Recovery of a top predator mediates negative eutrophic effects on seagrass

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A fundamental goal of the study of ecology is to determine the drivers of habitat-forming vegetation, with much emphasis given to the relative importance to vegetation of "bottom-up" forces such as the role of nutrients and "top-down" forces such as the influence of herbivores and their predators. For coastal vegetation (e.g., kelp, seagrass, marsh, and mangroves) it has been well demonstrated that alterations to bottom-up forcing can cause major disturbances leading to loss of dominant vegetation. One such process is anthropogenic nutrient loading, which can lead to major changes in the abundance and species composition of primary producers, ultimately affecting important ecosystem services. In contrast, much less is known about the relative importance of apex predators on coastal vegetated ecosystems because most top predator populations have been depleted or lost completely. Here we provide evidence that an unusual four-level trophic cascade applies in one such system, whereby a top predator mitigates the bottom-up influences of nutrient loading. In a study of seagrass beds in an estuarine ecosystem exposed to extreme nutrient loading, we use a combination of a 50-y time series analysis, spatial comparisons, and mesocosm and field experiments to demonstrate that sea otters (*Enhydra lutris*) promote the growth and expansion of eelgrass (*Zostera marina*) through a trophic cascade, counteracting the negative effects of agriculturally induced nutrient loading. Our results add to a small but growing body of literature illustrating that significant interactions between bottom-up and top-down forces occur, in this case with consequences for the conservation of valued ecosystem services provided by seagrass.

eutrophication | food web | estuary | resilience

Understanding the relative influence of "bottom-up" vs. "top-down" forces on vegetated assemblages has long been an important conceptual goal of the study of ecology (1–4). As many vegetated habitats have declined globally in past decades (5–8), with concurrent losses of valued ecosystem services, investigations of drivers of vegetation sustainability have also taken on applied significance and urgency in conservation science (9, 10). Human activities have altered bottom-up forces, for instance by increasing nutrient availability (11, 12), and top-down forces, by hunting and fishing of top predators (5, 13). Detecting the relative role of such alterations and interactions between them is critical for supporting key vegetated habitats and their ecosystem services.

Investigations of bottom-up and top-down forces in a single system can be challenging. Changes at the top of food webs have been demonstrated to affect vegetation in a diversity of ecosystems (5, 13–15). However, apex predators have been depleted or lost entirely across most of the natural world (5, 13), including many near-shore marine systems (5, 16). It is difficult to understand ecosystem-level effects of an apex predator if it is extremely rare or absent (17). Nearshore systems lacking apex predators have often undergone conspicuous changes in bottom-up forces resulting from human activities, so attention has focused on these latter changes, rather than on a potential role for apex predators or for interactions between top-down and bottom-up changes to the ecosystems. The few studies that have successfully investigated the relative importance of bottom-up and top-down factors on dominant vegetation over ecosystem scales have determined that strong interactions can occur (3, 18, 19).

Seagrasses are a globally distributed group of marine angiosperms that provide valued ecosystem services, such as fueling secondary production, creation of habitat for many other species (9), shoreline protection, and carbon sequestration from the surrounding seawater and overlying atmosphere (8, 10). Seagrass beds have declined in many regions of the world, often because of the smothering effects of algal epiphytes that are enhanced by nutrient loading (8, 20, 21). Furthermore, top-down consumer control, via mesograzers and small predators, has also been established as an important factor in regulating the interaction between seagrass and their algal competitors, especially in elevated nutrient loading and eutrophic conditions (16, 22–27). Mediation of competitive interactions between primary producers is directly controlled by herbivores, which have consistently demonstrated preferential consumption of algal epiphytes vs. seagrasses (27–30), thus benefitting rather than harming the dominant primary producer. Additionally, there is strong evidence from cage experiments that intermediate predators (such as fish and crabs) are capable of regulating grazer assemblages in seagrass beds (22, 23, 25, 26), leading to a trophic cascade that mediates the competition between seagrass and their epiphytes. Seagrass ecosystems thus provide an opportunity to examine bottom-up and top-down forces, and the interaction between them.

Significance

Many coastal vegetated ecosystems have declined, affected by human alterations to "bottom-up" forces such as nutrient loading from agriculture and by "top-down" forces such as overfishing of predators. Examining the interactions between such bottom-up and top-down changes is challenging, because top predators have disappeared from many of these ecosystems. A highly nutrient-loaded estuary in California recently colonized by a recovering sea otter population provided an unusual opportunity to examine these interactions. We demonstrate that top-down effects of sea otters mitigate negative effects of nutrient loading, enhancing growth of seagrass. Grazers that remove algae from seagrass are favored by decreased crabs resulting from otter predation. Recovery of top predators can thus support resilience of coastal vegetated ecosystems.

Recovery of top predator populations has the potential to restore trophic structure and ecosystem function to degraded ecosystems. We found an ideal study system to examine the potential role of recovering apex predators in mediating bottom-up effects, a nutrient-loaded and eutrophic estuarine ecosystem supporting eelgrass (*Zostera marina*) and recovering sea otters (*Enhydra lutris*). Sea otters are keystone species capable of structuring nearshore communities (kelp forests and soft-bottom) through their high predation pressure (31–33). We used a 50-y time series tracking ecosystem degradation and recolonization by sea otters, spatial comparisons between sites with varying sea otter predation and nutrient loading, and manipulative mesocosm and field experiments to investigate the interaction between bottom-up forces and a recovering top predator population.

Results and Discussion

Study System and Historical Trends. Elkhorn Slough is a highly nutrient-loaded (Fig. 1 *A* and *B*) and eutrophic (34) estuary on the central coast of California. The adjacent watershed is dominated by an agricultural landscape. Annual fertilizer sales in the watershed region increased from 200 tons nitrogen in the 1930s to 30,000 tons in 2005, which has resulted in an exponential increase in nutrient concentrations in Elkhorn Slough through time ($P < 0.0005$, $R^2 = 0.90$; Fig. 1*A* and Table S1*A*). We calculate that the current nutrient load to the Elkhorn Slough estuary is 407 kg N·ha^{-1}·y^{-1} (Table S1*B*), a load surpassing that of most global coastal waters considered highly eutrophic (20, 21, 35).

Our time-series analyses revealed remarkable expansion displayed by eelgrass in face of extreme nutrient loading (Fig. 1*A*) and concurrent loss of the adjacent salt marsh (36), which has been demonstrated to buffer the harmful effects of nutrient loading and eutrophication (20). Increases in nutrient concentrations as early as the 1970s (mean NO_3, 16.2 μM) began to exceed baseline levels reported from the 1920s (mean NO_3, 0.5 μM) (37) and concentrations from adjacent ocean sources (mean NO_3, 5.0 μM) (38). Nutrient concentrations more than doubled from 1971 (mean NO_3, 13.1 μM) to 1977 (mean NO_3, 29.6 μM). This increase in nutrients coincided with declines in eelgrass bed extent from 1965 to 1984 (Fig. 1*A*). However, the expected decline in eelgrass has reversed twice during the past three decades, in the first instance following initial recolonization of Elkhorn Slough by sea otters, and in the second instance following a sharp increase in otter abundance after a period of lower numbers. Before sea otters first colonized in 1984, eelgrass was at an all time low (2 ha), and nutrient concentrations, although still high, were an order of magnitude lower than the most recent period of eelgrass recovery (Fig. 1*A*). The otter density following the initial colonization was lower than the more recent period, yet their effect was probably sufficient to promote expansion of eelgrass in lower nutrient conditions, as sea otters are capable of greatly reducing their prey populations (i.e., crabs) in short time periods (<3 y) (39). Sea otter densities were significantly correlated with extent of eelgrass ($P < 0.019$, $R^2 = 0.52$; Table S1*C*), and since the initial sea otter recolonization in 1984, eelgrass bed extent increased by 600% (Fig. 1*A*). In a global context (Fig. 1*B*), this expansion of eelgrass in the setting of severe nutrient loading is anomalous; empirical evidence from other estuaries as well as modeling (20, 21, 35) predicts that Elkhorn Slough should have undergone dramatic seagrass loss, not expansion. However, following the most recent period of sea otter decline (2000–2004; Fig. 1*A*), the relationship between nutrient loading and seagrass loss was much closer to the model prediction from estuaries worldwide (Fig. 1*B*).

