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FOOD WEBS, RESILIENCE, AND FUNCTIONING OF AN ESTUARY UNDER MULTIPLE THREATS: LESSONS LEARNED FROM ELKHORN SLOUGH

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By

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ABSTRACT

Food webs, resilience, and functioning of an estuary under multiple threats, lessons learned from Elkhorn Slough

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Estuaries are among the most productive ecosystems on the planet and provide many important functions to the benefit of humans. Estuaries, however, are also under multiple anthropogenic threats, such as habitat degradation, pollution, eutrophication, and trophic downgrading through overharvesting of marine predators. These threats to estuaries come with a heavy cost through the loss of key ecosystem functions and services. The purpose of my dissertation was to characterize how anthropogenic threats affect estuarine communities in Elkhorn Slough, an estuary that provides habitat to a great diversity of organisms but is under threat through extreme nutrient loading and habitat modification.

In my first chapter I investigated how bottom-up (nutrients) and top-down (predation) forcing interact to influence seagrass beds. Using the recovery of sea otters (*Enhydra lutris*) I demonstrated that top predators can mediate the harmful effects of nutrient loading and eutrophication to eelgrass (*Zostera marina*) beds through the removal of crabs, which frees mesograzers to perform an important function: removing shade-causing algal epiphytes from seagrass leaves. I followed up on these results for my second chapter to develop a mechanistic understanding of eelgrass resilience in face of macroalgal blooms (*Ulva* spp.) that coincide with peak

eelgrass production. Using a series of field experiments I demonstrated that sea otters can promote both eelgrass and *Ulva* at a seagrass-macroalgal ecotone, a process that benefits eelgrass resilience by enhancing *Ulva*'s mesograzer assemblage that lowers the epiphyte load on eelgrass.

For my third and final chapter I used a 40 year data set of fish, water quality, and climate indices to determine the long-term effects of hypoxia on two important ecosystem services: the provision of biodiversity and nursery function. My results demonstrated that anthropogenic nutrient loading and subsequent hypoxia negatively impacted fish diversity and the nursery function for English sole (*Parophrys vetulus*). Despite ever increasing nutrient inputs, hypoxia was highly variable in time and space, and was mediated by climate, specifically El Niño events that increase flushing through increased precipitation as well as suppressing upwelling that brings hypoxic water from the deep sea. The suppression of hypoxia through El Niño events was a consistent pattern across estuaries in the northeast Pacific, providing important insight as to how climate change will affect anthropogenic threats to ecosystem services provided by estuaries.

My dissertation unravels some of the mysteries underlying ecosystem resilience in face of anthropogenic threats. Systems like Elkhorn Slough are critical for informing research and management as to how ecosystems function under intense stress. The rarity of available long-term data, along with the recovery of a foundation species - *Zostera marina* - and a model top predator – *Enhydra lutris* – made it possible to tease apart processes and mechanisms driving resilience over meaningful

time scales. Furthermore, my dissertation highlights the importance of studying systems where resilience and recovery are occurring, as they will provide insight to inform management and policy in a world of increasing anthropogenic threats and changing climate.

I dedicate this dissertation to my family – Alejandra, Isabela, Sam, and Kio – whose love and support have no bounds.

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INTRODUCTION

The last two decades in marine conservation research has revealed a troubling trend of ecosystem collapse at the hands of human population expansion and overexploitation (Jackson *et al.* 2001; Myers & Worm 2003; Lotze *et al.* 2006; Worm *et al.* 2006; Waycott *et al.* 2009). Human use of coastal zones and resource consumption has led to pollution, hypoxia, warming, acidification, and trophic downgrading, all of which compromise the ecosystem services provided by coastal ecosystems that humans depend on. The decline in coastal ecosystems and their services has generated urgency in the research, management, and conservation communities to understand ecosystem functioning and resilience.

Estuaries are one of the most imperiled of earth's ecosystems because they are often located near urban or agricultural centers, and thus in close proximity to associated threats that come with high human activity. The delivery of anthropogenic nutrients to coastal waters has been one of the main drivers of ecosystem declines (Cloern 2001; Diaz 2001; Rabalais *et al.* 2002, 2010; Valiela & Cole 2002; Burkholder *et al.* 2007). The process of eutrophication, or an increase in the delivery of organic material to a system (Nixon 1995), is the main product of anthropogenic nutrient loading and it can cause deleterious effects to ecosystem functions and services provided by estuaries. First, it can over stimulate algal production where it can cause shifts in vegetation dominance from a more favored seagrass state to one dominated by lower functioning ephemeral macro- and microalgae (Valiela *et al.* 1997; Nelson & Lee 2001; Valiela & Cole 2002; Hauxwell *et al.* 2003; Burkholder *et*

al. 2007). Second, the overproduction of algae can increase organic deposition to the benthos and increase respiration rates that ultimately cause hypoxia – the depletion of oxygen from the sediments and water column, with severe consequences for aquatic life (Paerl *et al.* 1998; Diaz 2001; Rabalais *et al.* 2002, 2010; Breitburg *et al.* 2009). Further complicating matters, along the California Current, intensifying upwelling over the last few decades brings hypoxic waters from the deep over the continental shelf and into estuarine waters (Grantham *et al.* 2004; Chan *et al.* 2008; Caffrey *et al.* 2010; Hessing-Lewis *et al.* 2011; Roegner *et al.* 2011; Booth *et al.* 2012; Sydeman *et al.* 2014), and therefore certain estuaries are challenged from hypoxia driven by both anthropogenic and oceanic sources.

