Consumers mitigate heat stress and nutrient enrichment effects on eelgrass *Zostera marina* communities at its southern range limit

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ABSTRACT: At the southern end of their range, eelgrass *Zostera marina* L. meadows in North Carolina, USA, are vulnerable to multiple stressors. These include eutrophication, which can promote the overgrowth of algal epiphytes, and heat stress, which causes significant seasonal die-offs during the summer. To quantify the relative influences of these ecosystem stressors on eelgrass biomass, we conducted a mesocosm experiment investigating interactive effects of heat stress (+1.5°C) and nutrient loading (+10 μM NH_4NO_3, 1 μM KH_2PO_4) on an amphipod–eelgrass system. Additionally, we manipulated consumer pressure from an abundant omnivorous fish (pinfish *Lagodon rhomboides*) to understand how top-down pressure interacted with bottom-up nutrient additions and warming. Pinfish consumed nearly all amphipods (which had no effect on epibiont load), prevented macroalgal accumulation, and mitigated eelgrass loss due to heat stress. Notably, when pinfish were absent from mesocosms (1) macroalgal biomass was high even though grazers were allowed to reach high densities (>600 ind. per 18 l mesocosm), and (2) warming caused an 80% reduction in eelgrass aboveground biomass. Macroalgal biomass was positively correlated with water temperature and also had a significant correlation with eelgrass aboveground biomass. Although nutrient concentrations were similar to nutrient pulses from local storm events, nutrient enrichment only increased epibiont load when combined with warming, and we observed no discernible interactive effects on aboveground biomass. We conclude that omnivorous consumers can play an important role in preventing macroalgal accumulation from exacerbating heat stress effects, although continued warming may cause significant regional habitat loss regardless of consumer presence as eelgrass reaches its extreme physiological limits.

KEY WORDS: *Zostera marina* · Warming · Eutrophication · Multiple stressors · Grazing · Top-down control

INTRODUCTION

Across marine systems, the interactive effects of multiple stressors are frequently more severe than the predicted cumulative effects when each stressor is considered independently (Crain et al. 2008). However, the magnitude and type of interaction (additive, antagonistic, or synergistic) is dependent on which community or trophic levels are being examined in addition to the unique stressor combination (e.g. warm-ing, eutrophication, salinity, habitat loss, acidification). In particular, individuals at the southern edge of a species' range are already near their thermal tolerance, and thus interactions between warming and anthropogenic stressors may accelerate local extinctions (Harley et al. 2006, Carilli et al. 2010). The resulting ecosystem effects may be most dramatic when a foundation species becomes extirpated from an area, because individuals that rely on habitat forming species will be displaced or die out (Doney et al. 2012).

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Eelgrass *Zostera marina* is a foundation species that is widely distributed across estuaries in the Northern Hemisphere, and between 33 and 65°N along the Western Atlantic (Thayer et al. 1984, Green & Short 2003). Coastal ecosystems are affected by climate-driven range shifts, eutrophication (due to agriculture, silvaculture and coastal development), and loss of top consumers (caused by overfishing), all of which may have negative effects on eelgrass biomass and distribution (Orth et al. 2006, Ralph et al. 2006). Considering the key ecosystem services seagrass meadows provide (Larkum et al. 2006) and that seagrass cover has declined at a global scale in recent decades (Waycott et al. 2009, Antón et al. 2011), it is important to understand the potential synergistic effects of these multiple stressors on eelgrass meadows.

At the southern limit of its range, the optimal temperature for eelgrass growth is 22°C (Penhale 1977, Lee et al. 2007) and since water temperatures are above 25°C for most of July and August along this boundary (Kenworthy 1981, Thayer et al. 1984), heat stress drives the near complete loss of aboveground biomass in many eelgrass meadows (Thayer et al. 1984). When water temperature exceeds the thermal optimum, photosynthetic rates decline and respiration rates increase, which reduces net photosynthesis, inhibits leaf growth and eventually causes mortality (Marsh et al. 1986). Seagrass at higher temperatures also require more light to maintain a positive carbon balance (Lee et al. 2007).With increasing durations of water temperature exceeding 30°C due to warming in the mid-Atlantic, models of eelgrass meadows indicate that seasonal declines in eelgrass cover will be magnified (Carr et al. 2012), which will exacerbate the loss of essential nursery habitat for fishes and crustaceans (Micheli et al. 2008).

Simultaneously, nutrient enrichment (another major stressor that works through bottom-up pathways) has been found to reduce eelgrass cover (Valiela et al. 1997, Hughes et al. 2004, Lotze et al. 2006, Burkholder et al. 2007, Waycott et al. 2009). Although a slight increase in nutrients can initially increase the biomass in eelgrass meadows in nutrient-limited systems, further enrichment (especially water column enrichment) increases the biomass of algal epiphytes, reduces light penetration to the blades, and ultimately reduces eelgrass biomass (Burkholder et al. 2007). However, if present, herbivorous mesograzers such as amphipods can control epiphyte growth, mitigating the negative effects of nutrient enrichment through top-down control (Hughes et al. 2004, Spivak et al. 2009b, Blake & Duffy 2010, 2012, Myers & Heck 2013).

Interactions between multiple stressors have been shown to disrupt bottom-up and top-down community regulation, and can have interactive effects on habitat forming species and associated fauna (Crain et al. 2008). Seagrass systems, including eelgrass meadows at the southern extent of their range, are a good model for exploring the interactive effects of environmental stressors and top-down mitigation of bottom-up impacts because the effects of individual stressors on eelgrass biomass and food web interactions have already been established (Hughes et al. 2004). Burkholder et al. (1992) examined the varying seasonal effects of eutrophication on eelgrass in experimental mesocosms in North Carolina, USA, and found that even low levels of nitrate enrichment (3.5 μM NO_3^--N) promoted the decline of eelgrass. In addition, Burkholder et al. (1992) determined that this loss was more pronounced in the summer months when water temperatures were highest. Further work indicated that increasing the average temperature by 3 or 4°C intensified the effects of water-column nitrate inhibition on eelgrass growth (Touchette & Burkholder 2002), suggesting that the effects of increasing temperature interact with nutrient enrichment. These studies are valuable for understanding the physiological effects of nutrient enrichment and warming within seagrass systems, but do not incorporate biological interactions (i.e. trophic top-down control).