If a sea otter-driven trophic cascade was contributing to the expansion of eelgrass beds, we hypothesized that the most likely trophic link between otters and mesograzers would be crabs, which are a common prey item for sea otters (40), and are the primary intermediate predator in sea otter diets. We examined otter foraging data from the past decade and determined that crabs of all species comprised 52% of the total diet of sea otters foraging on or near eelgrass beds in Elkhorn Slough, with crabs
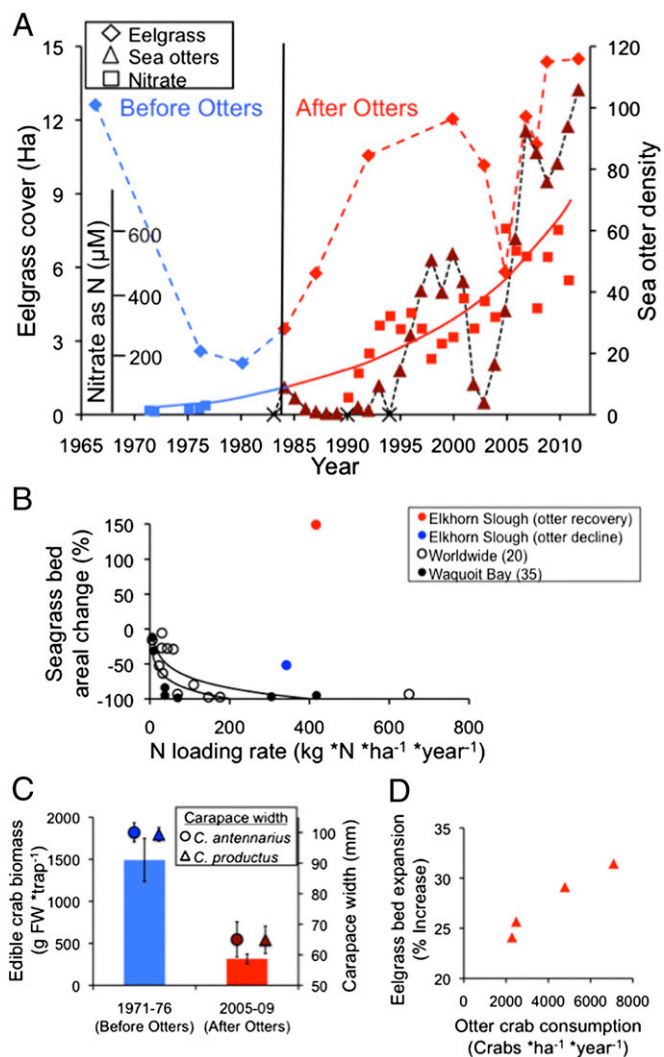

Fig. 1. Historical analysis of nutrients, eelgrass, sea otters, and crabs in Elkhorn Slough. (*A*) Fifty years of eelgrass declines and expansion driven by bottom-up nutrient loading and top-down sea otter-driven trophic cascade effects. Nitrate data ($N = 28$) represent the annual mean, and the solid line is a an exponentially modeled linear function of the entire data series (1971–2011; Table S1*A*). Sea otter ($N = 30$) and eelgrass ($N = 13$) data are represented by dotted lines to visually show trends. (*B*) Metaanalysis showing the relationship between land-derived nitrogen loads entering estuaries and percent change of seagrass estimated from areal surveys. Worldwide and Waquoit Bay, MA (an estuary with varying nutrient loading), data are redrawn from Burkholder et al. (21) (with permission from the publisher). Elkhorn Slough data (Table S1*B*) are not included in the log-linear relationship (solid lines), but are plotted for periods following sea-otter decline (2000–2004), and sea-otter recovery (2005–2010) to demonstrate departure from the model. (*C*) Results from crab surveys a decade before sea otter colonization (1971–1976) and two decades after sea otter colonization (2005–2009; Table S1*D*; *Methods* provides a sample size description). (*D*) Eelgrass bed expansion (2006–2012) at eelgrass beds ($n = 4$) in Elkhorn Slough (calculated as the percent increase in cover of eelgrass as a function of available eelgrass habitat, measured in hectares; Fig. S3) correlated with estimated sea otter predation on crabs in standardized 1-ha plots (Fig. S2) in each bed (Table S1*E*).

from the genus *Cancer* making up 43% of the sea otter diet (Fig. S1). Sea otters are well known to limit populations of their macroinvertebrate prey, including crabs (41), and thus we predicted that the expansion of otter populations in the estuary should have resulted in negative impacts on crabs. Indeed, we detected a significant decline in the biomass ($P < 0.0005$) and

size of crabs in the estuary (*Cancer antennarius*, $P < 0.0005$; and *Cancer productus*, $P < 0.0005$; Fig. 1*C*). Sea otters were most likely to cause declines in crab populations because sea otters were expanding during a period when other crab predators, namely sharks and rays, were in a state of decline, in part as a result of overfishing from four decades (1951–1995) of annual "shark derbies" (42). Additionally, leopard sharks (*Triakis semifasciata*), one of the most abundant top predators in the estuary, experienced a diet shift from crabs before otter colonization to fat innkeeper worms (*Urechis caupo*) after sea otter colonization, indicating an overall decline in crab availability (43). Furthermore, crab harvesting in Elkhorn Slough has declined in the past two decades compared with the 1970s when crab harvesting was common (44) and, in 2007, most of the estuary was declared a Marine Protected Area, thus eliminating all crab harvesting in and around the eelgrass beds. The offshore "rock crab" fishery, which includes both *C. antennarius* and *C. productus*, is a relatively small fishery compared with the much larger Dungeness crab (*Cancer magister*) fishery, and yielded only an average of 3,000 kg annually from 1960 to 2010 (45) and peaked in 1989 when eelgrass was in a period of recovery (Fig. 1*A*). The decrease in populations of other top predators and the lack of overharvesting of crabs in and around the estuary all suggest that the observed decline in *Cancer* crab biomass and size in Elkhorn Slough was a a result of sea otter predation.

To more closely examine the potential relationship among otters, crabs, and eelgrass, we quantified otter predation on crabs in each eelgrass bed in Elkhorn Slough from 2006 to 2012 (*SI Methods*) and correlated it with eelgrass bed expansion (i.e., percent increase in eelgrass cover) after recovery from the most recent decline (2000–2004) in which >50% of eelgrass was lost. Eelgrass expansion during the ensuing 6-y period was positively correlated with sea otter predation on crabs ($P = 0.021$, $R^2 = 0.96$; Fig. 1*D* and Figs. S2 and S3).

Combining results from historical analyses on the relationship among otters, crabs, and seagrass with previous published results on the control of algal epiphytes on seagrass by mesograzers (22–30) generated a hypothesized mechanism by which sea otters mediate bottom-up effects on seagrass. In our conceptual model, a four-level trophic cascade modulates negative algal epiphyte effects on eelgrass, with sea otters controlling intermediate predator crab populations, thereby releasing mesograzers from predation and enhancing their grazing effects on algal epiphytes (Fig. 2*A*).