Determining the resilience of ecosystems in face of increasing anthropogenic threats is a central goal in modern ecology and conservation biology (Scheffer *et al.* 2001; Côté & Darling 2010; Doak & Morris 2010; Thom *et al.* 2011; Micheli *et al.* 2012; Silliman *et al.* 2012). Identifying and determining the mechanism that supports population and ecosystem resilience is a powerful tool for guiding both managers as to where to invest in restoration and decision makers as to where policies need to be developed. However, ecosystems demonstrating recovery and resilience are becoming increasingly rare in nature due to the intensification of anthropogenic stress.

For this dissertation I have focused on an estuarine system along the central coast of California, Elkhorn Slough, which is under intense anthropogenic stress in the form of anthropogenic nutrient loading (Caffrey 2002; Hughes *et al.* 2011; Hughes *et al.* 2013), yet has demonstrated remarkable resilience through the rapid

expansion of seagrass, supporting the recovery of endangered sea otters, and providing important nursery grounds for several fish species of cultural, commercial and ecological importance including leopard sharks and English sole (Brown 2006; Carlisle & Starr 2009). The goal of this research was to explore the patterns and mechanisms driving the resilience in estuarine seagrass and fish despite the increasing threat of anthropogenic nutrient loading, eutrophication, and hypoxia (Caffrey 2002; Beck & Bruland 2000; Caffrey *et al.* 2010; Hughes *et al.* 2011).

The trophic downgrading of earth's coastal ecosystems over the last few centuries has challenged researchers to determine the relative role of top-down (consumers) versus bottom-up (resources) forces in driving ecosystem resilience and collapse (Jackson *et al.* 2001; Halpern *et al.* 2006; Estes *et al.* 2011; Ripple *et al.* 2014). The primary challenge is that it is difficult to determine the role of top predators in the ecosystem if the predator is absent or functionally depleted. In the first chapter of this dissertation I took advantage of the recovery of a top predator, the sea otter (*Enhydra lutris*), to determine its role in eelgrass (*Zostera marina*) beds that were under threat from severe nutrient loading and eutrophication in Elkhorn Slough. Through a series of field and mesocosm experiments, field surveys, and modeling of long-term monitoring data I was able to demonstrate that sea otters are capable of generating a trophic cascade that benefits eelgrass. In this system sea otters have depleted crab (primarily *Cancer* spp.) populations that have freed invertebrate mesograzers from predation, thus allowing the grazers to perform the important function of keeping eelgrass leaves clean of shade-causing algal epiphytes. The

results of this study emphasize the importance of the interactive effects of top-down and bottom-up forces on the persistence of dominant habitat forming vegetation, in this case I found that the top-predator meditates the harmful effects of eutrophication on seagrass through a trophic cascade (Hughes *et al.* 2013).

There is a century-old paradigm in seagrass ecology that macroalgal blooms cause declines in seagrasses through smothering, shading, and competition for space (Letts & Adeney 1908; Nelson & Lee 2001; Valiela & Cole 2002; Burkholder *et al.* 2007). However, in Elkhorn Slough I have documented that eelgrass has been rapidly expanding in areas that are dominated by the bloom-forming algae *Ulva* spp, seemingly defying a long-standing paradigm in seagrass ecology. For my second chapter, I hypothesized that sea otters are driving the shift in dominance of eelgrass by generating a trophic cascade at the seagrass-macroalgal ecotone, similar to the cascade described in Chapter 1 in the interior portions of the beds, where sea otters promote grazers and thereby shift the competitive balance in favor of eelgrass over macroalgae. To my surprise sea otters generated a trophic cascade that benefitted both eelgrass and *Ulva*. Using structural equation modeling (SEM), I determined that sea otters deplete crabs, which would otherwise eat *Ulva* and minimize the mesograzer assemblage at the seagrass-macroalgal ecotone, and in turn promote algal epiphyte growth on the eelgrass leaves leading to lower biomass and shoot production. The results of this study add to a growing body of literature that suggests macroalgae does not always negatively impact seagrass, and that it is context specific (Baden *et al.* 2010; Hessing-Lewis *et al.* 2011; Olyarnik & Stachowicz 2012; Thomsen *et al.* 2012;

Whalen *et al.* 2013). In this case eelgrass is able to escape smothering from *Ulva* through production of a taller canopy and benefits from *Ulva* through the delivery of important mesograzers due to the sea otter trophic cascade.

