

# Trophic cascades on the edge: fostering seagrass resilience via a novel pathway

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**Abstract** Despite widespread degradation, some coastal ecosystems display remarkable resilience. For seagrasses, a century-old paradigm has implicated macroalgal blooms stimulated by anthropogenic nutrient loading as a primary driver of seagrass decline, yet relatively little attention has been given to drivers of seagrass resilience. In Elkhorn Slough, CA, an estuarine system characterized by extreme anthropogenic nutrient loading and macroalgal (*Ulva* spp.) blooms, seagrass (*Zostera marina*) beds have recovered concurrent with colonization of the estuary by top predators, sea otters (*Enhydra lutris*). Here, we follow up on the results of a previous experiment at the seagrass interior, showing how sea otters can generate a trophic cascade that promotes seagrass. We conducted an experiment and

constructed structural equation models to determine how sea otters, through a trophic cascade, might affect the edge of seagrass beds where expansion occurs. We found that at the edge, sea otters promoted both seagrass and ephemeral macroalgae, with the latter contributing beneficial grazers to the seagrass. The surprising results that sea otters promote two potentially competing vegetation types, and a grazer assemblage at their boundary provides a mechanism by which seagrasses can expand in eutrophic environments, and contributes to a growing body of literature demonstrating that ephemeral macroalgae are not always negatively associated with seagrass. Our results highlight the potential for top predator recovery to enhance ecosystem resilience to anthropogenic alterations through several cascading mechanisms.

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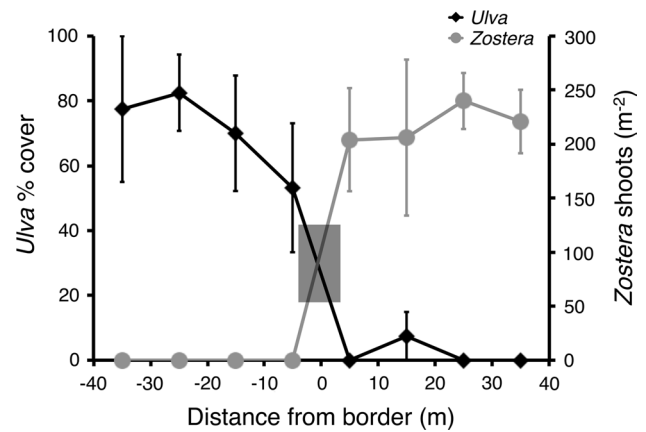
## Introduction

Determining the drivers and mechanisms of collapse and recovery in ecosystems is a fundamental goal of ecology and conservation (Holling 1973; Gunderson 2000; Lotze et al. 2006; Jones and Schmitz 2009; Bernhardt and Leslie 2013). The ecosystem ecology literature is dominated by studies of decline because of widespread degradation of many of the earth's ecosystems, and thus, fewer studies have focused on the two key components of ecosystem stability (Lotze et al. 2011): resistance or the ability of a system to withstand a perturbation, and resilience or the system's ability to recover from a perturbation (Tilman and Downing 1994). For marine vegetation, the majority of studies have reported overall declines (Jackson et al. 2001;

Orth et al. 2006; Waycott et al. 2009), yet there are several systems demonstrating resistance and resilience (Waycott et al. 2009; Lotze et al. 2011). Case studies that develop an understanding of the mechanisms driving resistance and resilience of coastal vegetation are the key for promoting recovery in other systems.

For seagrasses, a century-old paradigm supported by extensive empirical studies has implicated eutrophication resulting from anthropogenic nutrient loading in phase shifts from seagrass to ephemeral macroalgae (Letts and Adeney 1908; Valiela et al. 1997; Orth et al. 2006; Burkholder et al. 2007). There are two pathways that can cause the shift in dominance: (1) shading effects by epiphytic algae that decrease the photosynthetic output of seagrass leaves (Zimmerman 2010), and thus, indirectly favor macroalgae over seagrass, and (2) direct smothering of existing and newly formed eelgrass shoots by macroalgae (Nelson and Lee 2001). However, these two pathways are not mutually exclusive, and could act synergistically to decrease stability in seagrass. Conversely, the last few decades of research have generated theory on how recovery of top predators can enhance the stability of seagrass beds under threat from eutrophication (Valentine and Duffy 2006). Recently, results from experimental and long-term monitoring studies have demonstrated that stability in seagrass beds can be enhanced by the presence of top predators, even if the change in trophic structure was not originally responsible for seagrass decline (Moksnes et al. 2008; Baden et al. 2010; Hughes et al. 2013; Heithaus et al. 2014).

Elkhorn Slough is an estuary in central California that provides a rare example of seagrass recovery despite decades of increased anthropogenic nutrient loading and eutrophication (Hughes et al. 2013). Nutrient concentrations and loading in the estuary are some of the highest recorded for coastal systems, and are a continuous perturbation to the system. Experimental manipulation of trophic levels at the interior of an eelgrass (*Zostera marina*) bed showed shading by algal epiphytes strongly affects eelgrass condition, and that the presence of sea otters and their predation on crabs was a key driver of low epiphyte loads and the persistence of eelgrass, despite extreme nutrient loading. Macroalgae are rare in the interior of these beds (Fig. 1), but proliferate towards the edge where bed expansion occurs, and form a virtual monoculture upslope of the eelgrass edge. Previous results (Hughes et al. 2013) correlated bed expansion with otter density and foraging, yet the mechanisms behind the expansion were not elucidated. It is possible that the mechanism of sea otter control at the eelgrass bed edge vs. interior is the same, where sea otters reduce crabs thus releasing invertebrate epiphyte grazers from predation, which enhances eelgrass by reducing epiphytic growth. Alternatively, mechanisms of otter effects could be quite different at the edge than in the interior.