Top-down regulation also plays an important role in structuring eelgrass ecosystems. Several mesocosm studies have investigated whether increases in blue crab (secondary consumer) densities reduce herbivore densities and ultimately cause negative cascading effects on seagrass biomass (Duffy et al. 2005, Canuel et al. 2007, Douglass et al. 2007). These studies have demonstrated that crab presence can reduce amphipod abundance through consumption (Spivak et al. 2009b), or suppress amphipod grazing (Duffy et al. 2005), both of which increase epiphyte biomass. However, increases in epiphyte biomass, due to the presence of a secondary consumer, had no effect on eelgrass biomass. Pinfish *Lagodon rhomboides* is another secondary consumer that is extremely prevalent in seagrass habitats during summer months (e.g. comprised >80% of fishes in North Carolina seagrass trawl surveys; Baillie et al. 2014). The diet of juvenile pinfish consists predominantly of amphipods, and pinfish are thought to drive dramatic, seasonal amphipod declines. Indeed, as pinfish and other juveniles recruit to nursery eelgrass meadows, amphipod densities decline dramatically and remain low throughout the summer months (Nel-

son 1979). Since juvenile pinfish become omnivorous and consume algal epiphytes as they grow larger (Stoner 1979), they may also have a positive effect on seagrass biomass and productivity (Heck et al. 2000). The high densities of pinfish (along with other omnivorous secondary consumers) may play a critical role in controlling epiphyte loads when amphipods are at very low densities in eelgrass meadows in the summer months.

Extensive research has been conducted on the independent effects of nutrient enrichment, temperature, and food-web interactions on eelgrass, but opportunities remain to explore the cumulative effects of all 3 factors, particularly in explaining the future geographic distribution of this foundation species. To investigate the effects of top-down (secondary consumer pressure), bottom-up (nutrient enrichment) and abiotic stressors (warming), we aimed to determine (1) how heat stress and nutrient loading affect eelgrass biomass, (2) how the effects of heat stress and nutrient loading change with the removal of secondary consumer pressure, and (3) if there are any interactive or reciprocal effects between secondary consumer pressure and abiotic stressors in eelgrass meadows. We expected elevated water temperatures to enhance heat stress, and nutrient enrichment to increase epiphytic shading of the blades (both are processes that reduce aboveground biomass). Also, we anticipated that removing the secondary consumer would release grazers from top-down control and in turn reduce epiphytic cover and shading. This would, in our predictions, mitigate the effects of nutrient enrichment on eelgrass and prevent nutrient enrichment from exacerbating extreme heat stress effects.

MATERIALS AND METHODS

We conducted a 3-factor mesocosm experiment to explore how elevated temperature and nutrient loading affect eelgrass meadows, and whether removing secondary consumer pressure influences the direction or magnitude of these effects. To mimic natural eelgrass systems, we included a diverse community of amphipods found in local eelgrass meadows as our grazers, and manipulated the presence of pinfish, a regionally dominant secondary consumer. Temperature (ambient, +1.5°C), nutrient loading (ambient, +10 µM NH_4NO_3/1 µM KH_2PO_4) and consumer pressure (grazers + secondary consumers, grazers only) were crossed in a fully orthogonal design with 6 replicates.

We collected eelgrass cores (~26 cm diameter) from a monospecific eelgrass meadow in Middle Marsh, Back Sound, North Carolina in late July 2010. Cores were transplanted into 18 l experimental mesocosms in an outdoor flowing seawater system at the University of North Carolina at Chapel Hill's Institute of Marine Sciences in Morehead City. We rinsed cores with fresh water, wiped blades to remove all mobile invertebrates and macroalgae, and counted shoot density (mean ± SE: 29.2 ± 1.3 shoots) before placing eelgrass mesocosms in a continuous-flow, sand-filtered seawater system to prevent outside colonization of small invertebrates. A community of 50 amphipods, collected from water table cultures that mimicked local eelgrass grazer communities (predominately *Gammarus* spp. and *Ampithoe* spp.; Nelson 1979), were added to each mesocosm.

To understand how warming will potentially affect eelgrass biomass and its associated community, we conducted this experiment from 10 August through 21 September 2010, when seasonal temperatures and physiological heat stress were at peak levels. Heaters were added to mesocosms to obtain elevated temperatures; mock heaters were used in treatments with ambient water temperature to maintain consistent habitat complexity. The water temperatures of mesocosms and adjacent Bogue Sound were monitored 3 times daily with a hand-held thermometer to quantify the difference between heated and ambient water treatments and to ensure that ambient mesocosm water matched the water temperature at the same depth in the adjacent Sound.

Mesocosm nutrient concentrations were enriched by an addition of 10 µM NH_4NO_3 and 1 µM KH_2PO_4 (20:1 N:P) every 3 d to mimic storm nutrient pulses. These concentrations represent nutrient measurements following stormwater runoff events from long-term monitoring of coastal streams in the area (M. F. Piehler unpubl. data). Because our system experiences elevated nutrients following storm events as opposed to a constant supply of elevated nutrients, we chose to use this pulse design for water column nutrient enrichment instead of slow release fertilizer. NH_4NO_3 and KH_2PO_4 were dissolved in seawater and the concentrated solution was added to the mesocosms to increase nutrient concentration by 10 µM NH_4NO_3 and 1 µM KH_2PO_4. Water flow was shut off for 30 min immediately following the additions to allow nutrients to mix in the water column.