One of the more important functions of estuaries is the provision of fish habitat that promotes the ecosystem services of enhanced biodiversity and nursery function that supports regional fisheries (Beck *et al.* 2001, 2003; Nagelkerken *et al.* 2013; Sheaves *et al.* 2014). These important services, however, are compromised by anthropogenic threats, such as coastal hypoxia and climate change (Breitburg *et al.* 2009). Using a 40 year time series of fish, water quality, and climate monitoring I was able to determine the role of hypoxia on influencing fish diversity and nursery function in Elkhorn Slough. I found that areas that experience more severe hypoxia were poor habitat for fish by having decreased abundance of the two dominant species of flatfish: speckled sanddab (*Citharichthys stigmaeus*) and English sole (*Parophrys vetulus*), the latter of which uses the estuary as its primary nursery grounds serving an important regional offshore fishery (Brown 2006). Surprisingly, I found that hypoxia in the estuary is highly variable in time and space despite the decades long increase in anthropogenic nutrient loading. Periods of normoxia (normal oxygen conditions) resulted in higher fish diversity and flatfish abundance in the entire main channel of the estuary, whereas only the lower half of the estuary is available during hypoxia. For English sole, periods of hypoxia were associated with decreased recruitment, abundance, and fisheries landings in the offshore adult population. Finally, I determined that the variation in hypoxia was strongly regulated

by climate, namely El Niño. Whereas increases in El Niño intensity corresponded to decreased hypoxia through increased precipitation and flushing of the estuary, and to a lesser degree, suppression of upwelling in the lower estuary. Results from this study indicate that climate can mediate anthropogenic threats to important ecosystem services, in this case provision of biodiversity, nursery function, and fisheries production. These results emphasize the importance of incorporating long-term monitoring into models predicting the effects of climate change and anthropogenic stressors on valued ecosystem services.

My dissertation research takes full advantage of some remarkable long-term data sets, and the recovery and resilience of important populations to develop a mechanistic understanding of what drives ecosystem resilience in face of increasing anthropogenic threats. Future research can use this dissertation as a roadmap to identify and study other systems facing similar challenges as Elkhorn Slough, yet also benefitting from conservation such as trophic upgrading. Additionally, managers and decision makers can use results from this dissertation and studies like it to inform restoration and policy, especially as they relate to water quality, climate change, fisheries management, and the recovery of endangered top predators and imperiled ecosystems like seagrass.

1. Chapter 1 - Recovery of a top predator mediates negative eutrophic effects on seagrass

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1.1 ABSTRACT

A fundamental goal 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-year 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.

1.2 INTRODUCTION

Understanding the relative influence of bottom-up vs. top-down forces on vegetated assemblages has long been an important conceptual goal of ecology (Hairston et al. 1960; Power 1992; Silliman et al. 2005; Halpern et al. 2006). As many vegetated habitats have declined globally in past decades (Jackson et al. 2001; Scheffer et al. 2001; Lotze et al. 2006; Waycott et al. 2009), with concurrent losses of valued ecosystem services, investigations of drivers of vegetation sustainability have also taken on applied significance and urgency in conservation science (Bruno et al. 2003; Duarte et al. 2005). Human activities have altered both bottom-up forces, for instance by increasing nutrient availability (Vitousek et al. 1997; Valiela et al. 1997), and top-down forces, by hunting and fishing of top predators (Jackson et al. 2001; Estes et al. 2011). Detecting the relative role of such alterations and interactions between them is critical for supporting key vegetated habitats and their ecosystem services.

Investigations of both 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 (Jackson et al. 2001; Estes et al. 2011; Terborgh et al. 2010; Burkholder et al. 2013). However, apex predators have been either depleted or lost entirely across most of the natural world (Jackson et al. 2001; Estes et al. 2011), including many near-shore marine systems (Jackson et al. 2001; Heck and Valentine 2007). It is difficult to understand ecosystem-level effects of an apex predator if it is extremely rare or absent (Croll et al. 2005). 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 (Silliman et al. 2002; Silliman et al. 2005; Altieri et al. 2012).

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 (Bruno et al. 2003), shoreline protection, and carbon sequestration from the surrounding seawater and overlying atmosphere (Waycott et al. 2009; Duarte et al. 2005). Seagrass beds have declined in many regions of the world, often due to the smothering effects of algal epiphytes that are enhanced by nutrient loading (Waycott et al. 2009, Valiela and Cole 2002). 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 (Heck et al. 2000; Valentine and Duffy 2006; Heck and Valentine 2007; Moksnes et al. 2008; Baden et al. 2010; Lewis and Anderson 2012; Whalen et al. 2013). Mediation of competitive interactions between primary producers is directly controlled by herbivores, which have consistently demonstrated preferential consumption of algal epiphytes over seagrasses (Williams and Ruckelshaus 1993; Hughes et al. 2004; Duffy 2006; Whalen et al. 2013), 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 (Heck et al. 2000; Moksnes et al. 2008; Baden et al. 2010; Lewis and Anderson 2012), leading to a trophic cascade that mediates the competition between seagrass and their epiphytes. Seagrass ecosystems thus provide an opportunity to examine both bottom-up and top-down forces, and the interaction between them.

