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Overwintering strategies of bloom-forming *Ulva* species in Narragansett Bay, RI

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Abstract

Temperate coastal estuaries worldwide such as Narragansett Bay, Rhode Island are impacted by seasonal macroalgal blooms (e.g. *Ulva*) during warm months, while bloom-forming macroalgae are rarely encountered during winter. We assessed the ability of distromatic *Ulva* to overwinter via fragments, recruits, and/or microscopic propagules. We documented: a) small tissue fragments in sediment cores and the water column, b) recruits and microscopic propagules on field-based settlement tiles, and c) production of reproductive propagules, throughout the winter months. Laboratory culturing experiments indicated that both fragments and propagules are viable. Our data indicate that bloom-forming overwintering *Ulva* simultaneously utilize multiple reproductive strategies.

Key Words: macroalgal bloom, overwintering, propagules, *Ulva*

Macroalgal blooms in coastal areas are largely composed of fast-growing species that rapidly utilize resources, including many species in the genus *Ulva* (Valiela et al. 1997, Guidone et al. 2013, Smetacek and Zingone 2013). Macroalgal blooms have increased in frequency and duration over the past several decades due to several factors, including anthropogenic nutrient inputs (Rosenberg 1985, Nixon 1995, Teichberg et al. 2010). Free-floating macroalgal blooms can have devastating impacts upon the economy of coastal areas by fouling beaches and decreasing the productivity of fisheries. Blooms decrease dissolved oxygen within the water column contributing to hypoxic events, which can result in fish kills, suffocation of benthic fauna, and reduced shellfish recruitment (Deacutis and Oviatt 2004, Thomsen and McGlathery 2006). The ability of macroalgae to form these

blooms may be associated with their ability to grow vegetatively via fragmentation (e.g., Kamermans et al. 1998), in addition to reproduction via microscopic propagules.

Macroalgal blooms are a common occurrence within Narragansett Bay, Rhode Island, particularly in the shallow areas of the upper bay. Blooms in this system are frequently dominated by the green distromatic species *Ulva compressa* Linnaeus and *U. rigida* C. Agardh (hereafter referred to collectively as *Ulva* spp., as tubular *Ulva* species are not typically as abundant in this system), but can contain at least thirty different species (Guidone and Thornber 2013, Guidone et al. 2013). Blooms occur frequently in the late spring and summer, but are rarely observed in the late fall and winter (Newton and Thornber 2012, Guidone and Thornber 2013). Since *Ulva* spp. mats do not occur during winter months, *Ulva* spp. must utilize one or more overwintering strategies that permit them to propagate quickly once suitable environmental conditions return. In other regions, vegetative fragments of *Ulva* spp. are capable of surviving low temperatures (Zhang et al. 2010) while buried within sediments, which may reduce the impacts of freezing (Kamermans et al. 1998). In addition, Lotze et al. (2000) found that tubular *Ulva* spp. (formerly *Enteromorpha*) germlings (recruits) are able to survive the winter attached to hard substrates, leading to an early spring increase in *Ulva* spp. biomass. Furthermore, *Ulva* spp. may produce reproductive propagules (spores/zygotes) throughout the winter as a primary overwintering strategy (Liu et al. 2012). Any of these methods may explain seasonal summer ulvoid blooms within Narragansett Bay, RI. Here, we present data on the overwintering abilities of *Ulva* spp. as vegetative fragments, attached germlings, and reproductive propagules, with implications for the year-round persistence of *Ulva* spp.

The mean density of *Ulva* spp. fragments buried in sediments was 7.13 m^{-3} ± 2.12 (mean ±1 standard error) and did not vary significantly among sites or months ($F_{2,7}$ = 0.47, p = 0.64; $F_{3,6.4}$ = 2.22, p = 0.18; Fig. 1). The mean density of *Ulva* spp. fragments in the water column, averaged across all winter months was 131 m^{-3} ± 40. The mean length of fragments was 11.6 ± 5.7 mm and 55.2 ± 6.2mm (sediment and water column, respectively), although fragments as long as 330 mm were present. Buried fragments of *Ulva compressa* and *U. rigida* were healthy; when subsequently cultured in the laboratory for 1.5 weeks (see Fig. 1 legend for culturing details), their biomass remained constant (initial vs. final biomass, t_{16} = 0.65, p = 0.74; data were pooled between species, as they did not significantly differ).