Spatial Comparisons. To examine the importance of sea otters in estuarine eelgrass beds, we compared properties of eelgrass beds between Tomales Bay and Elkhorn Slough, CA, which are similar in many physical (46) and biological attributes but differ in the presence of sea otters and nutrient loading. Nitrate concentrations are lower in Tomales Bay (0–23 µM) (47) than they are in the eutrophic (34, 48) Elkhorn Slough (10–600 µM). Elkhorn Slough presently supports as many as 120 otters, but sea otters have yet to recolonize Tomales Bay. The reason for this difference is historical accident: southern sea otters recovered from a remnant population in central California after near extermination from the maritime fur trade industry. The current northern range extent is at Pigeon Point, ~185 km south of Tomales Bay as the otter swims (49), thus precluding the use of Tomales Bay by sea otters in the present day. However, prehistoric midden site records indicate that sea otters were once common in estuaries along the entire central California coast, including the Tomales Bay region (50).

We systematically sampled both estuaries for eelgrass aboveground and belowground biomass, algal epiphyte load, grazer biomass and density, and crab biomass and size. Eelgrass beds in Elkhorn Slough had significantly lower crab biomass ($P = 0.034$) and size [for both of the common large crab species, *C. antennarius* ($P = 0.034$) and *C. productus* ($P = 0.009$); Fig. 2*B*] and greater aboveground eelgrass biomass ($P = 0.035$) than Tomales Bay (Fig. 2*E*), as predicted for the estuary with otters present
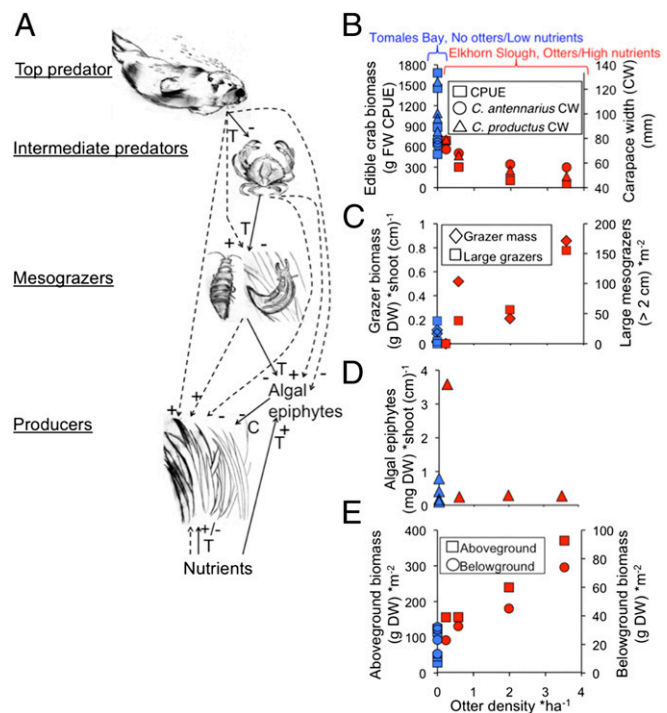

Fig. 2. (*A*) Interaction web of top-down and bottom-up effects in the eelgrass study system. The top predator is the sea otter (*E. lutris*), the mesopredators are crabs (*Cancer* spp. and *Pugettia producta*), the epiphyte mesograzers are primarily an isopod (*I. resecata*) and a sea slug (*P. taylori*), and algal epiphyte competitors of eelgrass primarily consist of chain-forming diatoms, and the red alga *Smithora naiadum*. Solid arrows indicate direct effects, dashed arrows indicate indirect effects, and the plus and minus symbols indicate positive and/or negative effects on trophic guilds and eelgrass condition. C, competitive interaction; T, trophic interaction. (Original artwork by A.C. Hughes.) (*B–E*) Survey results testing for the effects of sea otter density on eelgrass bed community properties (Tables S2 and S3). Elkhorn Slough (sea otters present and high nutrients) eelgrass beds ($n = 4$) are coded in red, and the Tomales Bay reference site (no sea otters, low nutrients) beds ($n = 4$) are coded in blue. (*B*) Crab biomass and size structure of two species of *Cancer* crabs; (*C*) grazer biomass per shoot and large grazer density; (*D*) algal epiphyte loading; and (*E*) aboveground and belowground eelgrass biomass. DW, dry weight; FW, fresh weight.

(Table S2). Crab biomass and sizes for Tomales Bay (Fig. 2*B*) were similar to those for Elkhorn Slough before the otter recolonization (Fig. 1*C*), further indicating that otters are controlling crab populations in Elkhorn Slough. Eelgrass belowground biomass, epiphyte loading, grazer biomass, and large mesograzer density (*Phyllaplysia taylori* and *Idotea resecata* >2 cm, the size class most likely to be consumed by crabs; Fig. 2 *C–E*) did not significantly differ between estuaries between Tomales Bay and Elkhorn Slough, but varied in the direction predicted by our model (Fig. 2*A*).

Remarkably, comparisons between Tomales Bay and Elkhorn Slough indicated that eelgrass can perform equally, if not better, in nutrient-loaded and eutrophic conditions (34) (Movie S1). High spatial variation of crabs, grazers, epiphytes, and eelgrass abundance characterize Elkhorn Slough, indicating the potential for a gradient in the key forcing processes (Fig. 2 *B–E*). Our analyses indicate that sea otters are a key driver of this variation (Table S3). Otter density across eelgrass beds within Elkhorn Slough was negatively correlated with crab biomass ($P = 0.043$, $R^2 = 0.92$) and size (*C. antennarius*, $P = 0.040$; $R^2 = 0.92$; and *C. productus*, $P = 0.061$, $R^2 = 0.88$; Fig. 2*B*). Large mesograzer density varied positively and significantly ($P = 0.041$, $R^2 = 0.92$; Fig. 2*C*) with increased sea otter density. Although the sea otter density gradient was not significantly correlated with grazer

biomass (Fig. 2C), the covarying trend was in the predicted direction (Fig. 2A). Algal epiphyte loads on seagrass significantly decreased with increased sea otter density ($P = 0.025$, $R^2 = 0.77$; Fig. 2D). Finally, eelgrass shoot density ($P = 0.003$, $R^2 = 0.99$), aboveground biomass ($P = 0.012$, $R^2 = 0.98$), and belowground biomass ($P = 0.013$, $R^2 = 0.97$; Fig. 2E) significantly increased with higher sea otter density.

Mesocosm and Field Experiments. To test the proposed mechanisms underlying the individual links in our ecological model (Fig. 2A), we conducted a series of mesocosm and field experiments. The mesocosm experiment supported the postulated food web links among crabs, mesograzers, epiphytes, and eelgrass. Mesocosms simulating low otter predation had decreased overall sea slug biomass and increased large (*P. taylori* >2 cm) sea slug mortality through observed predation by crabs (Fig. 3A), which led to increased algal epiphyte loads (Fig. 3B) and a net loss in eelgrass biomass and reduced rhizome elongation (Fig. 3C and Table S4). The reduced mortality rate of large sea slugs in the treatment mimicking high sea otter predation suggested that smaller crabs are inefficient predators, thereby releasing mesograzers from predation and increasing grazing efficiency.

We verified the underlying mechanism of the sea otter-driven trophic cascade effects on eelgrass by using a field cage experiment that tested for (*i*) no sea otter predation (crab inclusion), (*ii*) simulated sea otter predation (crab and otter exclusion), and (*iii*) actual sea otter predation (crabs and otters included). After 1 mo, grazer biomass and large grazer density (*P. taylori* and *I. resecata* >2 cm, the size class most likely to be consumed by crabs) were significantly greater (Fig. 3D) in the cages with simulated and actual sea otter predation (Table S5). As predicted, algal epiphyte loads were significantly lower (Fig. 3E), and aboveground and belowground eelgrass biomass (Fig. 3F) as well as shoot density (Table S5) was significantly greater in treatments with actual and simulated sea otter predation.