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 (Estes and Palmisano 1974; Kvitek et al. 1988; Estes et al. 1998). We used a 50-year 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.

1.3 RESULTS AND DISCUSSION

1.3.1 Study system and historical trends. Elkhorn Slough is a highly nutrient loaded (Figure 1. 1*A*,*B*) and eutrophic (Hughes et al. 2011) estuary on the central coast of California, U.S.A. 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 30000 tons in 2005, which has resulted in an exponential increase in nutrient concentrations in Elkhorn Slough through time ($P < 0.0005$, $R^2 = 0.90$) (Figure 1. 1. 1*A*) (Table 1.S1*A*). We calculate that the current nutrient load to the Elkhorn Slough estuary is 407 kg nitrogen $*ha^{-1} *year^{-1}$ (Table 1.S1*B*), a load surpassing that of most global coastal waters considered highly eutrophic (Valiela and Cole 2002; Hauxwell et al. 2003; Burkholder et al. 2007).

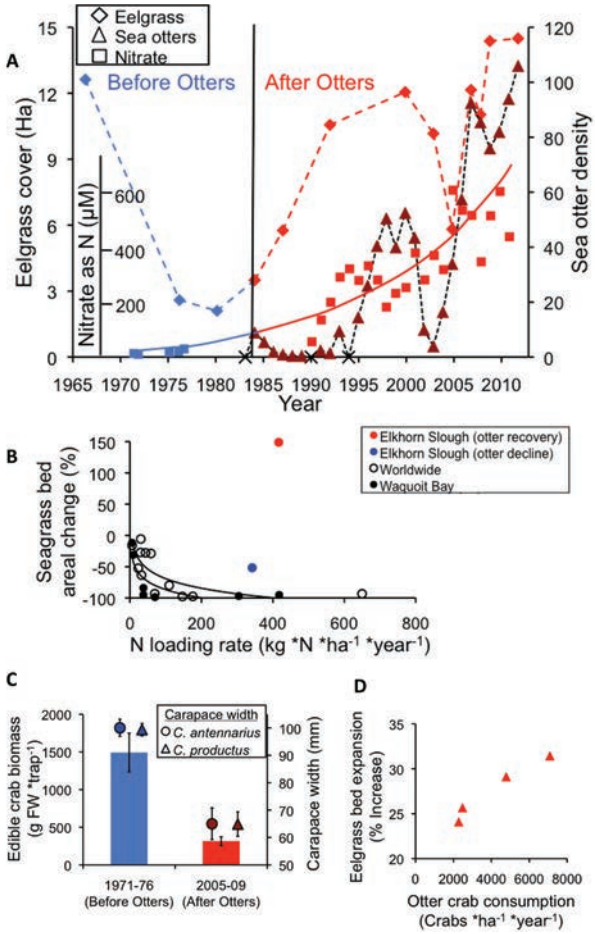

Figure 1.1. Historical analysis of nutrients, eelgrass, sea otters, and crabs in Elkhorn Slough. (*A*) 50 years of eelgrass declines and expansion driven by bottom-up nutrient loading, and top-down sea otter driven trophic cascade effects. Nitrate data (n = 28) represent the annual mean, the solid line is a an exponentially modeled linear function of the entire data series (1971-2011) (Table 1.S1*A*). Sea otter (n = 30) and eelgrass (n = 13) data are represented by dotted lines to visually show trends. (*B)* Meta-analysis showing the relationship between land-derived nitrogen loads entering estuaries and percent change of seagrass estimated from areal surveys. Worldwide and Waquoit Bay, MA, U.S.A. (an estuary with varying nutrient loading) data are redrawn from Burkholder et al. (2007) (with permission from the publisher). Elkhorn Slough data (Table 1.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 prior to sea otter colonization (1971-76) and two decades after sea otter colonization (2005-09) (Table 1.S1*D*; see *Methods* for sample size description). (*D*) Eelgrass bed expansion (2006-2012) at eelgrass beds (n = 4) in Elkhorn Slough (calculated as the % increase in cover of eelgrass as a function of available eelgrass habitat, measured in ha) (Figure 1.S3) correlated with estimated sea otter predation on crabs in standardized 1 ha plots (Figure 1.S2) in each bed (Table 1.S1*E*).