We found evidence of *Ulva* spp. germling recruitment from July through October 2012 on our monthly ('short-term') settlement tiles from Chepiwanoxet, while recruitment was only observed in September 2012 for Oakland Beach (Fig. 2A, B). Although germling biomass did not differ significantly between our two sampling sites or among months (January 2012 - March 2013; $F_{1,14}$ = 3.05, p = 0.10; $F_{14,14}$ = 1.04, p = 0.47, respectively), there was a significant interaction term ($F_{14,116}$ = 1.88, p = 0.03; see Table 1 for total algal recruitment). We found no correlation of *Ulva* biomass with other algal biomass (r^2 = 0.04), indicating that monthly *Ulva* recruitment is not linked to the recruitment of other algal species on our tiles.

By contrast, our 'long-term' settlement tiles exhibited much higher biomass of *Ulva* recruits (Fig. 2C, D; Table 1 for total algal recruitment). For the first tile deployment (deployed in September 2011, removed from January - May 2012), *Ulva* biomass reached a maximum in April-May 2012 at both sites, although it did not significantly differ between

sites or among months ($F_{1,4.0086} = 1.64$, $p = 0.27$; $F_{4,4} = 2.10$, $p = 0.24$). The biomass of *Ulva* recruits was positively correlated with the biomass of other algal recruits on the tiles ($r^2 = 0.30$, $p < 0.0001$).

For the second tile deployment (deployed in May 2012, removed from June 2012-March 2013), we found relatively low *Ulva* germling biomass (mean 0.01 $\pm$ 0.005 g · 100 cm^{-2}) that again did not significantly differ between sites or among months ($F_{1,10.68} = 3.63$ $p = 0.08$; $F_{9,9} = 0.97$, $p = 0.52$, respectively). For this deployment, the biomass of *Ulva* recruits was not correlated with other algal recruitment ($r^2 = 0.004$, $p = 0.54$). However, when we compare *Ulva* biomass from the two deployments (only including data from tiles in the field 4 months or more, to standardize among deployments), there was a nearly eightyfold difference in mean *Ulva* recruit biomass (0.78 g vs 0.02 g · 100 cm^{-2}) between the two deployments ($F_{1,109} = 18.67$, $p < 0.0001$), indicating significant year-to-year variability.

Short-term tiles deployed in the field for one month and then cultured in the laboratory under simulated spring conditions for two months indicated that distromatic *Ulva* spp. are producing reproductive propagules throughout the winter months, as distromatic blades were present every month. We found no significant differences among months or sites in *Ulva* recruit density ($p > 0.05$ for both; mean of 1.4 $\pm$ 0.65 recruits · 100 cm^{-2}; see Fig. 2 legend for methods).

Overall, our data indicate that *Ulva* spp. within Narragansett Bay utilize multiple mechanisms to survive the winter. Our data show that *Ulva* spp. are able to successfully overwinter as buried fragments, and we found that fragments are also present in the water column during the winter. Additionally, sediment fragment densities were consistent

throughout the winter months and among our three sample sites, although the lack of statistical significance among months may be due, in part, to high variability in fragment density and/or low replication. As our cultured fragments were viable throughout the winter months, in this system, *Ulva* spp. fragments can survive winter conditions and proliferate in the spring months when *Ulva* spp. blooms commence. These fragments may act in a similar way to terrestrial 'seed banks', providing a mechanism for species' persistence in years where there is reduced reproductive propagule production and/or recruit survival (Fenner and Thompson 2004).

Our settlement tile data support previously observed patterns of *Ulva* spp. biomass in Narragansett Bay, RI (Guidone and Thornber 2013). On long-term tiles, *Ulva* spp. reached maximum biomass from March through May 2012. By contrast, our short-term tiles show increased recruitment from mid summer to early fall 2012 at Chepiwanoxet, and in September 2012 at Oakland Beach. These patterns suggest that *Ulva* spp. are overwintering as microscopic germlings upon the benthos, likely settling during the late summer or early fall and initiating growth in the early spring when light levels, nutrient levels, and temperature are high enough to support growth (Lotze et al. 2000, Liu et al. 2012). This explains the overall lack of *Ulva* spp. biomass throughout the winter months and the rapidly forming blooms observed in mid spring (Guidone and Thornber 2013).