**Fig. 1** Profile of the eelgrass-macroalgal boundary based on 70-m transect surveys ( $n = 5$ ) from 2011 sampling across the eelgrass-macroalgal boundary. The grey box demonstrates where the experimental cages were placed along the edge. Error bars are  $\pm 1$  SE

For instance, through trophic pathways sea otters could decrease ephemeral macroalgal mats that would also promote eelgrass shoot production at the edge. The goal of this study was to determine the potential role of sea otters in generating a trophic cascade at the edge of eelgrass beds, and provide insight into the variable pathways that promote ecosystem resilience.

There are many cases of studies that have investigated top predator effects on vegetation through trophic cascades (Jackson et al. 2001; Estes et al. 2011) with the emphasis of looking at general effects on vegetation integrity. However, in most cases, the dynamics of the vegetation edge is essential for understanding expansion or contraction, and ultimately resilience (Konar and Estes 2003). The mechanisms driving vegetation integrity at the interior vs. edge may be inherently different due to a suite of factors, which include differences in consumer abundances and species, competition with other plant populations, and bottom-up factors, such as light availability (Lidicker 1999). These differences at the interior vs. edge of vegetation could shift key species interactions, and thus the role of trophic cascades as drivers of vegetation resistance and resilience.

## Materials and methods

Elkhorn Slough is characterized by extreme nutrient loading (Hughes et al. 2011, 2013, 2015), and it suffers from eutrophication in the form of dense macroalgal mats that cover the estuary's mudflats and shallow subtidal zones through the spring, summer, and fall (supplementary information, Fig. B1), co-occurring with periods of peak eelgrass productivity (Olesen and Sand-Jensen 1994; Zimmerman et al. 1995). Despite intense eutrophication,

eelgrass has experienced a rapid recovery since the early 1980s, tightly correlated with sea otter density and predation (Hughes et al. 2013). As of 2012, eelgrass beds in Elkhorn Slough covered 15 ha which is 60 % of the 1931 pre-commercial agricultural period, and up from an all time low of 2 ha in 1980, 4 years prior to the arrival of sea otters (Hughes et al. 2013). The fastest growing eelgrass beds are expanding at a rate of 8 m year<sup>-1</sup>, and are displacing areas of high macroalgal cover (supplementary information, Fig. B2). In Elkhorn Slough, eelgrass bed recovery has occurred through the new patch formation (recruitment of seedlings) and through the expansion of newly formed and existing beds. The eelgrass-macroalgal boundary is situated in the intertidal zone at -0.5 to 0.0-m mean lower low water (MLLW) and is set by the desiccation tolerance of *Zostera marina* (Boese et al. 2003), limiting its upper boundary, whereas *Ulva* can withstand higher desiccation stress (Gao et al. 2011) and therefore extends higher in the intertidal than eelgrass (Fig. 1; supplementary information, Fig. B2). Both eelgrass and *Ulva* are limited by light towards the lower end of their distribution, yet these light requirements differ (0.5–4 % surface irradiance *Ulva*; 11 % surface irradiance eelgrass) (Sand-Jensen 1988; Duarte 1991; Palacios and Zimmerman 2007), and, therefore, these differences in light requirements are not likely to set the eelgrass-macroalgal boundary, since eelgrass occurs lower in tidal elevation in Elkhorn Slough.

### Sea otter effects at the eelgrass edge

To test for sea otter effects at the edge of eelgrass beds, we conducted a predator exclusion experiment at the rapidly expanding edge of an eelgrass bed in July 2013 (Fig. 1; supplementary information, Fig. B2). We closely modeled this new experiment on one we had conducted in July 2012 at the interior of the eelgrass bed (Hughes et al. 2013). We estimated sea otter densities in this area 1 month prior to and during the experimental periods using weekly shore-based surveys. We counted sea otters occurring in the experimental eelgrass beds every 30 min during the 3-to 4-h surveys ( $n = 9$ ). Previous estimates of crab consumption in the experimental area determined that sea otters were capable of consuming ~8000 crabs ha<sup>-1</sup> year<sup>-1</sup> (Hughes et al. 2013), essentially eliminating predatory crab effects on small invertebrates that graze algal epiphytes from the eelgrass.

We established the caging experiment using a randomized block design that consisted of eight blocks and four treatments: (1) Low otter mimic—cages, including crab and excluding sea otters and other mesopredators (e.g., small fish and other unaccounted for predators) (-otters +crab-mesopredator), (2) High otter mimic—cages excluding sea otters, crabs, and mesopredators

(-otters-crab-mesopredator), (3) Actual otter predation—partial cage control that allowed access to both sea otters, crabs, and mesopredators, yet included the top of the cage to test for shading effects on eelgrass and *Ulva* (+otters+crab+mesopredator), and (4) Actual otter predation or cage-free control (+otters+crab+mesopredator).