Our time series analyses revealed remarkable expansion displayed by eelgrass in face of extreme nutrient loading (Figure 1.1*A*) and concurrent loss of the adjacent salt marsh (Van Dyke and Wasson 2005), which has been demonstrated to buffer the harmful effects of nutrient loading and eutrophication (Valiela and Cole 2002). 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) (MacGinitie 1935) and concentrations from adjacent ocean sources (Mean NO_3 = 5.0 µM) (Chapin et al. 2004). 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 1965-1984 (Figure 1.1*A*). However, the expected decline in eelgrass has reversed twice over 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 (Figure 1.1*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 years) (Garshelis et al. 1986). Sea otter densities were significantly correlated with extent of eelgrass ($P < 0.019$, $R^2 = 0.52$) (Table 1.S1*C*), and since the initial sea otter recolonization in 1984, eelgrass

bed extent increased by 600% (Figure 1.1*A*). In a global context (Figure 1.1*B*), this expansion of eelgrass in the face of severe nutrient loading is anomalous; empirical evidence from other estuaries as well as modeling (Valiela and Cole 2002; Hauxwell et al. 2003; Burkholder et al. 2007) predicts that Elkhorn Slough should have undergone dramatic seagrass loss, not expansion. However, following the most recent period of sea otter decline (2000-2004) (Figure 1.1*A*) the relationship between nutrient loading and seagrass loss was much closer to the model prediction from estuaries worldwide (Figure 1.1*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 (Tinker et al. 2008a), 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 from the genus *Cancer* making up 43% of the sea otter diet (Figure 1.S1). Sea otters are well known to limit populations of their macroinvertebrate prey, including crabs (Garshelis and Garshelis 1984), 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 (*C. antennarius:* $P < 0.0005$ and *C. productus*: $P < 0.0005$) (Figure 1.1*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 due to overfishing from four decades (1951-1995) of annual “shark derbies” (Carlisle et al. 2007). 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 (Kao 2000). Furthermore, crab harvesting in Elkhorn Slough has declined in the last two decades compared to the 1970s when crab harvesting was common (Nybakken et al. 1977), 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 to the much larger “Dungeness crab” (*C. magister*) fishery, and yielded only an average of 3000 kg annually from 1960-2010 (California Department of Fish and Wildlife) and peaked in 1989 when eelgrass was in a period of recovery (Figure 1.1*A*). The decrease in populations of other top predators and the lack of over-harvesting of crabs in and around the estuary all suggest that the observed decline in *Cancer* crab biomass and size in Elkhorn Slough was due to 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 2006-2012 (*SI Methods*) and correlated it with eelgrass bed expansion (% increase in eelgrass cover) after recovery from the most recent decline (2000-2004) where > 50% of eelgrass was lost. Eelgrass expansion during the ensuing 6-y

period was positively correlated with sea otter predation on crabs ($P = 0.021$, $R^2 = 0.96$) (Figure 1.1*D*, Figures 1.S2 and 1.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 (Williams and Ruckelshaus 1993; Heck et al. 2000; Hughes et al. 2004; Duffy 2006; Valentine and Duffy 2006; Heck and Valentine 2007; Moksnes et al. 2008; Baden et al. 2010; Lewis and Anderson 2012; Whalen et al. 2013) 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 (Figure 1.2*A*).

1.3.2 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, USA, which are similar in many physical (Largier et al. 1997) and biological attributes but differ in the presence of sea otters and nutrient loading. Nitrate concentrations are lower in Tomales Bay (0-23 µM) (Kimbro et al. 2009) than they are in the eutrophic (Caffrey et al. 2010; Hughes et al. 2011) Elkhorn Slough (10-600 µM). Elkhorn Slough presently supports up to 120 otters, but sea otters have yet to re-colonize 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, approximately 185 km south of Tomales Bay as the otter swims (Tinker et al. 2008b), 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 (Broughton 1999).