The decrease in *Ulva* spp. biomass between May and June 2012 on long-term tiles is likely due to *Ulva* spp. individuals becoming too large and dislodging from the settlement tiles. These dislodged individuals become free-floating thalli which may fragment, contributing to *Ulva* spp. bloom formation.

Our data provide increasing evidence for the multiple strategies that bloom-forming *Ulva* spp. can employ to survive harsh winter conditions. Macroalgal blooms are recognized as an increasing phenomenon and problem worldwide (Smetacek and Zingone 2013); while numerous environmental factors contribute to bloom formation (Valiela et al. 1997), the strategies that these algae employ during non-bloom periods are also critical to their interannual persistence.

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Table 1: Mean total wet mass (in grams) of all algal germlings upon 100 cm^2 settlement tiles (n = 5/treatment/site/date), from January 2012 - March 2013. Empty cells indicate zero algal biomass present; see Fig. 2 legend for method details.

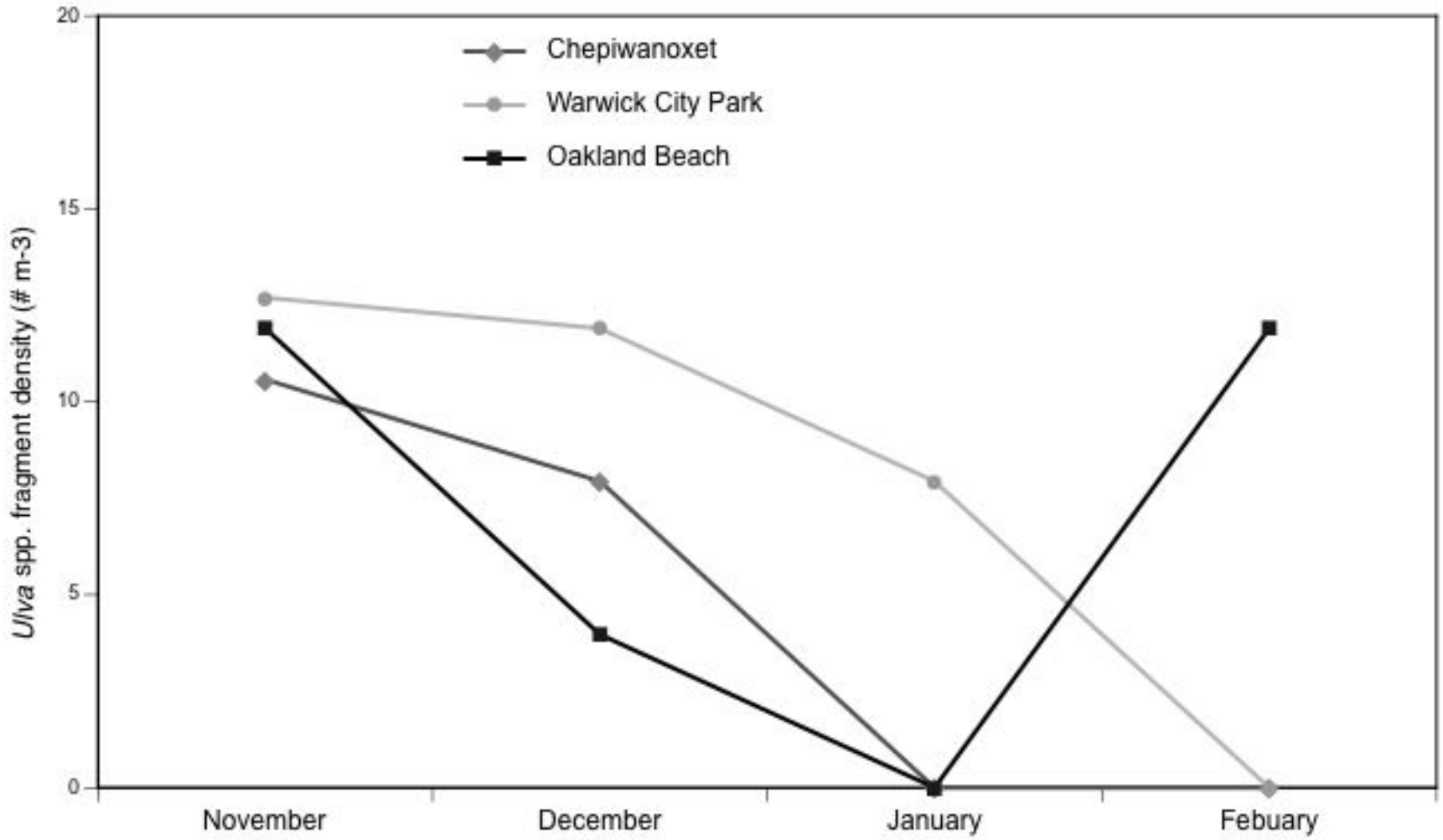
	Chepiwanoxet Point		Oakland Beach	
Month-Year	Short term	Long term	Short term	Long term
Jan.- 2012	4×10^{-5}	0.865		0.128
Feb.		1.740	1×10^{-4}	0.137
Mar.		9.037		0.927
Apr.		15.190		2.860
May.		4.081		3.773
Jun.	4×10^{-4}	5×10^{-4}	5×10^{-4}	3×10^{-4}
Jul.	0.093	0.488	0.001	0.004
Aug.	0.064	2.655	0.195	0.113
Sep.	5.368	33.694	0.377	4.181
Oct.	0.216	15.362	0.280	0.245
Nov.		3.229	3×10^{-4}	0.116
Dec.		3.741		0.242
Jan.- 2013		2.539	0.006	0.032
Feb.		2.194	5×10^{-5}	0.707
Mar.		0.543		4×10^{-4}