Conclusion

Taken together, these lines of evidence strongly indicate that complex top-down effects of sea otter predation have resulted in positive benefits to eelgrass beds, mitigating the effects of continuing and increasing nutrient loading in Elkhorn Slough. Our findings add to a growing body of literature in seagrass ecology (22–30) that highlights the importance of consumer controls in regulating the conflict between seagrasses and their algal epiphytes. In this case, the addition of an apex predator mediates species interactions at the base of the food web and counteracts the negative effects of anthropogenic nutrient loading. Our findings depart from a view of nature built largely around bottom-up control, which has been the dominant predictor in explaining seagrass loss for more than three decades (12, 20, 21, 35, 51, 52).

Here we have demonstrated that sea otters initiate a trophic cascade in estuarine ecosystems superficially similar to that in the more familiar kelp forest model (31, 33): in both cases, increases in sea otters result in increases in the dominant, habitat-forming coastal vegetation. However, the mechanism by which sea otter predation supports vegetated habitat differs fundamentally between kelp forests and estuaries: the estuarine trophic cascade involves four trophic levels, not three. The explanation for this apparent anomaly lies in two details of the natural history of the estuarine autotrophs and their herbivores: the herbivores preferentially feed on epiphytic algae vs. eelgrass, and the epiphytic algae can harm eelgrass through shading effects in the absence of herbivory (Fig. 2A). These indirect effects may be particularly pronounced in nutrient-loaded systems, which foster ephemeral algal growth. More broadly, multilevel trophic cascades involving indirect effects may be particularly important in systems with strong alteration of bottom-up controls (3, 16). Our findings highlight the importance of unraveling the potentially interactive nature of these key ecological processes when assessing the drivers of vegetated ecosystems.

Methods

Historical Trends. To detect correlations between eelgrass cover and bottom-up and top-down forces, we synthesized data from a variety of sources. We determined trends in the bottom-up influences on the Elkhorn Slough eelgrass beds by constructing a time series of nutrient concentrations in Elkhorn Slough. All samples were collected in the lower part of the estuary adjacent to the historical and present day distribution of eelgrass and sea otters. Surface
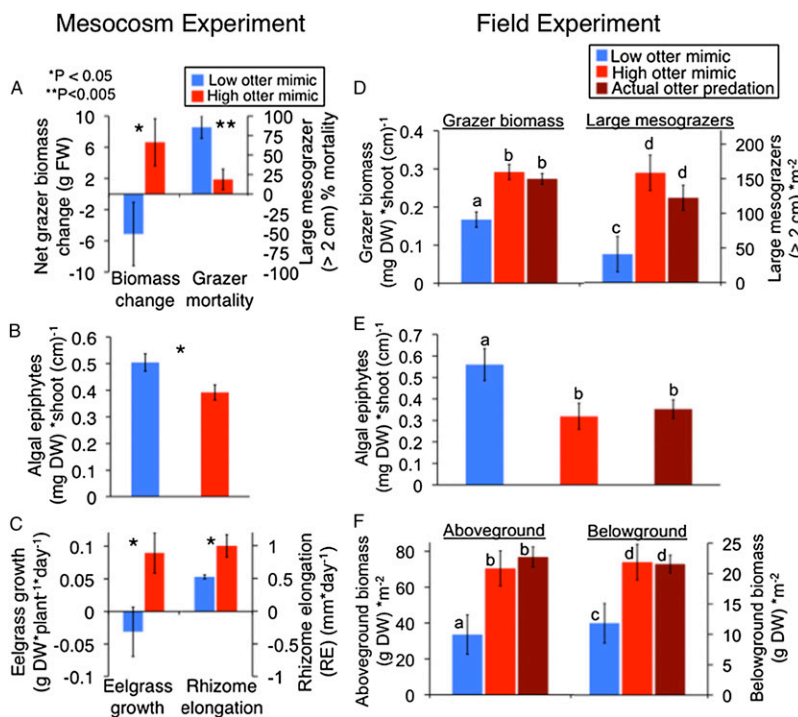

Fig. 3. Results from a 30-d mesocosm experiment (*A–C*) comparing the effects of simulated low (large crab treatment; *n* = 8) and high (small crab treatment; *n* = 7) sea otter predation on (*A*) net change in grazer biomass and grazer mortality, (*B*) algal epiphyte load, and (*C*) eelgrass growth and rhizome elongation (Table S4). (*D–F*) Results from a 30-d field cage experiment (Fig. S5) testing for the effects of simulated low sea otter predation (i.e., cages including crabs and excluding sea otters; *n* = 6), simulated high sea otter predation (i.e., cages excluding crabs and sea otters; *n* = 8), and actual high sea otter predation (which included (*i*) partial cage control that allowed access to both sea otters and crabs yet included the top of the cage to test for shading effects on the seagrass and (*ii*) cage-free plots; *n* = 16) on (*D*) grazer biomass and large grazer density, (*E*) algal epiphyte load, and (*F*) aboveground and belowground eelgrass biomass. Differences in lettering indicates significant differences based on randomized blocked ANOVA and Tukey HSD tests (Table S5). DW, dry weight; FW, fresh weight. Error bars are ±1 SEM.

water samples were collected monthly by hand and analyzed for nitrate as nitrogen (in micromolars; *SI Methods*). We modeled the increase in nitrate concentrations by correlating the year to the mean annual nitrate concentration ($N = 28$) by using regression analysis.

We mapped eelgrass cover in the estuary and quantified change through time by interpreting low altitude vertical aerial imagery acquired between 1966 and 2012. We only used years ($N = 13$) through which eelgrass cover could be determined with high confidence based on historical descriptions and recent ground surveys of distribution (*SI Methods*). To determine the long-term trends in sea otter densities in Elkhorn Slough, we used the standardized biannual census counts from the US Geological Survey (USGS) (www.werc.usgs.gov). This database has summarized sea otter abundance in Elkhorn Slough from 1-d surveys in the spring and fall from 1985 to 2012. Sea otters first entered Elkhorn Slough in 1984, so, for this year, we used a study by Kvitek et al. (32) to estimate the number of otter arrivals in the estuary. To determine the relationship between sea otter abundance and eelgrass cover, we used regression analysis by correlating eelgrass cover for all available years during the sea otter expansion period (1984–2012) with the mean annual sea otter density ($N = 10$).

We summarized land-derived nutrient loads from 2004 to 2012 and percent change in eelgrass during the most recent period of sea otter decline (2000–2004) and otter recovery (2005–2012). The nitrate load to Elkhorn Slough was determined from hourly measurements of nitrate concentration and water depth at the Land/Ocean Biogeochemical Observatory L01 mooring near the mouth of Elkhorn Slough (53). The volume flux past the mooring each hour was determined from the change in water depth and the observed bathymetry of the system above the mooring. The accuracy of these volume fluxes was independently assessed by comparison with a long-term set of Acoustic Doppler Current Profiler data collected at the L01 mooring (54). The total nitrate flux was then determined from the volume flux times the observed nitrate. The nitrate load from terrestrial sources was estimated as the volume flux times the fraction of any observed nitrate concentration greater than 30 μM. The 30-μM threshold was chosen because nitrate in surface waters of Monterey Bay never exceeds this value (55). The nitrate load from terrestrial sources is a minimum estimate because it ignores any nitrate from terrestrial sources when nitrate concentrations are less than 30 μM. However, the load estimated for terrestrial sources is 66% of the total load and cannot be seriously in error because there is also a nonnegligible load from ocean sources. The final annual load values were calculated by dividing nitrogen load (in kilograms) by the total wetland area (in hectares) for Elkhorn Slough (36). Finally, we used the mean nitrogen load from 2004 to represent the most recent period of eelgrass and sea otter decline, and the mean from 2006 to 2012 to represent the most recent period of eelgrass and otter recovery for a global comparison with other estuaries (20, 21, 35).