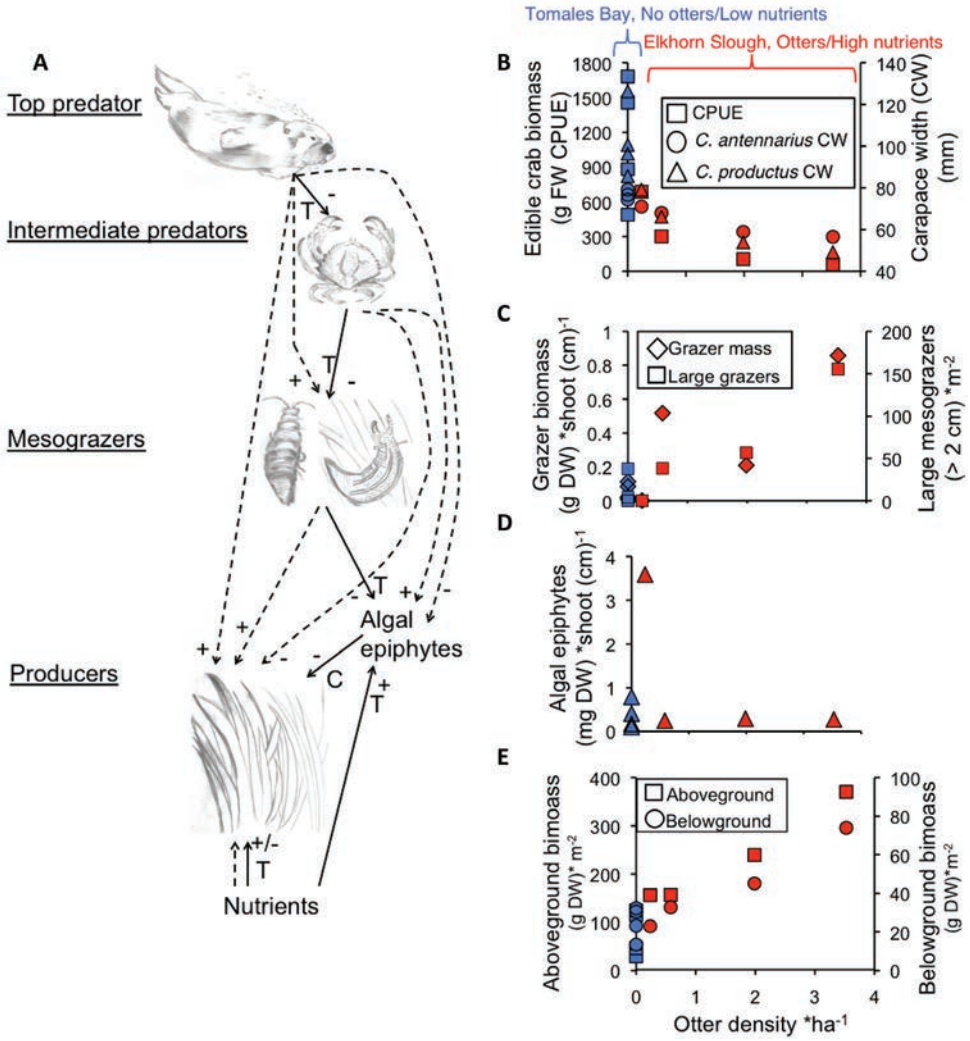

Figure 1.2. (*A*) Interaction web of top-down and bottom-up effects in the eelgrass study system. The top predator is the sea otter (*Enhydra lutris*), the mesopredators are crabs (*Cancer* spp. and *Pugettia producta*), the epiphyte mesograzers are primarily an isopod (*Idotea resecata*) and a sea slug (*Phyllaplysia taylori*), 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, the + and – indicate positive and/or negative effects on trophic guilds and eelgrass condition, T = trophic and C = competitive interactions, respectively. 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 red and the Tomales Bay reference site (no sea otters, low nutrients) beds (n = 4) are coded 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. Note: DW = dry weight, FW = fresh weight, and CPUE = Catch per Unit Effort, ha = hectares.

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, *Cancer antennarius* [$P = 0.034$] and *C. productus* [$P = 0.009$]) (Figure 1.2*B*) and greater aboveground eelgrass biomass ($P = 0.035$) than Tomales Bay (Figure 1.2*E*), as predicted for the estuary with otters present (Table 1.S2). Crab biomass and sizes for Tomales Bay (Figure 1.2*B*) were similar to Elkhorn Slough prior to the otter re-colonization (Figure 1.1*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) (Figure 1.2*C*-*E*) did not significantly differ between estuaries between Tomales Bay and Elkhorn Slough, but varied in the direction predicted by our model (Figure 1.2*A*).

Remarkably, comparisons between Tomales Bay and Elkhorn Slough indicated that eelgrass can perform equally, if not better in nutrient-loaded and eutrophic conditions (Hughes et al. 2011). High spatial variation of crabs, grazers, epiphytes, and eelgrass abundance characterize Elkhorn Slough, indicating the potential for a gradient in the key forcing processes (Figure 1.2*B*-*E*). Our analyses indicate that sea otters are a key driver of this variation. Otter density across eelgrass beds within Elkhorn Slough was negatively correlated with crab biomass ($P = 0.043$, $R^2 = 0.96$) and size (*C. antennarius*: $P = 0.040$, $R^2 = 0.92$ and *C. productus*: $P =$

0.061, R^2 = 0.88) (Figure 1. 2*B*) (Table 1.S3). Large mesograzer density varied positively and significantly (P = 0.041, R^2 = 0.92) (Figure 1.2*C*) with increased sea otter density. Although the sea otter density gradient was not significantly correlated with grazer biomass (Figure 1. 2*C*) the co-varying trend was in the predicted direction (Figure 1.2*A*). Algal epiphyte loads on seagrass significantly decreased with increased sea otter density (P = 0.025, R^2 = 0.77) (Figure 1.2*D*). Lastly, 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) (Figure 1.2*E*) significantly increased with higher sea otter density.

1.3.3 Mesocosm and field experiments. To test the proposed mechanisms underlying the individual links in our ecological model (Figure 1.2*A*) 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 (*Phyllaplysia taylori* > 2 cm) sea slug mortality through observed predation by crabs (Figure 1.3*A*), which led to increased algal epiphyte loads (Figure 1.3*B*) and a net loss in eelgrass biomass and reduced rhizome elongation (Figure 1.3*C*) (Table 1.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.