Prior to the experiment, we sampled *Ulva* biomass in 50 × 50 cm plots directly adjacent to the experimental plots to test for any pre-experimental differences among treatments and blocks. To standardize eelgrass, along with epiphyte and epigrazer (grazers of algal epiphytes) biomass on the eelgrass, we cut all eelgrass shoots to 20 cm, but above the meristem to ensure growth during the experiment. This allowed us to standardize eelgrass epigrazers and epiphytes by removing most of their biomass. We created a 1 × 1-m buffer zone by cutting all the shoots surrounding the 50 × 50-cm experimental area down to 20 cm to prevent shading by taller bordering shoots. We also severed rhizomes around the experimental cages to minimize the confounding effects of rhizomatic transport of carbon and nutrients. All treatments were spaced 2-m apart, and all blocks were spaced 8–10 m apart. Cages were constructed of a metal rebar frame wrapped with wire mesh with 2.5-cm openings to allow for the recruitment of algal epiphytes and mesograzers, yet restricting movement of most *Ulva* and mesopredators and all top predators. Other important mesopredators at the eelgrass-macroalgal edge were Pacific staghorn sculpin (*Leptocottus armatus*), shiner perch (*Cymatogaster aggregata*), and three-spined stickleback (*Gasterosteus aculeatus*) (Grant 2009), all whose size classes for both juvenile and adult life-stages were generally too large to access the predator exclusion cages. We placed cages directly over the eelgrass-macroalgal boundary, where ~50 % of the caged area was composed of eelgrass shoots and the other 50 % was composed of *Ulva* (supplementary information, Fig. B3). To ensure epigrazers were present during the experiment, we seeded all experimental plots with 20 large (>2-cm length) epiphyte grazers (Taylor's seahare, *Phyllaplysia taylori*), which were similar to naturally occurring densities from the interior (Hughes et al. 2013). Last, we placed one large (>80-mm carapace width—CW) *Cancer* sp. crab in the -otter+crabmesopredator cages. Unlike the Hughes et al. (2013) interior experiment, where one large (>60 mm CW) *Pugettia producta* crab and one large (>80 mm) *Cancer* sp. crab were placed in the -otter +crab-mesopredator cages, we only placed one large *Cancer* sp. crab in the -otter +crab-mesopredator cages for the edge experiment, as previous crab trapping and SCUBA surveys found few *Pugettia* crabs at the eelgrass edge. Although the crab density in the low otter mimic cages was high (4 m<sup>-2</sup>), this number was still similar or considerably lower compared to other predator cage experiments in seagrasses (Heck

et al. 2000; Baden et al. 2010; Lewis and Anderson 2012). Furthermore, crab predation was probably reduced, as we often noticed crabs buried in the sediment, which could have been a predator-mediated response to the high sea otter densities in the experimental area (Lowry and Pearse 1973; Bertness and Coverdale 2013).

The experiment started in July and lasted for 30 days. We maintained cages weekly by scrubbing them free of fouling organisms and drift algae. At the end of the experiment, we counted all shoots in the plots and harvested all of the eelgrass and *Ulva* biomass along with their respective epigrazer and epiphyte assemblages. We counted all epigrazers (>0.5 mm) on the *Ulva* and eelgrass, respectively, in each plot, and separated them into two size classes (small <2 cm length) and large (>2 cm length). All algal epiphytes were scraped from five representative eelgrass shoots from each plot, dried at 60 °C and weighed. For eelgrass, we combined the dry weights of both aboveground and belowground biomasses. Due to the difficulty in harvesting and separating out all live vs. dead eelgrass belowground tissue, we used a relative estimate of belowground biomass by selecting five shoots from each plot, and weighed out standardized 7-cm sections of their rhizome. We used the mean rhizome + root biomass and multiplied it by the final shoot density to estimate the belowground biomass for each plot. We also assessed nutrient concentrations during the experimental period using the Land/Ocean Biological Observatory L01 mooring, located 500 m from the experimental bed, which collects hourly nitrate measurements (Jannasch et al. 2008).

To test for differences among treatments, we compared epigrazer densities (combined from eelgrass and *Ulva*, in density per square meter), eelgrass algal epiphytes (in grams of dry weight per gram of shoot), new eelgrass shoot density (as percentage change), and eelgrass and *Ulva* biomass (in grams of dry weight per square meter) using a randomized blocked ANOVA with treatment (fixed) and block (random) as the dependent variables (SPSS software, version 22). We tested for the assumption of normality for the dependent variables using a Kolmogorov–Smirnov test, and log transformed when appropriate. To conserve the degrees of freedom and test for cage effects between cage controls, we first ran a full randomized blocked ANOVA model with all treatments and compared the control treatments using a Tukey HSD test. If there were no differences between controls (using a cutoff of  $P > 0.25$ ), then, we pooled the controls and reran a reduced randomized blocked ANOVA, and then compared differences among treatments with a Tukey HSD test. Given the challenges of multi-trophic experiments with low replication, we set our minimal detectable effect size to >25 % between treatments, and set  $\alpha$  to 0.10 to avoid type II errors incorrectly failing to reject the null hypothesis (Underwood 1997; Nakagawa and Cuthill 2007).

## Modeling the sea otter trophic cascade at the eelgrass edge

To further develop a mechanistic understanding on how sea otters can affect trophic dynamics at the eelgrass edge and interior, we used Structural Equation Modeling (SEM) (SPSS Amos software, version 23), which is a framework to test hypothesized relationships among variables and accounts for their shared and unique contributions. SEM has become a standard analytical tool in determining causal links in simplified food webs, such as eelgrass communities (Whalen et al. 2013; Alsterberg et al. 2013; Duffy et al. 2015), because it accounts for the unique and shared contributions of factors that can be intrinsically linked, and whose effects can be mediated by consumer pathways (Byrnes et al. 2011). Using results from our edge field experiment, results from the interior (Hughes et al. 2013), and results from previous eelgrass-consumer studies (e.g., Moksnes et al. 2008; Baden et al. 2010; Whalen et al. 2013), we developed a priori hypotheses about the significant pathways connecting sea otter trophic effects to eelgrass biomass and shoot formation. We used results from our 2013 predator exclusion-inclusion experiment at the eelgrass edge and results from the Hughes et al. (2013) experiment at the interior to populate the SEMs. The factors included were the exogenous binary variables of sea otters (0 s for exclusion plots, and 1 s for otter mimics and open plots) and mesopredators (small predatory fish and other unaccounted predators, 0 s for cage enclosures, and 1 s for control plots). For the other endogenous variables, we used the same measures as in the ANOVAs, but also included crabs from the crab inclusion cages whose carapace width was converted to biomass using a power function (Ofstedal et al. 2007), and for the eelgrass edge experiment, we separated out the densities of epigrazers from eelgrass and *Ulva*, respectively, because we were interested in their unique effects on eelgrass as separate paths. We tested for the assumption of normality for all continuous variables using a Kolmogorov–Smirnov test, and log transformed when appropriate.