Finally, we added 1 pinfish (52 ± 1.1 mm standard length, 3.7 ± 0.2 g) to half of the mesocosms and ran the experiment for 6 wk. This allowed time for amphipod abundance to approach carrying capaci-

ties representative of *in situ* conditions (Duffy & Harvilicz 2001) and for eelgrass to respond to heat stress in late summer. At the end of the experiment, we removed the pinfish and re-measured each individual's standard length and weight to quantify growth throughout the experiment. Amphipods were filtered from the water, sorted from eelgrass blades, and enumerated to quantify final densities.

To determine how elevated temperature, nutrient pulses, and pinfish presence affected eelgrass biomass, eelgrass was uprooted, shaken to gently remove grazers, and rinsed through a 0.5 mm sieve. We sorted eelgrass into above- and belowground portions after scraping each blade to remove all epibionts growing on blades. We also separated all macroalgae that had accumulated at the top of the mesocosm, spun it to remove excess water before measuring wet weight. All portions of the eelgrass were then dried in an oven at 60°C for 3 d and weighed. Finally, we homogenized all above- and belowground portions of the eelgrass from each mesocosm into a fine powder for C:N analysis to understand the effects of nutrient enrichment on nitrogen and carbon content of eelgrass.

We analyzed pinfish growth (change in standard length and weight) and final amphipod density, epibiont load, macroalgal biomass, above- and belowground biomass using generalized linear models (GLM) in JMP v.10.0. These analyses tested for the fully crossed effects of water temperature, nutrient enrichment, and pinfish presence, in addition to a block effect (mesocosm position on water table) on our independent variables, with significance level set at $\alpha < 0.05$. Three pinfish were lost at an unknown time during the experiment, thus we excluded these mesocosms from the analyses, reducing replication of the pinfish/nutrients/1.5°C treatment to n = 4, and the pinfish/ambient nutrients/1.5°C treatment to n = 5. Pinfish growth, amphipod density (Box-Cox power transformed), aboveground and belowground biomass met the assumptions of normal distribution; therefore, we used an identity link function for each GLM. Macroalgal biomass fit the Poisson distribution with a log link function. When the water table block effect was significant (macroalgal, aboveground, and belowground biomass), we determined if there were any interactive effects of block and our manipulated factors. However, there was never an interaction between water table block and temperature, nutrient, or consumer manipulation. Finally, we

ran correlations to determine the relationships between macroalgae, average mesocosm temperature, and aboveground biomass across and within secondary consumer treatments.

RESULTS

Mesocosm water quality

Throughout the 6 wk experiment, the water temperature in control mesocosms did not differ from that of Bogue Sound (average temperature difference: 0.1 ± 0.9°C). Water temperature extremes ranged from 22.8 to 36.6°C in ambient treatment mesocosms and 25.2 to 39.7°C in heated mesocosms, while the average temperature over the whole study was 27.4 ± 0.1°C in ambient treatments and 29.0 ± 0.1°C in heated treatments (Fig. 1). The difference between ambient and heated temperature treatments varied according to time of day. In the morning the water temperatures were on average 1.7 ± 0.1°C warmer in the +1.5°C treatment and only 0.8 ± 0.1°C warmer in the evening. Salinity averaged 33.7 PSU, but Hurricane Earl affected our experiment when it impacted eastern North Carolina on 2 September 2010, which resulted in a large fresh water influx and power outage (less than 3 h) that disrupted water flow. Although this led to a system-wide decrease in water temperature, heated temperature treatments remained higher than control temperatures following the storm, and we did not observe any mass mortality of eelgrass, amphipods, pinfish or macroalgae associated with this perturbation.
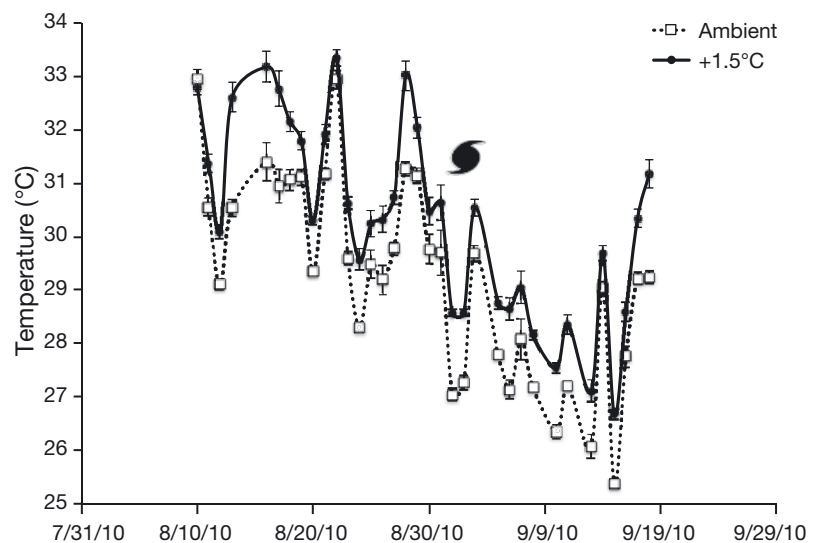

Fig. 1. Average daily temperature of heated (+1.5°C) and ambient water treatments across the duration of the experiment. Hurricane symbol represents the arrival of Earl on 2 September 2010. Dates given as month/day/year

Table 1. Analysis of response of eelgrass community to the individual and interactive effects of consumer presence (Pin), nutrient enrichment (Nut), and warming (Temp) using generalized linear models (df = 5 for treatments and 2 for all block analyses). Significant treatment effects at $\alpha < 0.05$ are indicated in **bold**