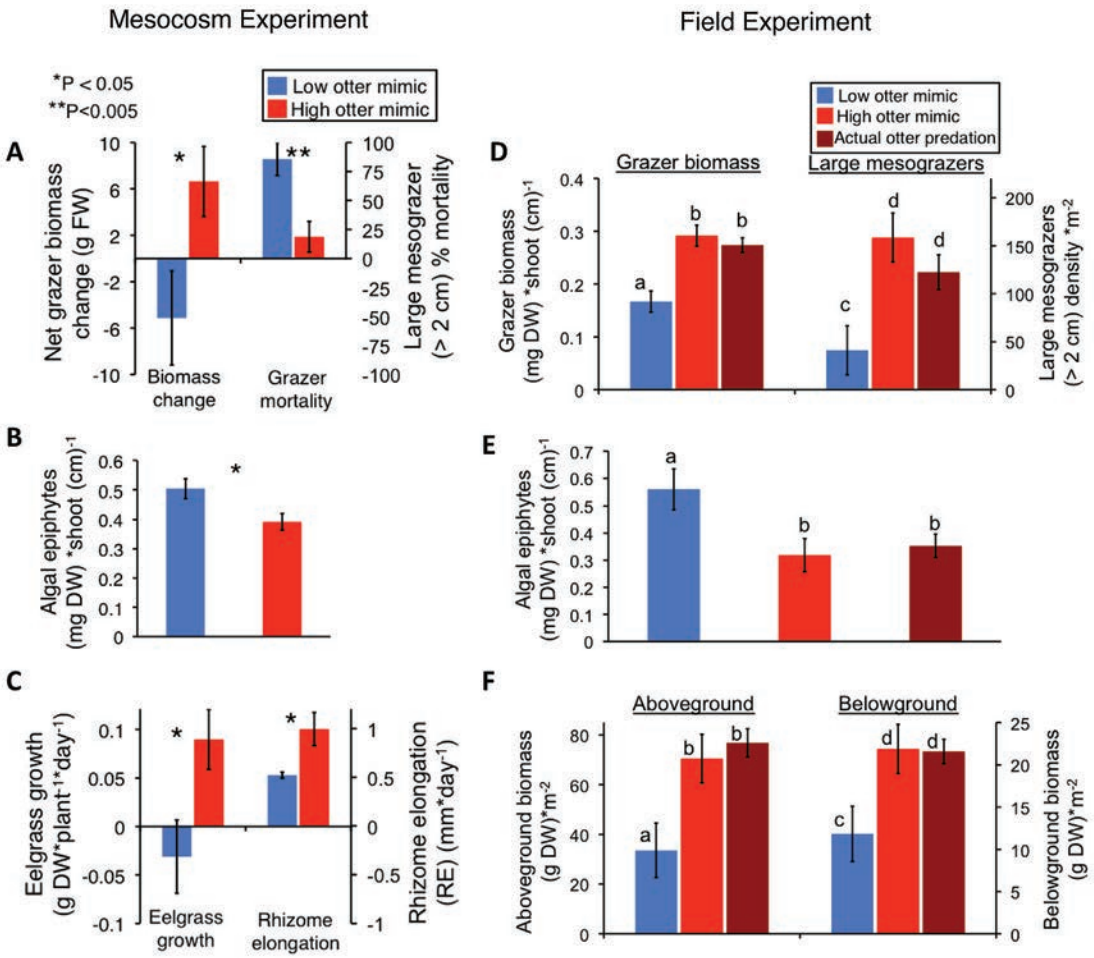

Figure 1.3. Results from a 30 d mesocosm experiment, (*A*-*C*) comparing the effects of simulated low (large crab treatment) (n = 8) and high (small crab treatment) (n = 7) sea otter predation on (*A*) net change in grazer biomass and grazer mortality; (*B*) algal epiphyte load, and (*C*) eelgrass growth and rhizome elongation (Table 1.S4). (*D*-*F*) Results from a 30 d field cage experiment (Figure 1.S5) testing for the effects of simulated low sea otter predation (+Crabs -Otters) (n = 6), simulated high sea otter predation (-Crabs -Otters) (n = 8), and actual high sea otter predation (which included: (1) 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 (2) 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's HSD tests (Table 1.S5). Note: DW = dry weight, FW = fresh weight, and error bars are ± 1SEM.

We verified the underlying mechanism of the sea otter-driven trophic cascade effects on eelgrass using a field cage experiment that tested for: no sea otter predation (crab inclusion), simulated sea otter predation (crab and otter exclusion), and actual sea otter predation (crabs and otters included). After one month, grazer biomass and large grazer density (*Phyllaplysia taylori* and *Idotea resecata* > 2 cm, the size class most likely to be consumed by crabs) were significantly greater (Figure 1.3*D*) in the cages with simulated and actual sea otter predation (Table 1.S5). As predicted, algal epiphyte loads were significantly lower (Figure 1.3*E*), and aboveground and belowground eelgrass biomass (Figure 1.3*F*) as well as shoot density (Table 1.S5) was significantly greater in treatments with actual and simulated sea otter predation.