Using a backward stepwise approach, we first ran our hypothesized SEMs, and reduced them by removing non-significant ( $P > 0.10$ ) pathways with the least support one at a time. Model evaluation was done using a  $\Delta$ AIC (Akaike Information Criterion) approach. We compared hypothetical and reduced SEMs using  $\Delta$ AIC (AIC difference) scores, where scores greater than two points from the model with the lowest AIC score, were considered to not be supported, and the model with the lowest AIC score was selected as the final (Burnham and Anderson 2002). We also tested our hypothetical and reduced SEMs for model fit using a  $\chi^2$  test which tests for differences between observed and estimated covariance matrices,  $\chi^2$  tests with



a  $P > 0.05$  were determined to have a good fit (Byrnes et al. 2011; Alsterberg et al. 2013; Whalen et al. 2013).

Using a nested approach, we used the models to explore the potential mechanisms behind eelgrass expansion by incorporating models with and without *Ulva* and pathways with and without direct crab effects on eelgrass. While we did not observe any indications of herbivory or mechanical destruction of eelgrass by crabs, we conservatively included a potential direct effect in the model. The models without *Ulva* and without crabs, respectively, were compared to full models using model averaging under the context of AIC.

We developed a priori hypotheses based on results from Hughes et al. (2013) at the eelgrass interior, the results presented herein for our edge experiment, as well as previous results from the literature describing consumer dynamics in eelgrass-macroalgal systems (Baden et al. 2010; Whalen et al. 2013) (supplementary information, Fig. B5). First, we developed an SEM for the interior ( $N = 30$  plots) (using results from Hughes et al. 2013) and hypothesized (supplementary information, Fig. B5) that sea otter predation on crabs generated a trophic cascade that enhanced epigrazer densities on the eelgrass leading to decreased algal epiphyte loading and enhanced eelgrass resilience by increasing biomass and shoot densities. We also built in linkages going from eelgrass to epigrazers to hypothesize that a positive feedback from enhanced eelgrass could promote greater epigrazer densities. Next, we developed an SEM for the eelgrass edge ( $N = 32$  plots) without *Ulva* and hypothesized (supplementary information, Fig. B5) that sea otter predation on crabs generates a trophic cascade that enhances epigrazer densities on the eelgrass leading to decreased algal epiphyte loading and enhanced eelgrass resilience by increasing biomass and shoot densities. We also built in linkages from eelgrass to epigrazers to hypothesize that a positive feedback from enhanced eelgrass could promote greater epigrazer densities.

Next, we developed an additional SEM for the edge where we hypothesized (supplementary information, Fig. B5) that if *Ulva* had effects on eelgrass resilience (a function of increased biomass and change in shoot density); then, it was most likely mediated through direct and indirect consumer pathways. Specifically, we hypothesized that sea otters reduce crabs, which can consume *Ulva* and its grazer assemblage, thus leading to increased *Ulva* in the presence of otters. Increased *Ulva* abundance could enhance epigrazer densities, which consume algal epiphytes growing on nearby eelgrass leaves, thus leading to increased eelgrass biomass and shoot formation. In addition, we hypothesized that *Ulva* could directly facilitate eelgrass likely through amelioration of desiccation stress. Alternatively, we hypothesized that *Ulva* could directly smother and shade existing and new eelgrass shoots, thereby negatively

affecting seagrass. Furthermore, we hypothesized that sea otters could simultaneously, through the reduction of crabs, generate a trophic cascade leading to enhanced epigrazer densities on the eelgrass, similar to the one generated in the interior of the bed away from the eelgrass edge (Hughes et al. 2013). For the SEMs, we accounted for the potential effects of other mesopredators (fish and other predators not accounted for in open controls) by including them as epigrazer-reducing pathways in the models. We included the three types of predators (sea otters, crabs, and mesopredators) in the SEM by assigning correlation between their errors based on hypothesized relationships and experimental design, where sea otters negatively correlated with crabs and positively correlated with other mesopredators, and crabs negatively correlated with other mesopredators. For the SEM with *Ulva* included, we also included correlated errors between other mesopredators and *Ulva* biomass, since open cages and plots were accessible to both mesopredators and floating *Ulva* mats.