Factors	Pinfish Weight		Length		Amphipod density		Biomass Epibiont		Macroalgae		Aboveground		Belowground	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Pin					93.11	**<0.001**	2.54	0.111	1010.11	**<0.001**	1.63	0.201	0.00	0.953
Nut	3.95	**0.047**	1.37	0.243	1.16	0.281	2.56	0.110	0.00	0.999	0.07	0.794	0.01	0.926
Pin × Nut					0.87	0.352	0.17	0.681	0.00	1.000	0.81	0.369	0.50	0.478
Temp	2.12	0.145	0.94	0.331	0.09	0.759	0.08	0.773	0.00	1.000	11.22	**0.001**	0.12	0.728
Pin × Temp					2.42	0.120	0.09	0.764	0.00	0.999	7.49	**0.006**	0.19	0.659
Nut × Temp	2.22	0.136	0.84	0.358	1.10	0.294	7.75	**0.005**	0.64	0.424	0.14	0.707	0.12	0.725
Pin × Nut × Temp					0.11	0.743	2.89	0.089	0.57	0.452	0.00	0.988	3.75	0.053
Block	5.32	0.070	1.79	0.409	1.16	0.561	0.95	0.623	992.47	**<0.001**	22.95	**<0.001**	11.42	**0.003**

Secondary consumer

To understand how secondary consumer pressure, nutrients, and warming potentially interact and affect eelgrass biomass, we first examined if there were any effects of the 2 stressors on pinfish. Although there were no differences in growth measured as change in standard length (final standard length 57.8 ± 0.8 mm, growth 1.5 ± 0.2 mm), nutrient enrichment significantly increased growth measured in weight of pinfish ($\chi^2 = 3.95$, p = 0.047; Table 1) from 1.19 ± 0.21 g in ambient nutrient treatments to 1.76 ± 0.27 g in nutrient enrichment treatments (Fig. 2A). Increased water temperature had no effect on growth (standard length or weight).
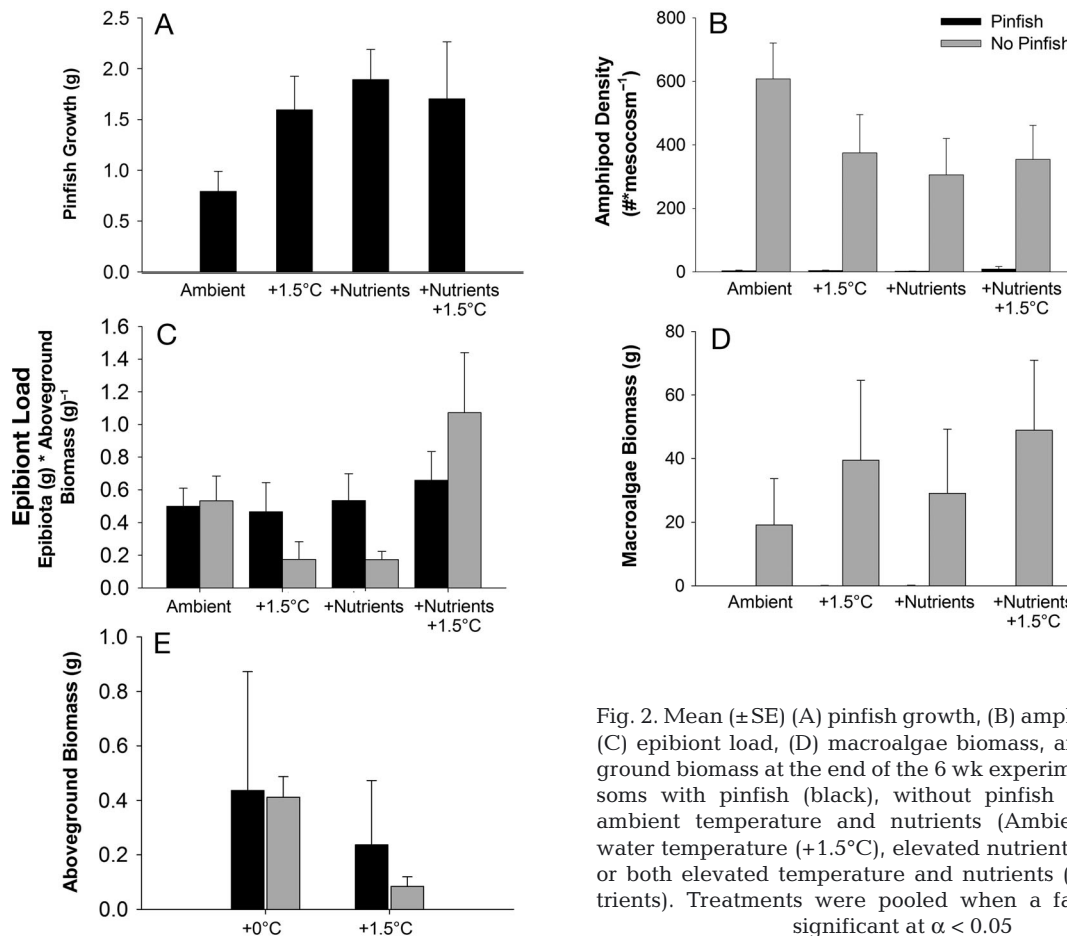

Fig. 2. Mean (±SE) (A) pinfish growth, (B) amphipod density, (C) epibiont load, (D) macroalgae biomass, and (E) aboveground biomass at the end of the 6 wk experiment in mesocosms with pinfish (black), without pinfish (gray), under ambient temperature and nutrients (Ambient), elevated water temperature (+1.5°C), elevated nutrients (+Nutrients) or both elevated temperature and nutrients (+1.5°C, +Nutrients). Treatments were pooled when a factor was not significant at $\alpha < 0.05$

Grazer density

Removing secondary consumer pressure released amphipods from top-down control. Pinfish consumed nearly all amphipods when present (4.0 ± 1.5 amphipods remaining at the end of the experiment), but with no secondary consumer present, amphipod densities reached 607.5 ± 113.0 ind. mesocosm^{-1} ($\chi^2 = 93.11$, p < 0.001; Fig. 2B). Nutrient enrichment ($\chi^2 = 1.16$, p = 0.281) and warming ($\chi^2 = 0.09$, p = 0.759) had no effect on final amphipod density.