We tested for the effects of long-term otter predation on the Elkhorn Slough crab population by comparing two time periods: 1971 to 1976 (a decade before otter immigration) and 2005 to 2009 (two decades after otter immigration). Data were collected from a similar region in the lower part of the estuary directly adjacent to the present-day and historical distributions of eelgrass and sea otters (*SI Methods*). We calculated crab biomass caught in standardized crab traps by converting the carapace width values of each crab to an edible biomass by using a power function (56), and summed up the total biomass for each trap. To ensure independence among samples, we used the mean crab mass per trap per day [$n = 17$ (1971–1976), $n = 26$ (2005–2009)] and mean daily carapace width for the two most abundant crab species, *C. antennarius* [$n = 14$ (1971–1976), $n = 12$ (2005–2009)] and *C. productus* [$n = 14$ (1971–1976), $n = 11$ (2005–2009)]. We compared crab biomass and size among the two time periods by using an independent-samples t test.

We estimated eelgrass bed expansion within Elkhorn Slough as the percent change in eelgrass cover (in hectares) from 2006 to 2012 as a function of otter predation over the same survey period. Georeferenced aerial imagery from May 24, 2006, and May 5, 2012, was used to conduct object-based classification of the surface area extent of eelgrass beds (Fig. S3). Areas of suitable habitat for eelgrass were spatially delineated by using high-resolution (2 m) multibeam bathymetry from 2005 and 2011 and aerial Light Detection and Ranging (2 m) from 2004 and 2011 to create continuous digital elevation models in ArcMap version 10.1 (Environmental Systems Research Institute, Redlands, CA). To measure crab predation by sea otters, we used observational data on sea otter foraging collected between 1999 and 2012 by field staff of the Monterey Bay Aquarium and USGS. This data set comprised >10,000 observed feeding dives recorded from tagged and untagged sea otters feeding in the main channel of Elkhorn Slough. We analyzed these data by using a previously described Monte Carlo simulation algorithm for

estimating prey-specific consumption rates from observational data while accounting for sampling uncertainty (57). By multiplying the mean estimated consumption rate by the average density of otters in each eelgrass bed (Fig. S2), we calculated the rate of crab predation (in crabs per hectare per year) in each of the four eelgrass beds (*SI Methods*). Eelgrass bed expansion was calculated by subtracting the percent coverage of eelgrass within the available habitat in 2006 by the percent coverage of eelgrass within the available habitat in 2012 for each of the four eelgrass beds (Fig. S3). We used linear regression to determine the relationship between eelgrass bed expansion as a function of sea otter predation ($n = 4$).

Spatial Comparisons. To determine eelgrass condition and community structure at eelgrass beds with varying sea otter densities, we sampled across 100-m transects at the only four large beds in Elkhorn Slough (36° 48′ 45″ N, 121° 46′ 10″ W; Fig. S2) and four Tomales Bay beds (38° 11′ 53″ N, 122° 56′ 30″ W). All transects bisected the central portion of each bed as well as the standardized 1-ha sea otter foraging/crab survey area (as detailed later; although Tomales Bay had no sea otter surveys, as none were present). Elkhorn Slough eelgrass beds were sampled in July and August 2012, and Tomales Bay beds were sampled in August 2012. At each bed, we systematically sampled eelgrass every 10 to 12 m by using 0.50 × 0.50-m quadrats. Within each quadrat ($N = 8$), we counted all eelgrass shoots and collected five shoots along with >7 cm of their rhizome and root material. All shoots were scraped free of algal epiphytes and all grazers were removed and counted. All grazers, epiphytes, and eelgrass were dried at 60 °C for 24 h and weighed.

We quantified crab densities, biomass, and sizes at Elkhorn Slough and Tomales Bay. At Elkhorn Slough, a single crab trap was placed in each of the four eelgrass beds during the month of July 2012. The same method was used to sample crabs at the four eelgrass beds in Tomales Bay for 1 wk in August 2012. We calculated the catch per unit effort (CPUE) for each daily trapping effort by converting the carapace width values of each crab to an edible biomass using a power function (56), and summed up the total biomass for each CPUE. CPUE was standardized to the total soak time (in hours) for each daily sampling effort. The mean CPUE and mean daily carapace width for the two most abundant crab species, *C. antennarius* and *C. productus*, were used in the final regression analysis ($N = 4$; *SI Methods*).

To determine variation in sea otters among the four beds in Elkhorn Slough, we surveyed otter densities in the eelgrass survey beds during summer 2012. We counted all otters within each bed at the start of observations and at 15-min intervals. Observation periods were 1 to 2 h and were performed weekly to twice weekly at each bed during the study period (May 15 to July 29, 2012).

Eelgrass community-dependent variables [CPUE and *C. antennarius* and *C. productus* carapace width (in millimeters), grazer biomass (in milligrams per centimeter of shoot), large grazer density (in number per square meter), algal epiphytes (in milligrams per centimeter of shoot), shoot density (in shoots per square meter), and eelgrass aboveground and belowground biomass (in grams per square meter)] from beds at Tomales Bay were compared with Elkhorn Slough ($n = 4$) by using an independent-samples t test. We used regression analysis to determine the relationship between otter density and the dependent variables among beds in Elkhorn Slough ($n = 4$).

Mesocosm and Field Experiments. To test whether the predicted top-down mechanisms were valid, we conducted a mesocosm experiment. The mesocosms consisted of transplanted eelgrass and mesograzers with standardized sizes, densities, and biomass. Mesocosms were subjected to two treatments: small crabs (mimicking crab populations under heavy otter predation) and large crabs (mimicking low otter predation; Fig. 1C). We measured response parameters after 30 d at the various trophic levels, including mesograzer (sea slug) biomass and mortality, epiphyte biomass, and eelgrass biomass and rhizome elongation, which are important indicators of condition and growth rates in seagrass (58) (*SI Methods*). All shoots were scraped free of algal epiphytes, and all grazers were removed and counted. All grazers, epiphytes, and eelgrass were dried at 60 °C for 24 h and weighed. We used an independent-samples t test to determine differences among small ($n = 8$) and large ($n = 7$) crab treatments.

We next conducted a field experiment to validate results from the mesocosm experiment in a nutrient-loaded estuarine environment (Fig. S4), and to include an actual sea otter predation treatment. By using a randomized block design, we placed enclosures (cages) on an eelgrass bed in Elkhorn Slough with high sea otter densities, in four different treatments: (*i*) simulated low otter predation (closed cage containing two large crabs), (*ii*) simulated high otter predation (closed cage without crabs), (*iii*) actual sea otter predation in the enclosure (cage open to otter and crab predation),

ECOLOGY

and (*iv*) actual sea otter predation without an enclosure (to serve as control for cage effects; *SI Methods* and Fig. S5). Eelgrass shoot lengths were standardized and each cage was seeded with 20 large mesograzers. We used ANOVA to test for treatment effects ($N = 8$) on grazer mass, algal epiphyte mass, shoot density, and aboveground and belowground eelgrass biomass, as well as density of large (>2 cm) mesograzers. Finally, we tested for differences among individual treatments by using a Tukey honestly significant difference (HSD) test (*SI Methods*).

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Supporting Information

Hughes et al. 10.1073/pnas.1302805110

SI Methods

All analyses in this study had α set at 0.10 to avoid type II errors that falsely fail to reject the null hypothesis (1) given the challenges of large-scale field sampling and experiments with low replication. All statistics were run by using SPSS software (version 20; IBM, Armonk, NY).

Time Series Analysis. *Historical nutrient sources and concentrations.* To determine trends in the bottom-up influences on the Elkhorn Slough eelgrass beds, we constructed a time series of nutrient concentrations in Elkhorn Slough. Elkhorn Slough is surrounded by a highly agricultural watershed in Monterey County, CA. The fertilizer in row crops causes nutrient runoff into the county receiving waters and ultimately Elkhorn Slough (2). We constructed the time series from several data sources: fertilizer sales (California Department of Food and Agriculture annual reports on tonnage of nitrogen fertilizer sales, 1930–2005), nitrate data from 1970 to 1971 (3), 1974 to 1976 (4), and 1977 from the California Central Coast Regional Water Quality Control Board; and 1989 to 2011 Elkhorn Slough National Estuarine Research Reserve (ESNERR).