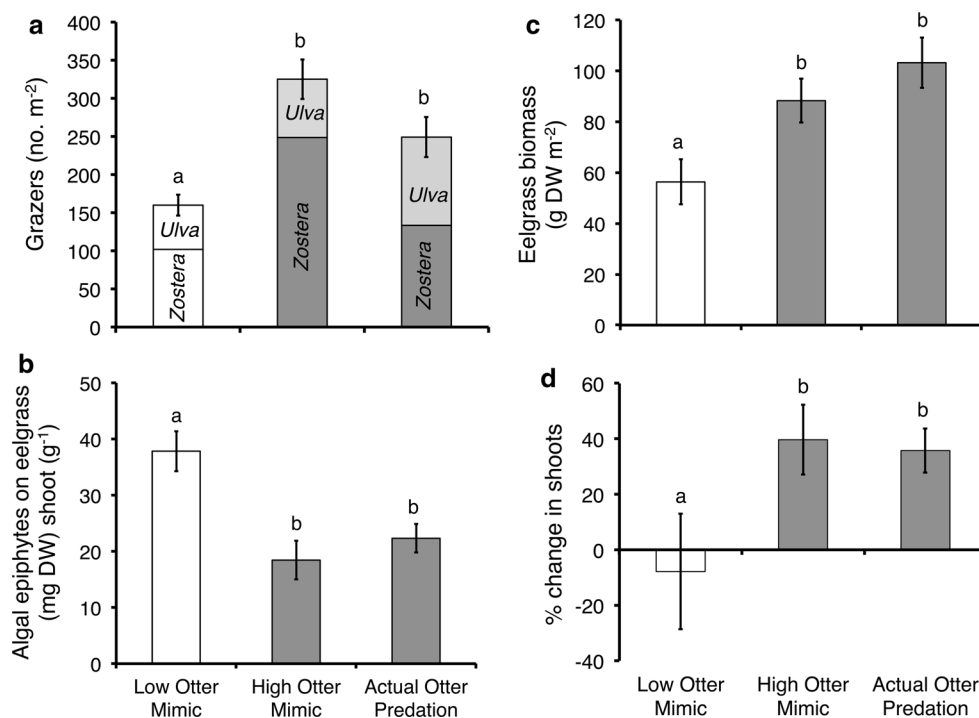
We note the differences and similarities between the interior and exterior experiments, but acknowledge that these experiments were conducted in different years; therefore, we cannot rule out the possibility that differences are due to temporal rather than spatial factors.

## Results

### Sea otter effects at the eelgrass edge

Sea otter density was high (mean =  $2.4 \text{ otters ha}^{-1} \pm 0.9$  SD) during the experimental period at the edge and was comparable to eelgrass beds with high sea otter densities reported by Hughes et al. (2013). We regularly observed otters accessing our experimental cage areas. In addition, nutrient concentrations were high during the experimental period, and often exceeded  $100\text{-}\mu\text{M NO}_3$  (supplementary information, Fig. B4). Nutrient concentrations were greater than background concentrations from the adjacent nearshore, which rarely exceed  $30\text{-}\mu\text{M NO}_3$  (Chapin et al. 2004), and were sufficient to stimulate algal epiphyte growth on the eelgrass leaves as well as growth by *Ulva*. There were no pre-experimental significant differences among treatments and blocks for *Ulva* biomass ( $P > 0.10$ ). However, there were significant differences among blocks ( $F_{7,22} = 2.472$ ,  $P = 0.049$ ) for preliminary shoot densities, yet no differences among treatments ( $P > 0.10$ ); therefore, we standardized the data using the percentage change in shoots as the response variable for the post-experimental analysis.

Our cage experiment at the eelgrass edge showed that sea otters can generate a trophic cascade that enhances epigrazer densities and decreases algal epiphytes. After



**Fig. 2** Results from 30-day field cage experiments at the eelgrass edge testing for the effects of simulated low sea otter predation (i.e., cages, including crab and excluding sea otters;  $n = 8$ ), simulated high sea otter predation (i.e., cages excluding crab and sea otters;  $n = 8$ ), and actual high sea otter predation (which included (i) partial cage control that allowed access to both sea otters, crab, and mesopredators, yet included the *top* of the cage to test for *shading* effects on

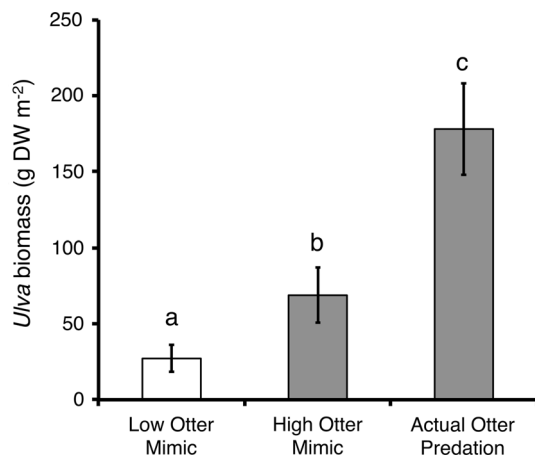
the eelgrass and (ii) cage-free *plots*;  $n = 16$ ) on **a** epigrazer density, **b** algal epiphyte load on the eelgrass, **c** eelgrass biomass, and **d** the % change in shoots. Differences in lettering indicate significant differences based on randomized blocked ANOVA and Tukey HSD tests (supplementary information, Table A1). DW = dry weight. Error bars are  $\pm 1$  SE

1 month, epigrazer densities were significantly greater at the eelgrass edge (80 % increase) (Fig. 2a) in the cages with simulated and actual sea otter predation (supplementary information, Table A1) compared to the low otter mimic treatment. Furthermore, 35 % of epigrazers were found within *Ulva* at the edge (Fig. 2a), indicating that *Ulva* could potentially enhance the epigrazer assemblage for eelgrass. In general, there were a few large (>2 cm) grazers, as only 8 % of epigrazers found on eelgrass at the edge were of the larger size classes, which differed from previous results from the interior, where large size classes dominated the epigrazer assemblage (Hughes et al. 2013). Difference in size classes between the interior and edge could have been due to the mixture of habitats, where the *Ulva* assemblage was dominated primarily by several species of smaller gammarid amphipods, along with the isopods *Idotea resicata* and *I. wosnenskii* and polychaete worms; whereas the eelgrass epigrazer assemblage was dominated primarily by small (<2 cm) *Idotea* spp. and the seahare *Phyllaplysia taylori*. Algal epiphyte loads, composed primarily of diatoms, were significantly lower (46 %) (Fig. 2b) in treatments with actual and simulated sea otter predation compared to the low otter mimic (supplementary information, Table A1).