Epibiont load

We expected the increase in amphipod densities due to pinfish removal to reduce the epibiont load, but found that pinfish presence had no effect on epibiont load ($\chi^2 = 2.54$, p = 0.111). However, nutrient enrichment and temperature had an interactive effect on epibiont load ($\chi^2 = 7.75$, p = 0.005; Fig. 2C). When mesocosms did not receive nutrient enrichment, increasing water temperature had no effect on epibiont load (0.515 ± 0.090 epibiota (g) * aboveground biomass (g)$^{-1}$ in ambient treatment), whereas under nutrient loading conditions, warming increased epibiont load to 0.888 ± 0.237 epibiota (g) * aboveground biomass (g)$^{-1}$. Epibiont community composition was not quantified, but we observed no obvious trends across treatments. Epiphytes consisted predominantly of fleshy algae (both turf microalgae and *Ulva intestinalis*); however, some calcareous algae were also present.

Macroalgal biomass

Although removing pinfish and releasing amphipods from top-down control did not increase epibionts, removing pinfish did cause an increase in the macroalgae *U. intestinalis*, which overgrew seagrass in mesocosms (Fig. 2D). Macroalgae was almost completely absent (0.03 ± 0.02 wet g) when pinfish were present, but biomass reached 34.11 ± 10.10 wet g when pinfish were removed ($\chi^2 = 1010.11$, p < 0.001). Additionally, macroalgal biomass when pinfish were absent was positively correlated (significant at $\alpha < 0.10$) with average mesocosm water temperature ($\rho = 0.258$, p < 0.087).

Eelgrass biomass and nutrient content

There was a significant interaction between water temperature and pinfish presence on aboveground

eelgrass biomass ($\chi^2 = 11.22$, p = 0.001; Fig. 2E), with the loss of pinfish exacerbating effects of heat stress on aboveground biomass (Fig. 2E). Increased water temperature had no significant effect on aboveground biomass when pinfish were present, but significantly reduced biomass from 0.24 ± 0.089 g when pinfish were present to 0.08 ± 0.035 g when pinfish were absent. Aboveground biomass was also negatively correlated with macroalgal biomass ($\rho = -0.365$, p = 0.014). There were no significant differences in belowground biomass across treatments.

Although we did not find an effect of nutrients on final epibiont load, macroalgal biomass, or eelgrass biomass, nutrient enrichment significantly increased the nutrient content of the eelgrass blades (Fig. 3A, Table 2), decreasing the C:N ratio from 24.03 to 21.75 ($\chi^2 = 4.32$, p = 0.038). This was due to an increase in nitrogen content ($\chi^2 = 4.01$, p = 0.045; Table 3) in the blades from 1.85 ± 0.10 to 2.06 ± 0.07 % N.
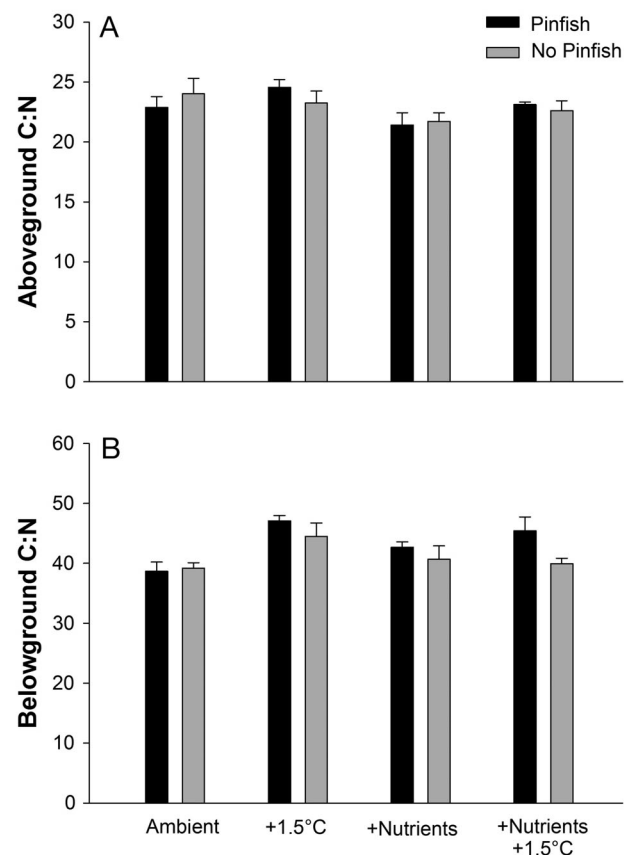

Fig. 3. Mean ($\pm$SE) nutrient content of (A) above- and (B) belowground eelgrass at the end of the 6 wk mesocosm trial with (black) or without a pinfish (gray) under ambient temperature and nutrients (Ambient), elevated water temperature ($+1.5°C$), elevated nutrients (+Nutrients) or both elevated temperature and nutrients ($+1.5°C$, +Nutrients)

Table 2. Analysis of the response of above- and belowground eelgrass carbon and nitrogen content to the individual and interactive effects of consumer presence (Pin), nutrient enrichment (Nut), and warming (Temp) using a generalized linear model. Significant treatment effects at α < 0.050 are indicated in **bold**; df = 5 for all treatments and 2 for block analyses

| Factors | Aboveground | | | | | | Belowground | | | | | |
| | C:N | | % Carbon | | % Nitrogen | | C:N | | % Carbon | | % Nitrogen | |
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Pin	0.21	0.645	0.00	0.971	0.21	0.646	3.39	0.066	1.87	0.172	0.61	0.434
Nut	4.32	**0.038**	0.49	0.483	4.01	**0.045**	0.00	0.999	0.01	0.914	0.02	0.882
Pin × Nut	0.01	0.934	1.19	0.276	0.07	0.790	0.89	0.347	0.77	0.380	0.21	0.645
Temp	2.07	0.150	5.94	**0.015**	4.37	**0.037**	6.64	**0.010**	1.41	0.235	3.99	**0.046**
Pin × Temp	2.19	0.139	1.85	0.174	1.23	0.268	1.33	0.248	1.35	0.245	0.37	0.541
Nut × Temp	0.84	0.360	0.35	0.552	1.19	0.275	3.51	0.061	0.01	0.935	3.74	0.053
Pin × Nut × Temp	0.33	0.565	2.53	0.112	0.48	0.486	0.02	0.896	0.07	0.786	0.59	0.442
Block	2.39	0.303	0.96	0.617	2.85	0.240	7.93	**0.019**	6.30	**0.043**	20.62	**<0.001**

