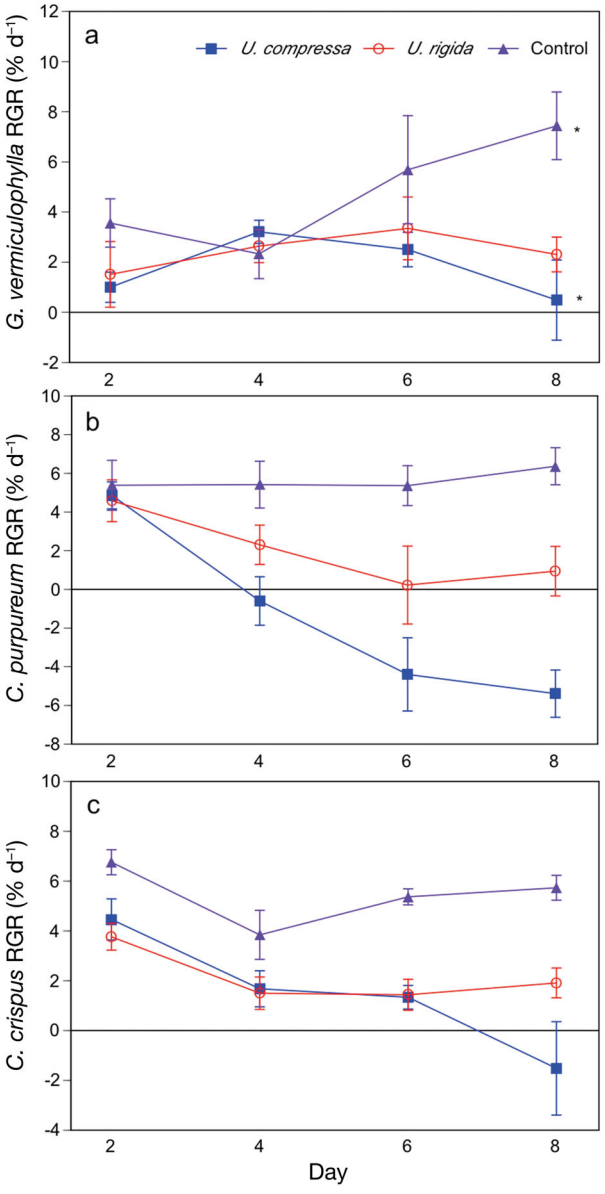

Fig. 1. Relative growth rate (RGR) of (a) *Gracilaria vermiculophylla*, (b) *Cystoclonium purpureum* and (c) *Chondrus crispus* co-cultured with *Ulva compressa*, *U. rigida*, or alone (control). Asterisks (*) denote a statistically significant difference based on Tukey's HSD post hoc comparisons (p < 0.05). The results of post hoc comparisons for *C. purpureum* and *C. crispus* are available in Tables 2 & 3, respectively. Data are means ± SE

tips co-cultured with *G. vermiculophylla* had an average RGR of 3.79 ± 0.90% d^{-1}.

The overall RGR 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%

Table 2. Mean ± SE relative growth rate (RGR) of *Cystoclonium purpureum* tips cultured with *Ulva 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})
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 ± SE relative growth rate (RGR) of *Chondrus crispus* tips cultured with *Ulva 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})
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}

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 d 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 (n = 48).

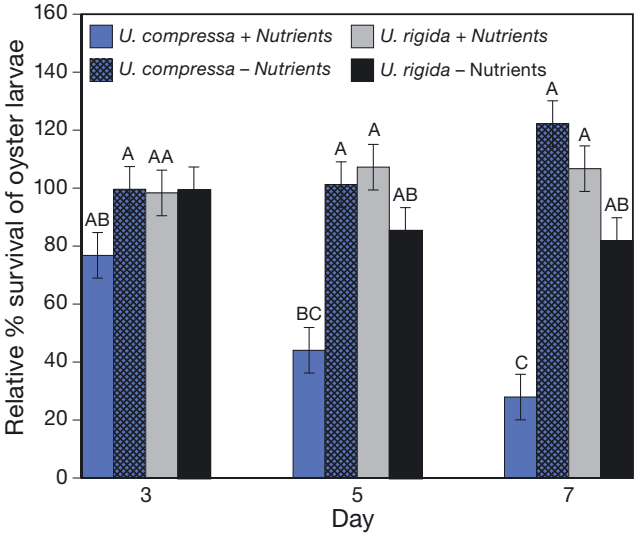

Fig. 2. Relative % survival of eastern oyster larvae exposed to exudate from *Ulva compressa* and *U. rigida* grown under nutrient-replete (+ Nutrients) or nutrient-deplete (– Nutrients) conditions. Bars with same letter are not statistically different based on Tukey's HSD post hoc comparisons ($p < 0.05$). Data are means ± SE

Relative % 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%; $F_{1,2} = 47.4$, $p = 0.008$; Fig. 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 d 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 d of culture (Fig. 2). This pattern was consistent after 7 d, when oyster survival in the *U. compressa* + nutrients treatment was less than 30% (Fig. 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 2 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 >3000 g m^{-2} in the intertidal and >8000 g 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 *Gracilaria 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 *U. 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 mesocosm 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 3 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 d of co-culture, respectively. *C. crispus* grown in the presence of both *Ulva* species had lower growth rates, but only began to lose mass after 8 d of co-culture with *U. compressa*. Interestingly, the non-native *G. vermiculophylla* appeared to be the least affected of the 3 macroalgae tested; *G. vermiculophylla* did not experience a significant reduction in growth rate in either *Ulva* treat-

ment until 8 d 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* 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 requires 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 & 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, *U. intestinalis* has been shown to raise the pH of rockpools to a level (>10) where seaweeds cannot utilize external carbonic anhydrase (CA) to convert HCO_3^- to CO_2 for use in photosynthesis, and therefore become carbon limited (Björk et al. 2004). *C. crispus* utilizes HCO_3^- only through external CA and becomes bleached when growing in rockpools dominated by *U. intestinalis* due to high pH (Björk et al. 2004). Although pH was only measured in the first *C. 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 *G. 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 to 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 United States (USDA 2014), when cultured under eutrophic conditions. Approximately two-thirds of US coastal waterways, including Narragansett Bay, are considered degraded by an excess of nitrogen 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 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 3 anonymous reviewers for their insightful feedback that greatly improved the manuscript.

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Editorial responsibility: Peter Steinberg, Sydney, New South Wales, Australia

Submitted: April 26, 2017; Accepted: March 8, 2018
Proofs received from author(s): April 25, 2018