Finally, our results showed that the sea otter trophic cascade promotes eelgrass health through enhanced biomass at the eelgrass edge. Eelgrass biomass was enhanced (70 %) by the simulated and actual presence of sea otters (Fig. 2c) (supplementary information, Table A1). Changes in shoot densities, a primary indicator of eelgrass resilience, were enhanced by sea otters. Plots with simulated and actual sea otter predation had a net gain in shoots (38 % on average), whereas plots excluding sea otters had a net loss (8 %) (Fig. 2d), indicating that sea otters are promoting bed resilience at the edge.

While the evidence did show benefits from sea otters to eelgrass at the edge, to our surprise, real and simulated otter predation also benefitted macroalgae. There was significantly greater *Ulva* biomass (Fig. 3) in treatments with actual (556 %) and simulated (153 %) sea otter predation at the end of the one-month experiment compared to the otter-free mimic treatment (supplementary information, Table A1). We note that *Ulva* was significantly greater in the actual sea otter predation cages compared to simulated otter predation, likely because actual sea otter cages were open to the accumulation of floating *Ulva*, which we observed to accumulate towards the eelgrass edge at the

end of the experiment. We observed that *Ulva* in general did not form mats that smothered the older eelgrass shoots, but *Ulva* was instead interspersed between older and taller shoots at the edge. On average, *Ulva* biomass exceeded eelgrass biomass in the treatments with actual sea otter predation, indicating either a neutral or positive effect of *Ulva*



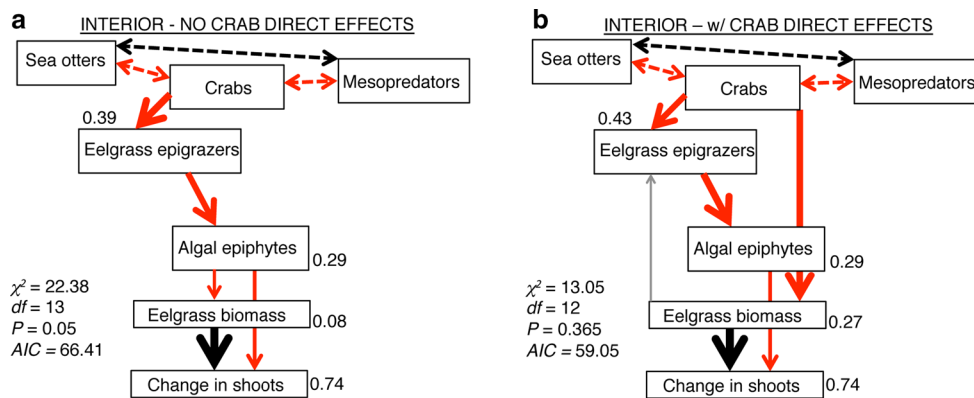
**Fig. 3** Results from a 30-day field cage experiment at the eelgrass edge testing for the effects of simulated low sea otter predation (i.e., cages, including crab and excluding sea otters and mesopredators;  $n = 8$ ), simulated high sea otter predation (i.e., cages excluding crab, sea otters and mesopredators;  $n = 8$ ), and actual high sea otter predation (which included (i) partial cage control that allowed access to both sea otters, crab, and mesopredators, yet included the top of the cage to test for shading effects on the eelgrass and (ii) cage-free plots;  $n = 16$ ) on *Ulva* biomass. Differences in lettering indicate significant differences based on randomized blocked ANOVA and Tukey HSD tests (supplementary information, Table A1). DW = dry weight. Error bars are  $\pm 1$  SE

on eelgrass resilience in the otter treatments. Despite the greater *Ulva* biomass, the macroalgal canopy was shorter (mean = 14.1 cm  $\pm$  5.4 SD) relative to the eelgrass canopy at the end of the experiment (mean = 60.6 cm  $\pm$  11.1 SD), leaving one side of the eelgrass edge exposed to mesopredators.

### Modeling the sea otter trophic cascade at the eelgrass edge

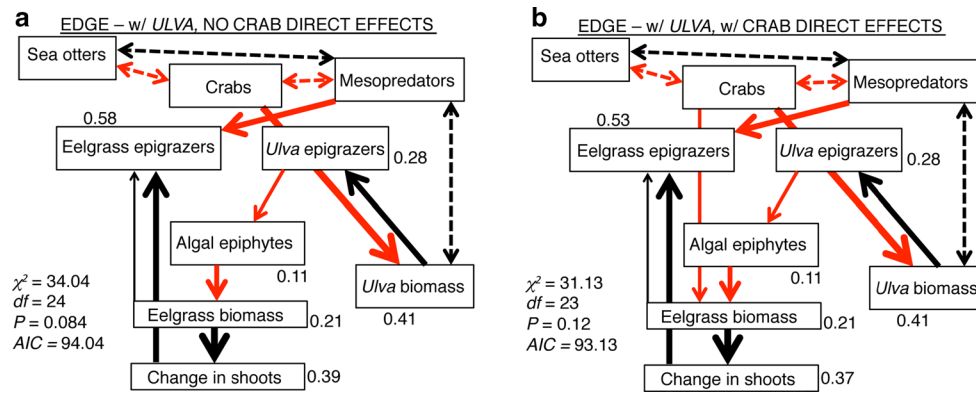
Our SEMs helped elucidate the different pathways by which sea otters can promote eelgrass stability and resilience at both the eelgrass interior and the edge. First, our final SEM model (without *Ulva*) supported our hypothesized pathways (supplementary information, Fig. B5) at the eelgrass interior (Fig. 4a) (supplementary information, Table A2) (Hughes et al. 2013), where the reduction in crabs enhanced the epiphyte grazer assemblage leading to a reduction in algal epiphytes that enhanced both eelgrass biomass and shoot densities, and therefore eelgrass resistance to nutrient loading. However, the most supported interior model included the additional pathway of crabs reducing eelgrass biomass along with eelgrass epigrazers (Fig. 4b).