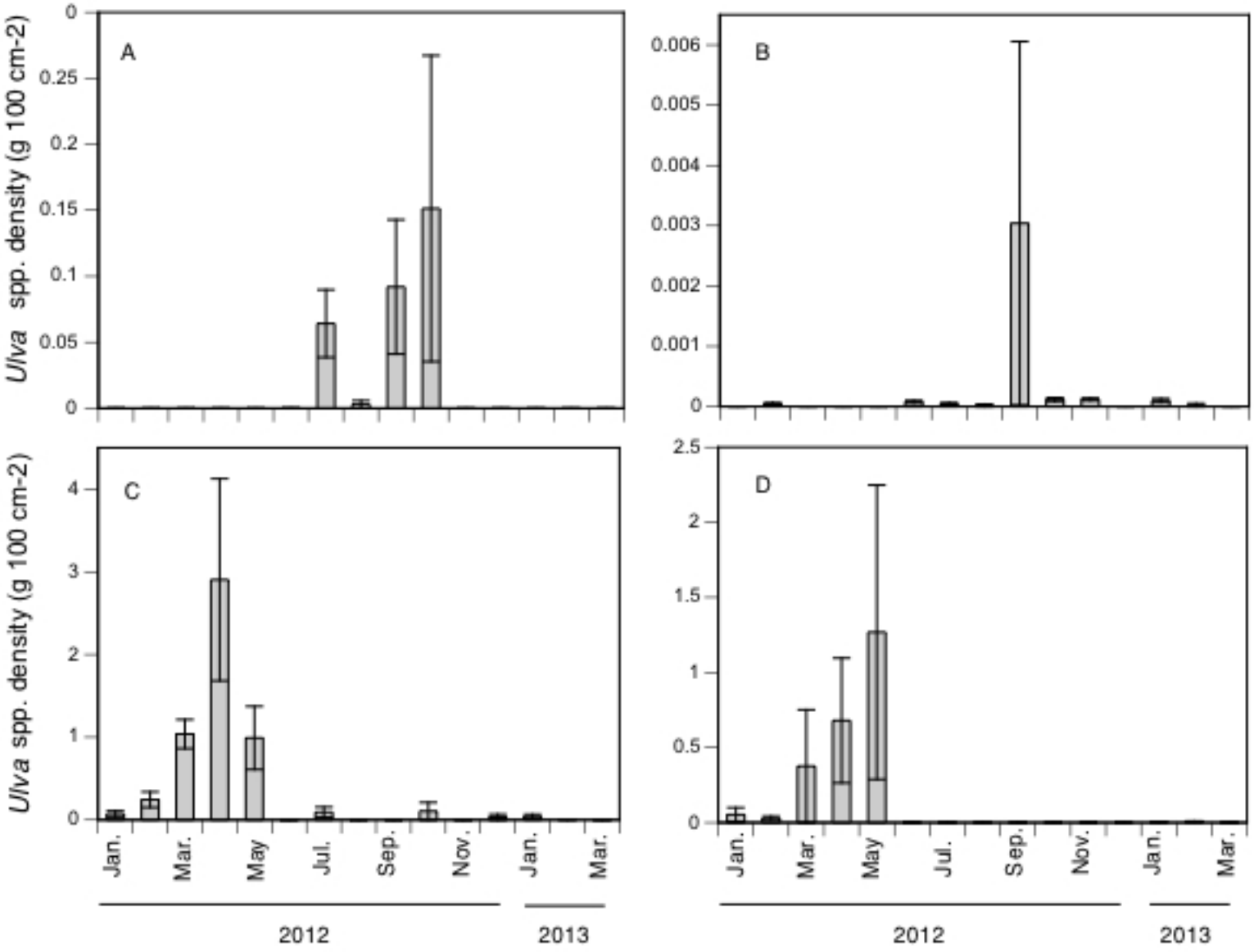
Figure Legends

Figure 1: Mean density of distromatic *Ulva* spp. fragments (± one standard error) in sediment core samples from November 2010 - February 2011. N = six cores (166 cm^3 each) were collected monthly at each of three bloom-impacted sites in Narragansett Bay, RI (Warwick City Park, Oakland Beach, and Chepiwanoxet Point -- all in Warwick, RI) during spring low tide. At each site, we laid out two 30 m transects. Along each transect, we collected one core from 1 m subtidal, one core at the water line, and one core 9 m horizontally away from mean lower low water (all cores were collected below the *S. alterniflora* zone). We counted all distromatic *Ulva* spp. fragments in each core and determined a collective wet mass. Other algal thalli were weighed together, per sample, and we recorded all genera present (fragments were frequently too small for accurate species-level designations). A subtidal net sweep (0.08 m^{-3}) was conducted at the same time to quantify suspended *Ulva* fragments. *Ulva* fragment viability was determined in January and February 2011. Using filtered seawater, we cultured ten distromatic *Ulva* spp. fragments (or fewer, depending upon availability, from field collected samples, with an initial size of at least 5 x 5 mm), per month. Fragments were cultured for 1.5 weeks to assess viability, using broad-spectrum growth lamps, on a 16:8 hour light:dark cycle, at 21-22 °C**.** All data were analyzed using JMP v 10.0 (www.jmp.com).

Figure 2: Mean *Ulva* spp. density ± one standard error, on 100 cm^2 PVC settlement tiles. A) short-term tiles at Chepiwanoxet Point; B) short-term tiles at Oakland Beach Cove; C) long-term tiles at Chepiwanoxet Point; D) long-term tiles at Oakland Beach Cove (n = 5 tiles for each tile type/month/location). To test if *Ulva* spp. overwinter as attached germlings, we deployed tiles covered in Grainger 3M tread medium resilient safety walk at