Historical eelgrass cover. We mapped eelgrass cover in the estuary and quantified change through time by interpreting low-altitude vertical aerial imagery acquired between 1966 and 2012 by several agencies, primarily the California Department of Fish and Wildlife. The photographs were scanned, georeferenced using ERDAS Image Analysis 1.1 (ERDAS/Intergraph, Norcross, GA), and habitat polygons were manually digitized using ArcView GIS 3.3 (ESRI, Redlands, CA). Precise delineation of eelgrass patches from individual photographs was challenging for a variety of reasons, including varied tidal heights and water clarity, the presence of solar glint, and the similar appearance of macroalgae. Therefore, we visually identified 13 y through which eelgrass cover could be determined with high confidence based on historical descriptions and present-day ground surveys of distribution. We combined all polygons from each year to characterize the eelgrass extent. This methodology produced unequal intervals but allowed us to accurately assess trends. Intervals were shorter in the later years when sea otter expansion was occurring as a result of increased quality of imagery and accuracy of ground truthing.

Historical crab densities and sizes. We tested for the effects of long-term otter predation on the Elkhorn Slough crab population by comparing two time periods: 1971 to 1976 (a decade before otter immigration) and 2005 to 2009 (two decades after otter immigration). The 1970s data set was from a study by Nybakken et al. (4) and the Monterey Bay National Marine Sanctuary's Sanctuary Integrated Monitoring Network, and the 2005 to 2009 data set was from the ESNERR. We used data from a similar region in the lower part of the estuary directly adjacent to the present-day and historical distributions of eelgrass and sea otters. The crab traps used in the two studies both had >20-cm openings to allow for maximum crab sizes. Crabs from the 1970s were caught by using standard recreational traps (0.1587 m^3) composed of nylon or wire mesh wrapped around a circular metal frame and baited with fish, mussels, squid, or shrimp. More recent surveys from 2005 to 2009 used smaller sized traps (0.0621 m^3) than those in the 1970s, were constructed of nylon mesh wrapped around a rectangular metal frame, and were baited with anchovies. There were differences in the hours spent crab trapping, 2 to 8 h for 1971 to 1976 and 24 h for 2005 to 2009. However, we found that crab traps from the 1970s generally reached saturation in the 2- to 8-h sampling period, and traps from 2005 to 2009

rarely became saturated. Therefore, we did not correct for the differences in soak time to avoid any erroneous inflation of data from the 1970s that would bias our results. We did standardize crab traps to 1970s sizes.

Otter density and foraging observations for predation correlations with eelgrass bed expansion. To quantify spatial differences in sea otter density and predation pressure, we used existing data on sea otter distribution and abundance available from standardized biannual censuses conducted by the US Geological Survey Western Ecological Research Center and the California Department of Fish and Wildlife (GIS-compliant data from these censuses are available at www.werc.usgs.gov). Counts have been conducted twice annually (in spring and fall) since 1985, with most of the sea otter habitat in Elkhorn Slough counted by pairs of shore-based observers equipped with 10× binoculars and 50× Questar spotting scopes (Questar) and inaccessible areas surveyed by airplane (a Partenavia single-engine plane with three observers). The location, behavior, and habitat type of every sighted otter was recorded onto detailed maps (1:24,000) and later digitized into a GIS. Each annual count thus provides a snapshot of sea otter distribution: to account for otter mobility, we applied a kernel smoothing algorithm to these data to create a sea otter density "surface" within Elkhorn Slough. Specifically, by using the most recent 5 y of census count data (2007–2012), during which period sea otter numbers have been approximately stable, we fit a 2D kernel density smoother by using the Spatial Analyst toolbox in ArcGIS 10.1 (ESRI), and using a 2.5-km smoothing window. The resulting surface provided localized estimates of average otter density (i.e., otters per square kilometer) throughout Elkhorn Slough, and we averaged these values for each of the four eelgrass beds in standardized 1 ha plots that encompassed both eelgrass community and crab survey areas in 2012 (Fig. S2).

To measure crab predation by sea otters, observational data were recorded from feeding otters by using high-powered (50–80×) telescopes (Questar). Otters were selected haphazardly for data collection (i.e., without regard for location, status, or prey type), and ~10,200 feeding dives (occurring in 248 independent feeding bouts) were recorded between 1999 and 2012. For each dive, observers recorded subsurface dive duration, interdive surface interval, success of the dive (i.e., whether prey were captured), prey type (prey were identified to lowest possible taxonomic level), prey size (estimated relative to the sea otters' paw width), number of prey items consumed, and handling time per prey item consumed. The resulting data set was analyzed by using a Monte Carlo simulation-based algorithm described elsewhere (5), which results in bias-corrected estimates of diet composition and rate of biomass consumption by species, with associated measures of uncertainty. The results of this analysis show that crabs of the genus *Cancer* were the most commonly consumed prey type, making up ~43% (±2.1%) of the biomass consumed by otters in the slough (Fig. S1). A typical sea otter consumed 4.69 g (±0.26) of edible crab biomass per minute of foraging effort. The mean carapace width of *Cancer* crabs captured on feeding dives where the prey size could be reliably estimated was 59.3 mm (N = 1,112 crabs). A power function was used to convert mean carapace size (in millimeters) to corresponding estimates of mean edible biomass (in grams) and total biomass per crab [edible biomass = 0.0077 * carapace diameter$^{0.2265}$ ($R^2 = 0.97$) and edible biomass is 65% of total mass (6)]. Assuming an average of 30% of the time spent feeding, this translates to 2,030 g of crab biomass consumed per day, or 30.0 crabs. This estimate, based on observed feeding behavior, is very close to an independent

estimate of crab consumption that can be calculated based on metabolic requirements of sea otters, which must consume 25% of their body mass each day (7). Based on metabolic requirements, a 20-kg adult female otter consuming a diet of 43% crabs would require 2,150 g of edible biomass or 32 crabs per day. If only 35 sea otters feed primarily within Elkhorn Slough, they would be expected to consume more than 400,000 crabs (or 40,000 kg) every year. We multiplied the per-capita crab consumption rates by the average otter density in each of the four eelgrass beds to estimate mean crab predation rates in 1-ha areas of each bed from 2007 to 2012.

We used a cross-validation technique to corroborate estimates of sea otter density and crab predation rates in Elkhorn Slough eelgrass beds. By using linear regression, we correlated the relative frequency of occurrence of sea otters in each bed ($N = 4$) based on 2012 summer sea otter surveys (*Spatial Comparison* and Fig. 2) to the more long-term estimates of sea otter density and crab predation in each bed from 2007 to 2012 (as detailed in the previous paragraphs on estimation of otter predation rates; Fig. 1*D*). These two independently derived estimates were highly correlated ($P = 0.002$, $R^2 = 0.997$), thus validating our estimates of spatial variation in sea otter density and predation effects on crabs.

Spatial Comparisons. Traps used were shrimp pots ($61 \times 61 \times 23$ cm; 0.0856 m^3) composed of galvanized metal and a mesh size of 22 mm. Traps were modified to increase the tunnel size to ~200 mm to allow capture of crabs of all size classes, but preventing sea otters from reaching in to grab them. Traps were baited with anchovies and replenished every 2 to 3 d. Crab traps were checked daily. Each crab was identified to species, and size was measured at the widest point of the carapace. Crabs were released >100 m away from the traps after measurement.