Applying the same interior model to the eelgrass edge produced poor model fit ( $P < 0.05$ ) (supplementary information, Table A3), there was not a trophic pathway leading to enhanced eelgrass biomass and shoot formation, indicating that alternative pathways affected eelgrass resilience. Our final SEM for the eelgrass edge, including *Ulva* (Fig. 5a, b), identified which paths best fit the observed patterns and supported our model of hypothesized pathways



**Fig. 4** Final SEMs ( $n = 30$  plots) testing for the effects of sea otters on eelgrass resistance and resilience as a function of biomass and change in shoots from the Hughes et al. (2013) eelgrass interior experiment. Model **a** was a nested model without the pathway from crabs to eelgrass, model **b** included that pathway. The non-significant ( $P > 0.05$ )  $\chi^2$  test indicated good model fit for both competing models; however, AIC values indicated that model **b** had the most support. Arrow widths are proportional to standardized regression weights.

Numbers next to endogenous variables indicate  $R^2$ . Grey arrows indicate negative correlations, black lines indicate positive correlations, and dotted grey arrows were non-significant paths in the final model. Dashed double-headed arrows were correlations of error between endogenous variable error terms and exogenous variables. See supplementary information, Table A2 for (a) AIC model selection, (b) model averaging, and (c) coefficient values, standard errors, and standardized path coefficients for nested and full SEMs



**Fig. 5** Final SEMs ( $n = 32$  plots) testing for the effects of sea otters on eelgrass resistance and resilience as a function of biomass and change in shoots from the eelgrass edge experiment. Model **a** was a nested model without the pathway from crabs to eelgrass, model **b** included that pathway. The non-significant ( $P > 0.05$ )  $\chi^2$  test indicated good model fit for both competing models, and AIC values indicated that both models **a**, **b** had good support. Arrow widths are proportional to standardized regression weights. Numbers next to

endogenous variables indicate  $R^2$ . Grey arrows indicate negative correlations, black lines indicate positive correlations. Dashed double-headed arrows were correlation of error between endogenous variable error terms and exogenous variables. See supplementary information, Table A3 for (a) AIC model selection, (b) model averaging, and (c) coefficient values, standard errors, and standardized path coefficients for nested and full SEMs

(supplementary information, Fig. B5). The paths that best supported increased eelgrass biomass and shoot formation were generated by sea otters, where sea otter consumption of crabs enhanced *Ulva* biomass and its associated epigrazer assemblage at the edge, and in turn contributed to the reduction of epiphyte loads on eelgrass leading to greater eelgrass biomass and shoot production (Fig. 5a, b). In addition, the direct effects of crabs on eelgrass biomass also indicated a negative effect; however, this could have been an experimental artifact, as we did not see a significant net reduction in shoots in the low otter mimic cages (Figs. 2, 5b). There was no direct effect of *Ulva* on eelgrass in this model; the algal epiphytes exerted a much stronger direct influence than macroalgae. Unlike the interior of the eelgrass bed (Fig. 5a, b) (Hughes et al. 2013), sea otter consumption of crabs at the edge did not affect eelgrass epigrazers. However, our SEM indicated that mesopredators (most likely fish) at the edge caused declines to epigrazers on the eelgrass, further demonstrating the importance of *Ulva* in maintaining the epigrazer assemblage at the eelgrass edge.

There were five other pathways in our original hypothesis that were non-significant in the model output and thus removed from the final edge model. First, we found no evidence that crabs and other mesopredators directly impacted *Ulva* epigrazers, but instead crabs reduced *Ulva* epigrazers through *Ulva* consumption, likely a result of switching to scavenging when invertebrate prey became limited (MacGinitie 1935). Second, we found no evidence that *Ulva* mass enhanced the eelgrass epigrazer assemblage, but *Ulva*-associated epigrazers did reduce the epiphytes on eelgrass. Therefore, epigrazers associated with *Ulva*

were likely to consume algal epiphytes on eelgrass, but not remain on the eelgrass itself. The larger-sized epigrazer assemblage associated with the eelgrass could explain why smaller *Ulva* epigrazers could be feeding on eelgrass epiphytes, but were not retained in the eelgrass either through competition for space or seeking refuge in the *Ulva* canopy. Finally, the paths leading to direct facilitation of eelgrass by *Ulva* were non-significant and removed from the final SEM.

## Discussion

Here, we have demonstrated that although trophic cascades at the edge of an ecosystem can produce similar end results as in the interior (Hughes et al. 2013), the mechanism can greatly differ. The experiment by Hughes et al. (2013) at the eelgrass interior showed how sea otters can play an important role in maintaining the health of eelgrass by preventing loss of eelgrass shoots; thus, increasing eelgrass resistance to stress. In contrast, in our experiment at the eelgrass edge, we demonstrated that sea otters can enhance eelgrass resilience by promoting *Ulva* and its epigrazer assemblage that are important for removing algal growth on the eelgrass, thus leading to eelgrass expansion. In the short-term, sea otters promoted both types of vegetation, but in the long-term eelgrass appeared to be the stronger competitor, as it expanded into *Ulva* dominated areas (supplementary information, Fig. B2). Surprisingly, these contrasting pathways of trophic control at the edge and interior (Hughes et al. 2013) were operating just tens of meters apart (supplementary information, Fig. B2).