Table 3. Nutrient content (mean ± SE) of eelgrass measured as percent carbon and nitrogen of aboveground biomass averaged within each treatment

| Treatment | Aboveground | | | | Belowground | | | |
	% Carbon	SE	% Nitrogen	SE	% Carbon	SE	% Nitrogen	SE
Ambient	37.76	0.21	1.85	0.10	31.15	0.90	0.93	0.02
Ambient, pinfish	37.51	0.31	1.93	0.07	30.74	1.29	0.93	0.03
+1.5°C	37.25	0.16	1.88	0.09	30.77	1.61	0.81	0.04
+1.5°C, pinfish	36.90	0.38	1.76	0.06	32.11	0.61	0.81	0.05
Nutrients	38.23	0.22	2.06	0.07	30.37	1.41	0.88	0.03
Nutrients, pinfish	37.73	0.21	2.08	0.11	31.31	0.86	0.88	0.08
+1.5°C nutrients	36.59	1.25	1.89	0.08	30.63	0.87	0.91	0.07
+1.5°C, nutrients, pinfish	37.77	0.14	1.91	0.03	32.82	1.42	0.84	0.01

DISCUSSION

In this study, water temperature was the key factor in controlling eelgrass biomass (Fig. 4). Elevating water temperature by approximately 1.5°C (averaged over a diel cycle) magnified seasonal heat stress and led to a significantly greater loss of eelgrass biomass than in ambient temperature treatments. Nutrient enrichment alone did not cause an increase in epibiont or macroalgal biomasses as expected, but did increase the nutrient content of the eelgrass blades, and interactively with warming, increased epibiont loads. Changes in blade nutrient content did not result in discernible effects propagating up the food chain through amphipod density, but did cause an increase in pinfish biomass. Dramatic differences in grazer densities caused by pinfish consumption had no effect on epibiont or macroalgal biomass; however, we did find that pinfish played a critical role in eelgrass systems by directly preventing macroalgal accumulation. Pinfish consumed almost all macroalgae in the mesocosms, and when pinfish were absent, macroalgae proliferated and presumably further reduced eelgrass biomass through shading (Sand-Jensen 1977). Our re-

sults suggest that warming could reduce seasonal eelgrass habitat through heat stress, which would be compounded by an increase in macroalgae. However, as our study demonstrated, there is also potential for secondary consumers to mitigate macroalgal accumulation through top-down pressure.

We conducted this experiment when abiotic stress was greatest at the end of the growing season, when (at the southern limit of its range) eelgrass meadows annually die-off in the summer (Kenworthy 1981, Thayer et al. 1984). Since all treatments were undergoing heat stress, low eelgrass biomass may have prevented us from detecting clear interactions between stressors because heat-stress dominated the response of aboveground biomass. However, since a small increase in water temperature enhanced the decline of eelgrass biomass dramatically, it is likely that warming will continue to cause declines, shift the growing season earlier, and eventually move the species' range poleward. Recent studies have determined that every 1°C increase results in a 5 to 6 d forward shift in the eelgrass growing season (Clausen et al. 2014). Some eelgrass meadows at their southern range limit have already experienced a reduction
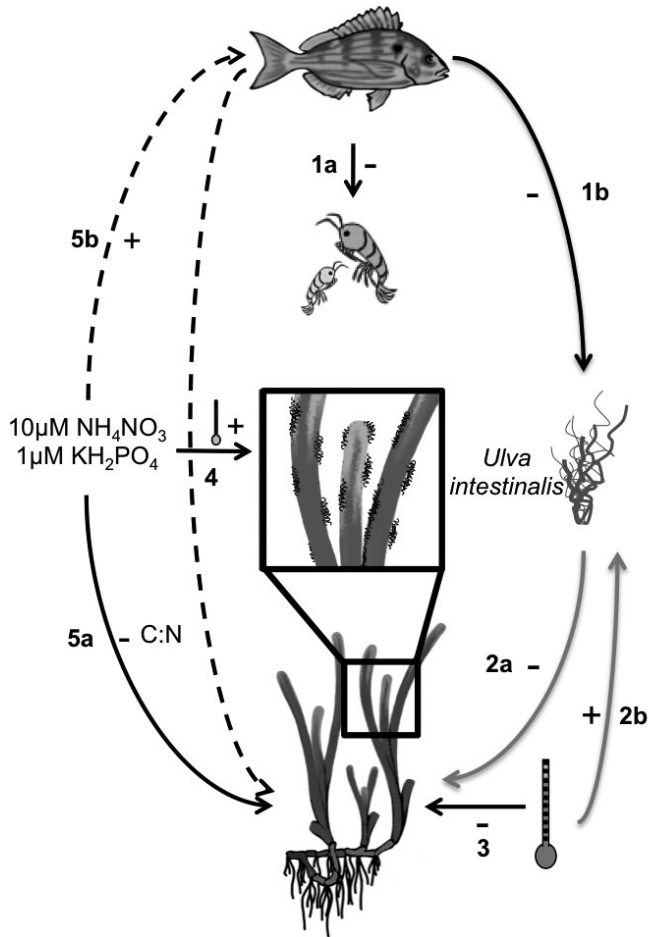
Fig. 4. Effects of secondary consumers, increased temperature, and nutrient enrichment on an eelgrass community. Pinfish removal released both (1a) amphipods and (1b) macroalgae from consumer control. Increases in amphipod density had no cascading effects on eelgrass biomass, (2a) while an increase in macroalgal biomass was correlated with lower aboveground biomass ($p < 0.1$). (2b) The quantity of macroalgal biomass that accumulated when pinfish were absent was correlated with individual mesocosm average water temperature ($p < 0.05$). (3) Increased water temperature reduced aboveground biomass, but heat stress and biomass reduction was greatest when pinfish were absent. (4) Pulsed nutrient enrichments increased epibiont load only when temperature was elevated and had no discernable cascading effects on eelgrass biomass. Nutrient enrichment also (5a) decreased the C:N ratio of eelgrass blades, and (5b) increased pinfish biomass. Solid black arrows: significant effects at $\alpha < 0.05$; gray arrows: correlations; dashed arrows: indirect effects