Mesocosm and Field Experiments. *Mesocosm experiment.* To determine the predatory role of crabs on grazers and eelgrass, we conducted a mesocosm experiment. Our experiment took place between April 11 and May 8, 2012. In an outdoor laboratory space, we created 15 mesocosms made from 5-gallon plastic buckets. The bottom of each bucket had 10 cm of sterilized sand mixed with 50 g of sediment collected from Elkhorn Slough to introduce native microbial communities to the mesocosm. Each bucket received a continuous supply of fresh sand-filtered seawater (50 cm^3·s^{-1}). We inserted the water-supply tubing into the middle of each mesocosm's water column to ensure mixing. To ensure no water flowed over the top of the buckets, five holes were drilled 2 cm below the rim. The top of the buckets were covered with black garden mesh that reduced photosynthetically active radiation (PAR; Li-193 underwater PAR sensor; LI-COR, Lincoln, NE) to 50% ambient light. In both the mesocosms and Elkhorn Slough eelgrass populations, PAR at the canopy was ~50% ambient during midday conditions.

We collected terminal shoots along with their rhizome and root tissue, sea slugs, and crabs from Elkhorn Slough and transported them to Long Marine Laboratory, Institute of Marine Science, University of California, Santa Cruz. The mesocosms all contained six eelgrass shoots. The density of eelgrass in the buckets scaled to 113 shoots·m^{-2}, which was within the range of eelgrass density during summertime conditions in Elkhorn Slough [132 ± 50.8 (SD)]. Before placement in mesocosms, we measured the biomass, leaf number, and shoot length of each plant, as well as the total plant and sea slug biomass, and compared the means among mesocosms and treatments to ensure there were no preexperimental biases ($P > 0.10$ for all variables). We standardized all shoot lengths to 20 cm and rhizome lengths to 10 cm. All shoots were wiped clean of epiphytes by using cotton pads. During the experiment, algal epiphytes (primarily diatoms) recruited to eelgrass leaves from propagules already occurring in

the seawater system. By using zip ties, we attached zinc bolts to the plants as anchors to ensure that crabs did not free them from the sediment.

Three sea slugs, *Phyllaplysia taylori*, were added to each mesocosm. We added in two large (>2 cm) and one small (<2 cm) sea slug, as 2 cm was a clear break in the size distribution of sea slugs and this ratio was similar to the distribution [$70.0\% \pm 8.8$ (SE)] of size classes found in Elkhorn Slough. Additionally, large grazer densities in the buckets scaled to 38·m^{-2}, which were in the range reported from the field [61 ± 60.5 (SD)]. The variation in size also allowed for us to investigate the predatory effects of crabs on the large mesograzer size class. We then randomly selected mesocosms that would have simulated low otter predation [a single large crab, *Cancer* spp. (carapace >80 mm) or *Pugettia producta* (carapace >60 mm); $n = 7$] or simulated high otter predation [a single small crab, *Cancer* spp. (carapace <40 mm) or *P. producta* (carapace <20 mm); $n = 8$]. Algal epiphyte (primarily chain forming diatoms) propagules came naturally from spores in the seawater supply or the transported Elkhorn Slough sediments. At the conclusion of the experiment, we harvested all eelgrass and sea slugs for processing. All shoots were scraped free of algal epiphytes, and all grazers were removed and counted.

Field experiment. We experimentally field-tested the effect of sea otters on eelgrass by using a caging experiment in an Elkhorn Slough eelgrass bed. The bed had high sea otter densities in the experimental area during the experimental period [$x = 3.53$·ha$^{-1} \pm 3.40$ (SD), based on daily counts]. Nitrate concentrations were high ($x = 10.09$ μmol) during the experimental period and peaked at 189 μmol toward the beginning weeks (www.mbari.org/lobo/loboviz.htm; Fig. S4). The occurrence of high sea otter densities and foraging pressure as well as high nutrient concentrations made ideal conditions to test the relative effects of top-down predation and bottom-up nutrient loading on eelgrass productivity.

In July 2012, we established the caging experiment by using a randomized block design that consisted of eight blocks and four treatments: cages including crab and excluding sea otters (+crabs −otters), cages excluding crabs and sea otters (−crabs −otters), a partial cage control that allowed access to both sea otters and crabs yet included the top of the cage to test for shading effects on the seagrass (+crabs +otters), and a cage-free control (open control). The +crab −otter cages simulated an otter-free environment, the −crabs −otters cages simulated a high predatory otter environment, and the +crabs +otters and open control allowed for natural predation by sea otters and crabs to occur. During the experiment, we observed both sea otters and crabs accessing both control treatments.

Cages were constructed using metal rebar welded to form $50 \times 50 \times 50$-cm cages (Fig. S5). For +crabs −otters and −crabs −otters cages, chicken-wire mesh (2.5×2.5 cm) was wrapped around all sides accept for the bottom to allow for seagrass to grow. The chicken wire allowed small grazer (sea slugs and crustaceans) access, yet prevented movement by crabs and otters. Additionally, hogwire mesh was wrapped around 25-cm rebar extensions that were driven into the sediment to prevent crabs from burrowing out or into the cages. Finally, a 20-cm hogwire skirt was wrapped around the bottom of the cages to prevent otters from accessing the cages by digging. Hogwire is commonly used in farming applications to cage livestock and prevent entrance from unintended animals of various sizes. The hogwire mesh on the experimental cages had 15-cm-wide rectangles with the connecting wires between them spaced from 2 to 2.5 cm for the vertical crab control portion (pushed into the mud) and from 2.5 to 5 cm for the horizontal otter control section (laying flat on the mud). The partial cage control (+crabs +otters) was open on all sides except for the top, which consisted of the chicken-wire mesh to test for cage effects on the eelgrass yet allowing access by crabs and otters.

The open control had no cage and therefore permitted access to grazers, crabs, and otters. We measured PAR during high tide during maximum light attenuation and found only a slight (17%) reduction of PAR in cages vs. open controls. The mean PAR in cage treatments was 329.1 μmol photons·m^{-2}·s^{-1}, and was 37% the subsurface PAR; both these values are well within saturating light levels for *Zostera* spp. (8, 9).

Before cage installation, eelgrass shoots were standardized in all treatments by cutting them to 20 cm. This allowed for us to control for grazers by removing the entire population. We counted shoots in treatments within blocks to ensure there were no preexperimental differences ($P > 0.10$) in shoot densities. A 1-m × 1-m buffer zone was created by cutting all the shoots surrounding the experimental 50 × 50-cm area to prevent shading by taller bordering shoots. All treatments were spaced 2 m apart, and all blocks were spaced 10 m apart along a transect that bisected the eelgrass bed.

After cages were installed, all treatments were seeded with 20 large (>2 cm) sea slugs to standardize the grazer densities. The other common mesograzer, the isopod crustacean *Idotea* spp., are swimmers that readily accessed all treatments (B.B.H., unpublished observation), so we did not seed them into the treatments. Finally, we added one *Cancer* spp. (80–100 mm) crab and one *P. producta* (60–80 mm) crab to each +crabs −otters cage. The crab size was selected based on size selection of otters (Figs. 1*C* and 2*B*) and size-related grazer predation rates as determined by the mesocosm experiment (Fig. 3*A*). The sizes for each crab species were in the high range for Elkhorn Slough. *Cancer* crabs are benthic carnivores, whereas *Pugettia* are canopy-dwelling omnivores primarily feeding on algae yet switching to invertebrates in the absence of algae (10, 11). Mesocosm experiments determined that both species of crabs eat sea slugs in seagrass systems and can significantly reduce their densities (Fig. 3*A*). Otter foraging surveys conducted in the eelgrass beds during the experimental period determined that the crabs constitute a high proportion of total prey consumed (~45%), 60% *Cancer* and 40% *Pugettia*, respectively.