Chepiwanoxet and Oakland Beach Cove, Warwick, RI. At each site, 25 'long-term' settlement tiles were placed 30 cm below mean lower low water in September of 2011 and five were removed each month, starting in January 2012. From December 2011 onward, five additional tiles were placed in the field each month and collected the next month ('short-term' tiles). We quantified the biomass and number of individuals in each genus present on each tile. Settlement tile collection continued until all initial 'long-term' tiles were collected (May 2012). At that time, sixty more long-term tiles were deployed at each site, and processed in an identical manner (due to tile loss from extreme weather events, sampling ended prematurely in March 2013). We assessed *Ulva* spp. reproductive propagule production from November 2012 – February 2013 by placing five additional settlement tiles in the field at each site, monthly, for 30 days. Tiles were then cultured in the laboratory for two months, with simulated spring water conditions (18-19ºC, 22-28 psu). After two months, all attached germlings were counted and identified to genus on each tile. None of the 'cultured' tiles had visible recruits at the time of field collection.


Chepiwanoxet
Warwick City Park
Oakland Beach
Ulva spp. fragment density (# m-3)
20
15
10
5
0
November
December
January
Febuary


A
B
C
D
Ulva spp. density (g 100 cm-2)
Ulva spp. density (g 100 cm-2)
0
0.25
0.2
0.15
0.1
0.05
0.006
0.005
0.004
0.003
0.002
0.001
4
3
2
1
2.5
1.5
0.5
Jan.
Mar.
May
Jul.
Sep.
Nov.
Jan.
Mar.
2012
2013