in biomass over the last 20 or 30 yr as temperatures have increased by 1.4°C, and water quality has been degraded between 1984 and 2005 (Micheli et al. 2008). Shoal grass *Halodule wrightii*, a tropical species with a northern range limit also in North Carolina, may be able to survive in warmer summers, but

there has been no change in its density in the last few decades and it has not begun to colonize eelgrass barrens (Micheli et al. 2008). Continued eutrophication of coastal estuaries may facilitate shoal grass colonization of old eelgrass meadows as was seen in former turtle grass meadows following bird guano enrichment in southern Florida (Fourqurean et al. 1995). *Ruppia maritima* (widgeon grass) may also do well under eutrophication because it has a physiological mechanism to prevent excessive nitrate uptake and grows well in enriched systems (Burkholder et al. 1994). However, even if shoal or widgeon grasses are able to colonize eelgrass meadows, there will be significant shifts in the nursery value of seagrass meadows in North Carolina since these grasses support a different community of fishes and crustaceans relative to eelgrass (Micheli et al. 2008, Baillie et al. 2014).

Increased temperatures have also been shown to strengthen herbivore–algae interactions (O'Connor 2009) as metabolic rates of consumers increase at a greater rate than those of primary producers and result in a trophic skew defined by increased consumer:producer biomass (López-Urrutia et al. 2006, Carr & Bruno 2013). However, the balance between herbivores and primary producers in response to increased temperatures can be dependent on absolute temperature and nutrient supply. For instance, recent work has found that top-down interactions only strengthen until temperatures reach an organism's thermal optimum where predator metabolism is greatest, after which metabolic rates (and therefore interaction rates) weaken (Englund et al. 2011). Furthermore, experimental work has shown that increasing temperatures only shift phytoplankton–zooplankton systems toward greater relative abundance of consumers in eutrophic environments (O'Connor et al. 2009). When examining our pinfish–amphipod–epibiont system, we also observed an interaction between warming and nutrients; however, we found that warming increased epibiont load under eutrophic conditions as opposed to strengthening the top-down interaction. Because temperatures periodically exceeded the optimum in heated treatment (30 to 33°C for pinfish; Muncy 1984), reduced metabolic rates may have weakened the top-down interaction and resulted in greater epibiont loads (Englund et al. 2011).

Unlike the dramatic effects of warming, we found only moderate effects of nutrient enrichment on the eelgrass community. We expected nutrient loading to exacerbate the effects of heat stress on aboveground biomass through algal overgrowth or nitrate induced carbon limitation. Burkholder et al. (1992) documen-

ted 75 to 95 % shoot mortality relative to un-enriched controls under pulsed nitrate enrichment, and as there were no differences in epiphyte load between treatments, they attributed die-off to nitrate toxicity exacerbated by heat stress. We did not see any effects of nutrient toxicity compounding heat stress; rather, nutrient enrichment had positive effects on eelgrass nutrient content, increasing the nitrogen content in the blades. The pulse design of our experiment, reflecting measured nutrient concentrations from local stormwater runoff events, prevented eelgrass blades from constantly having to reduce nitrate and maintain carbon stores in the blades as in previous experiments that used a press design and higher nutrient concentrations (Burkholder et al. 1992, 1994). Nutrient enrichments also significantly increase the biomass of pinfish. We observed no change in amphipods, epibionts, or macroalgae across pinfish treatments, but did observe a significant increase in pinfish biomass. We suggest that the nutrient enrichment caused enhanced primary production in nutrient enrichment treatments, causing pinfish to consume more resources and increase final pinfish biomass. Alternatively, the nutrient content, or quality, of the resources available may have been higher in nutrient enrichment treatments and increased pinfish biomass.

Across marine systems, positive effects of grazing in reducing nuisance macroalgae can be equal in magnitude to the negative effects of water column nutrient enrichment on seagrass biomass (Hughes et al. 2004). In both eelgrass mesocosms and field experiments, epiphyte loads have rapidly responded to water column nutrient enrichment, but amphipods have been found to control fast epiphyte growth, preventing nutrient loading from reducing eelgrass biomass (Neckles et al. 1993, Douglass et al. 2007, Spivak et al. 2009a). Unfortunately, we did not successfully quantify epibiont loads early in the experiment (2 to 4 wk), so we do not know if nutrient enrichment caused high initial epiphyte loads before consumers grazed down epiphytes (as found previously). The limited response in epibiont load may also be caused by having a full community of grazers, which has been found to increase stability in response to stressors such as nutrients and warming (Spivak et al. 2009a, Blake & Duffy 2010, 2012, Alsterberg et al. 2013). Additionally, our system was more complex than previous work because there were multiple consumers (pinfish, amphipods) feeding on multiple resources (epibionts, macroalgae, etc.). Spreading consumption across multiple consumers may have also limited differences in final epibiont load across treatments and prevented epibionts from shading and reducing eelgrass aboveground biomass.