The experiment lasted for 1 mo. During that period, we used self-contained underwater breathing apparatus (SCUBA) to in-spect cages for crabs, sea slugs, and structural integrity, as well as scrubbing cages to clean off any fouling material twice weekly. There was no evidence of crabs escaping or sea otters entering cage enclosure treatments during the course of the experiment. We observed otters inhabiting the experimental area daily throughout the 1-mo experiment and observed otters freely accessing the cages during SCUBA surveys. Two weeks into the experiment, we added an additional *Cancer* crab to one of the + crabs −otters cages as a result of a mortality of the original *Cancer* crab. After 1 mo, we counted all shoots and harvested five shoots from all the treatment replicates. All shoots were scraped free of algal epiphytes, and all grazers were removed and counted. All grazers, epiphytes, and eelgrass were dried at 60 °C for 24 h and weighed.

We compared grazer (in grams of dry weight per centimeter of shoot), algal epiphyte (in grams of dry weight per centimeter of shoot), shoot density (in number of shoots per square meter), and aboveground (shoot density times mean shoot mass, in grams of dry weight per square meter) and belowground [shoot density times mean (rhizome + root) mass (standardized to 7 cm), in grams of dry weight per square meter] biomasses, as well as density of large (>2 cm) mesograzers (in number per square meter), using a randomized blocked ANOVA (SPSS software, version 19), using treatment (fixed) and block (random) as the dependent variables. Total shoot biomass was calculated by multiplying shoot density by the mean shoot mass for each replicate. Shoots in two of the + crabs −otters replicates had been damaged by the crabs and were not used in the final analysis for algal epiphytes and eelgrass biomass estimates. All other replicates were not disturbed during the experiment. We tested for the assumption of normality for the dependent variables by using a Kolmogorov–Smirnov test. To conserve degrees of freedom, we first compared control treatments (+crabs +otters and open control) by using the randomized block ANOVA to determine if there were significant differences. If not, the two treatments were pooled as one control for the final randomized blocked ANOVA design. Finally, we compared differences among individual treatments by using a Tukey honestly significant difference test.

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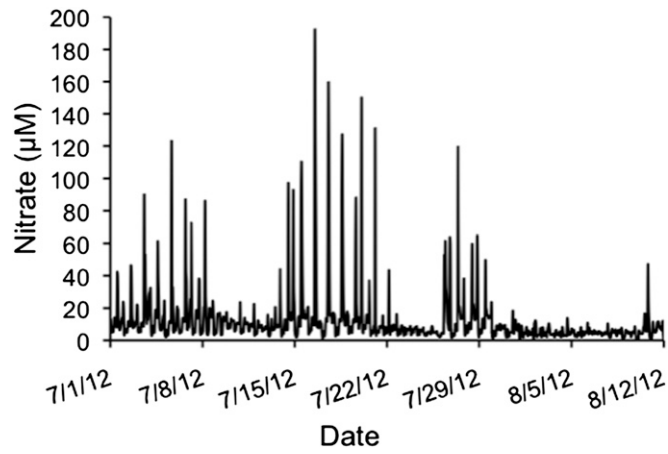
Fig. S1. Predation by sea otters by prey type from ~10,200 feeding dives (occurring in 248 independent feeding bouts) recorded between 1999 and 2012 in Elkhorn Slough.

Fig. S2. Survey locations in Elkhorn Slough ($n = 4$). Rectangular areas are 1-ha plots that encompassed eelgrass community transects and crab surveys for 2012, as well as otter crab predation estimates for 2007 to 2012. Otter densities were determined by using biannual surveys (2007–2012) and calculated by using a 2D kernel density smoother.

Fig. S3. Object based image analysis of the extent of eelgrass beds in 2006 and 2012. Eelgrass bed expansion was compared over time across four sites: (*A*) outfall, (*B*) area 4, (*C*) crop circles, (*D*) seal bend. High resolution (1 m) digital elevation models (DEMs) of the Elkhorn Slough main channel from 2005 and 2011 were used to estimate the changes in the area (in hectares) of habitat of depths where eelgrass can persist (0–2 m at North American Vertical Datum of 1988). The DEMs were created from multibeam bathymetric surveys and vessel-based Light Detection and Ranging surveys. Vertical uncertainties for the 2005 survey and 2011 survey were ±0.11 m and ±0.03 m, respectively. Eelgrass bed expansion was correlated with sea otter consumption rates (2007–2012) in the same beds (Fig. S2 and Table S1*D*).

Fig. S4. Nitrate data showing elevated concentrations during the 2012 survey and field experimental period in Elkhorn Slough. Hourly data were collected in situ using an in situ UV spectrophotometer nitrate sensor attached to the Monterey Bay Aquarium Research Institute's Land/Ocean Biogeochemical Observatory sensor mooring (1).

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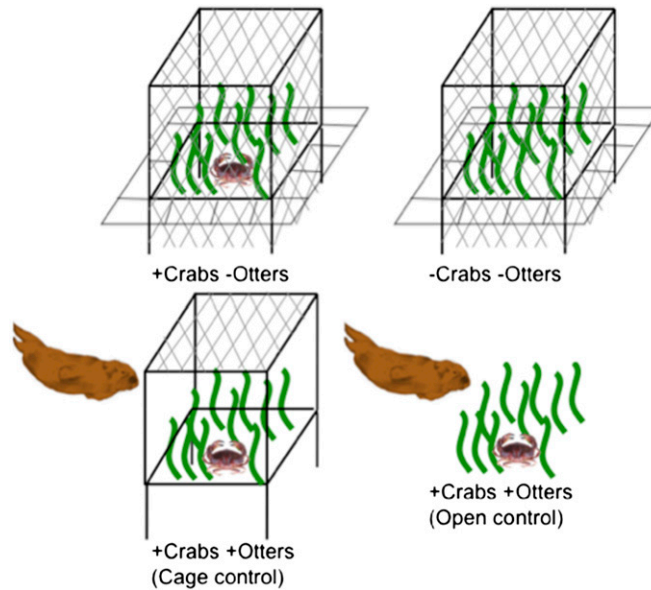
Fig. S5. Cage experimental design (50 cm × 50 cm × 50 cm), testing for the trophic cascade effects of crabs (+crabs −otters), the simulated trophic cascade effects of sea otters (−crabs −otters), and the direct effects of sea otters (partial cage control and open control) on eelgrass communities (grazers and algal epiphytes) and growth (individual and total shoot biomass per cage).

Elkhorn Slough

Tomales Bay
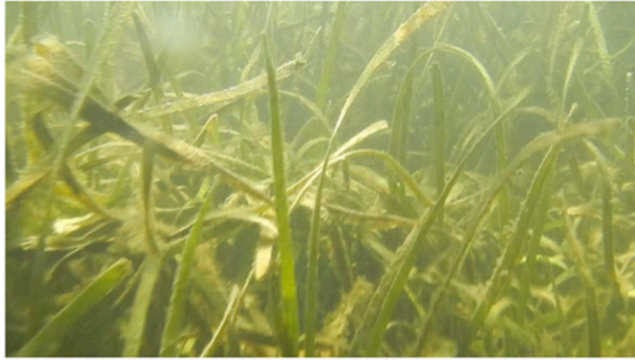
Movie S1. Underwater video from summer 2012 showing differences in *Zostera marina* seagrass beds in two California estuaries: Elkhorn Slough (high nutrient concentrations, high sea otter abundance) and Tomales Bay (lower nutrient concentrations, no sea otters). The first video is of an Elkhorn Slough eelgrass bed with high sea otter density, and is characterized by upright vegetation, relatively free of epiphytes, and containing large grazers (sea slugs, *P. taylori*). The second video is of Tomales Bay (no sea otters), where the eelgrass has extensive epiphyte growth, the leaves are sloughed over and noticeably shorter, and the bed has notably lower grazer abundances.

Movie S1

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)
Table S4 (DOCX)
Table S5 (DOCX)