The difference in trophic pathways was likely due to epigrazer limitation at the edge, where fish predation could have been more prevalent (Fig. 2a), as caging experiments from Hughes et al. (2013) suggested that crabs, and not fish, were the important predators in the interior. Furthermore, it has been demonstrated from other seagrass systems that fish predation generally increases towards the edge of patches (Macreadie et al. 2010; Duffy et al. 2013). The modeled mechanism from the interior did not explain patterns in epiphyte loading nor eelgrass responses when applied to the bed edge (Fig. 4a). However, when *Ulva* was included in the edge SEM, this revealed a new pathway by which sea otters enhance eelgrass resilience by increasing *Ulva* and thereby supply of epigrazers. Promoting epigrazers in two overlapping vegetation types could be especially important for eelgrass seedling recruitment and new patch formation, another mode of eelgrass expansion that has occurred in the presence of sea otters (Brent Hughes, pers. obs.), where grazers in these small patches could be much more exposed to predation, since most of the patch could be edge habitat (Macreadie et al. 2010). This example provides insight into how trophic cascades drive both resistance and resilience, and suggests that research in vegetated foundation species, such as kelp forests (Konar and Estes 2003), terrestrial forests, and grasslands (Lidicker 1999) should consider both the dynamics in the interior and exterior.

We have attributed differences between the interior and edge experiments to spatial factors, but given that the experiments were conducted in separate years, we cannot rule out the possibility that differences were due to temporal rather than spatial factors. However, the differences observed are consistent with spatial contrasts (e.g., *Ulva* cover and the herbivore assemblage) between center and edge, and not with any temporal differences observed across years; therefore, it seems likely that contrasts between dynamics at the center and edge are behind the difference in experimental results. Our results are also consistent with other studies examining trophic control of seagrasses; there is mounting evidence of grazer control of epiphytes (Hughes et al. 2004; Whalen et al. 2013; Duffy et al. 2015), and predator control of grazers (Moksnes et al. 2008; Baden et al. 2010; Duffy et al. 2013). Our study further emphasizes the need of large repeatable experiments, such as in Duffy et al. (2015) to elucidate processes at the edge and interior of habitat-forming vegetation.

Few studies have demonstrated the role of top predators in generating trophic cascades at both the interior and edge of habitat-forming vegetation. However, one study showed major spatial differences in consumer assemblages (crabs and snails) and top-down regulation at the edge vs. interior of salt marsh systems (Silliman and Bertness 2002), where differences operated on relatively small spatial scales. Most of the marine examples that have demonstrated trophic

cascades at the vegetation edge come from salt marsh systems, where strong consumer fronts can drive marsh dieback (Altieri et al. 2012; Silliman et al. 2013; Bertness and Coverdale 2013), and therefore are critical for determining resilience in those systems. We demonstrate that species interactions at the edge are important determinants of seagrass bed resilience, while interactions at the interior are important for maintaining stability and resistance to disturbance (Hughes et al. 2013).

The sea otter trophic cascade at the eelgrass edge sheds light on seagrass-macroalgal interactions. Surprisingly, the sea otter-driven trophic cascade enhanced both competing vegetation types, eelgrass, and macroalgae, yet this did not result in negative interactions between the two. Recent evidence has emerged that macroalgal blooms can, at times, have neutral or positive effects on seagrass performance (Hessing-Lewis et al. 2011; Thomsen et al. 2012), contrary to the long-standing paradigm (Letts and Adeney 1908; Valiela et al. 1997; Nelson and Lee 2001; Orth et al. 2006; Burkholder et al. 2007). Despite competitive interactions that may exist between macroalgae and seagrass, these neutral/positive effects of macroalgae on seagrass occur when there are enhanced epiphyte grazers in seagrass due to trophic control (Baden et al. 2010). Furthermore, drift macroalgae have been shown to indirectly facilitate seagrasses through the delivery of important epigrazers that preferentially feed on algal epiphytes growing on seagrass leaves, thus reducing seasonal dieback (Whalen et al. 2013). There is also emerging evidence that trophic structure, especially the variation in relative abundance of mesopredators and top predators, greatly influences epigrazer populations and their ability to control algal epiphytes and the persistence of seagrass (Moksnes et al. 2008; Baden et al. 2010; Hughes et al. 2013; Reynolds et al. 2014). Therefore, trophic structure has the potential to dictate the direction and interaction of regime shifts between macroalgae and seagrass in anthropogenically nutrient-loaded systems.

Recovery of degraded ecosystems without direct restoration intervention is relatively rare. Determining the mechanisms that enhance such resilience of imperiled ecosystems in the face of environmental changes can inform the management of collapsing systems. Results from our study, combined with results from Hughes et al. (2013), have demonstrated how the recovery of endangered sea otters can restore food webs and increase both the resistance and resilience of seagrass beds under threat from overgrowth of epiphytic algae and macroalgal blooms fostered by anthropogenic nutrient loading. Despite the widespread trophic downgrading of global ecosystems (Estes et al. 2011), there is hope for systems where the conservation (Heithaus et al. 2014) or restoration (Hughes et al. 2013) of top predator populations can result in a trophic upgrade that benefits the ecosystem.

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