Previous studies have found that omnivorous pinfish dampen trophic cascades because they consume both mesograzers and macroalgae (Bruno & O'Connor 2005). We collected pinfish that were representative of the sizes present in the Sounds in July when the experiment began. The average pinfish length (52 ± 1.1 mm) was within the omnivorous size class, where 30 % of their diet likely consists of epiphytes in addition to mesograzers (Stoner 1979); however, their diet is also determined by the relative abundance of macrophytes (Stoner 1979). Instead of having large effects on epibionts through cascading effects of amphipod reduction, pinfish had the strongest impact on eelgrass by consuming the macroalgae growing on top of the eelgrass in the mesocosms (Hughes et al. 2004, Heck & Valentine 2007). Continued nutrient enrichment (Hauxwell et al. 2003) and warming (Blake & Duffy 2012) will favor ephemeral and epiphytic algae over eelgrass biomass. Large ephemeral algal mats are present within eelgrass meadows and amphipod densities are high in spring and early summer before juvenile fishes (>80 % pinfish in eelgrass meadows) arrive, after which amphipod density and macroalgal biomass quickly decline and remain low until pinfish migrate out of coastal sounds in winter. Our study suggests that pinfish may play an important, possibly underappreciated, role in controlling macroalgal accumulation, because even at high densities, amphipods in our experiment were unable to graze as much macroalgae as a single omnivorous pinfish.

Conducting a mesocosm experiment may have produced some artefacts that must be considered when making any extrapolations to natural eelgrass meadows. For instance, mesocosms may have exacerbated the quantity of macroalgae beyond what could feasibly accumulate *in situ*. In eelgrass meadows, macroalgae would be swept away by tidal currents, while lower flushing rates, higher temperatures, and increased attachment area may have facilitated macroalgal accumulation in our experimental mesocosms. Although post hoc laboratory measurements of light attenuation found that macroalgae reduced light penetration and presumably reduced photosynthesis, light levels at the highest macroalgal biomass were above 25 % of ambient light, and therefore did not completely prevent eelgrass photosynthesis (see Fig. A1 in the Appendix). We do note that pinfish are very abundant in North Carolina eelgrass meadows, where macroalgal accumulation is rare in the sum-

mer, and therefore the macroalgae we observed in pinfish-absent treatments may simply highlight that in the field, this consumer provides strong top-down control, consistent in space and time, against macroscopic producers such as *Ulva*. We are also confident that macroalgal consumption by pinfish was not simply an artefact of starvation, as mesocosms contained ample epibiont biomass available for grazing and we did not see any evidence of pinfish bites on eelgrass. These observations suggest a preference for macroalgae by omnivorous pinfish in our mesocosm trials.

Among top-down control, nutrient enrichment, and warming, our mesocosm results imply that warming is the primary factor controlling eelgrass biomass at its southern limit. Continued warming may cause die-backs to occur earlier, persist longer, and ultimately result in a local loss of essential nursery habitat. We also found that top-down control can reduce algal overgrowth of eelgrass by consuming macroalgae, preventing macroalgal shading from exacerbating heat stress. Future studies should test these findings *in situ* and further quantify how warming may affect the timing of seasonal die-offs and the corresponding negative effects on eelgrass communities due to loss of this foundation species.

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Appendix. To understand how *Ulva* reduces light in mesocosm setting, we created a light attenuation curve for macroalgal biomass (Fig. A1). We collected algae from the flowing seawater system and measured light attenuation after adding *Ulva* to the surface of the mesocosm in 10 g increments. Ambient light levels were 1574 $\mu E\ m^{-2}\ s^{-1}$, and with 60 g of *Ulva* at the surface of the mescosm (the maximum biomass found in eelgrass mesocosms), light was reduced to 26% of surface light. These values are above the minimum light requirements for eelgrass reported along the mid-North Atlantic (Lee at al. 2007) and are similar to values measured in eelgrass meadows in Back Sound, North Carolina, USA ($435.5 \pm 5.6\ \mu E\ m^{-2}\ s^{-1}$; F. J. Fodrie unpubl. data)
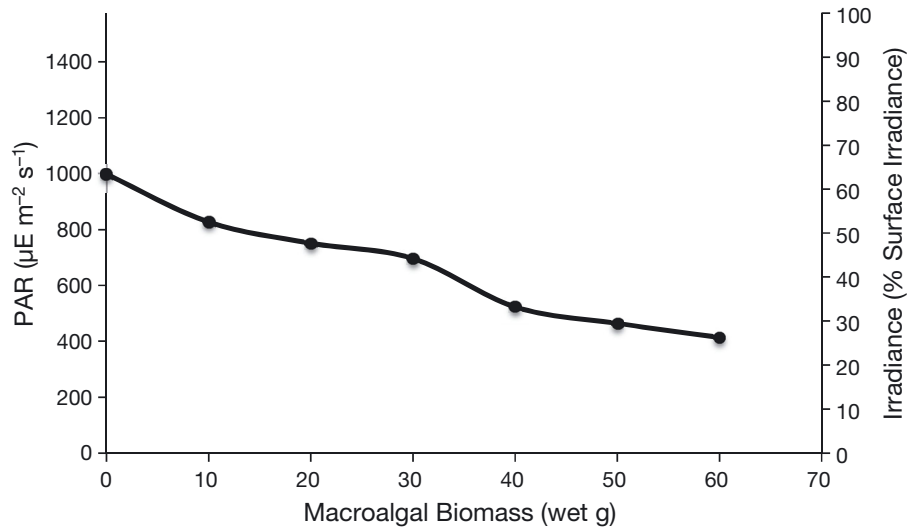

Fig. A1. Relationship of photosynthetically active radiation (PAR) ($\mu E\ m^{-2}\ s^{-1}$) and irradiance with increasing macroalgal biomass (wet g) in experimental mesocosms measured in July 2014