1.4 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 (Williams and Ruckelshaus 1993; Heck et al. 2000; Hughes et al. 2004; Duffy 2006; Valentine and Duffy 2006; Heck and Valentine 2007; Moksnes et al. 2008; Baden et al. 2010; Lewis and Anderson 2012; Whalen et al. 2013) 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 over three decades (Sand-Jensen 1977; Orth and Montfrans 1984; Valiela et al. 1997; Valiela and Cole 2002; Hauxwell et al. 2003; Burkholder et al. 2007).

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 (Estes and Palmisano 1974; Estes et al. 2011): 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, not three trophic levels. 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 over eelgrass, and the epiphytic algae can harm eelgrass through shading effects in the absence of herbivory (Figure 1.2*A*). These indirect effects may be particularly pronounced in nutrient-loaded systems, which foster ephermeral algal growth. More broadly, multi-level trophic cascades involving indirect effects may be particularly important in systems with strong alteration of bottom-up controls (Silliman et al. 2005; Heck and Valentine 2007). Our findings highlight the importance of unraveling the potentially interactive nature of these key ecological processes when assessing the drivers of vegetated ecosystems.

1.5 METHODS

1.5.1 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 both eelgrass and sea otters. Surface water samples were collected monthly by hand and analyzed for Nitrate as nitrogen (µM) (*SI Methods*). We modeled the increase in nitrate concentrations by correlating the year to the mean annual nitrate concentration (n = 28) 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 bi-annual census counts from the U.S. Geological Survey (USGS) (http://www.werc.usgs.gov/). This database has summarized sea otter abundance in Elkhorn Slough from one-day surveys in the spring and fall from 1985-2012. Sea otters first entered Elkhorn Slough in 1984, and so for this year we used a study by Kvitek et al. (1988) 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 2004-2012 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 LOBO L01 mooring near the mouth of Elkhorn Slough (Jannasch et al. 2008). 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 to a long-term set of Acoustic Doppler Current Profiler data collected at the L01 mooring (Nidzieko and Monosmith 2013). 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 above 30 µM. The 30 µM threshold was chosen because nitrate in surface waters of Monterey Bay never exceeds this value (Johnson et al. 2006). 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 non-negligible load from ocean sources. The final annual load values were calculated by dividing nitrogen load (kg) by the total wetland area (ha) for Elkhorn Slough (Van Dyke and Wasson 2005). 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 2006-2010 to represent the most recent period of eelgrass and otter recovery for a global comparison with other estuaries (Valiela and Cole 2002; Hauxwell et al. 2003; Burkholder et al. 2007).

We tested for the effects of long-term otter predation on the Elkhorn Slough crab population by comparing two time periods: 1971-76 (a decade before otter immigration) and 2005-2009 (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 using a power function (Oftedal et al. 2007), 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 [1971-76], n = 26 [2005-09]) and mean daily carapace width for the two most abundant crab species *Cancer antennarius* (n = 14 [1971-76], n = 12 [2005-09]) and *C. productus* (n = 14 [1971-76], n = 11 [2005-09]). We compared crab biomass and size among the two time periods using an independent samples t-test.

We estimated eelgrass bed expansion within Elkhorn Slough as the percent change in eelgrass cover (ha) from 2006-2012 as a function of otter predation over the same survey period. Georeferenced aerial imagery from 24 May 2006 and 5 May 2012 was used to conduct object-based classification of the surface area extent of eelgrass beds (Figure 1.S3). Areas of suitable habitat for eelgrass were spatially

delineated using high resolution (2 m) multibeam bathymetry from 2005 and 2011 and aerial Light Detection And Ranging (LiDAR) (2 m) from 2004 and 2011 to create continuous digital elevation models in ArcMap v.10.1 (Environmental Systems Research Institute, Redlands, California, U.S.A.). To measure crab predation by sea otters, we utilized 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 using a previously-described Monte Carlo simulation algorithm for estimating prey-specific consumption rates from observational data while accounting for sampling uncertainty (Tinker et al. 2012). By multiplying the mean estimated consumption rate by the average density of otters in each eelgrass bed (Figure 1. 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 (Figure 1.S3). We used linear regression to determine the relationship between eelgrass bed expansion as a function of sea otter predation (n = 4).

1.5.2 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 (Figure 1.S2) (36˚ 48’ 45” N,

121˚ 46’ 10” W) 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 (see below) (although Tomales Bay had no sea otter surveys, since 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 10-12 m using 0.25 x 0.25 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 hr 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 one week 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 (Oftedal et al. 2007), and summed up the total biomass for each CPUE. CPUE was standardized to the total soak time (hrs) for each daily sampling effort. The mean CPUE and mean daily carapace width for the two most abundant crab species *Cancer 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 the summer of 2012. We counted all otters within each bed at the start of observations and at 15 minute intervals. Observation periods were 1-2 hours and were performed weekly to twice weekly at each bed during the study period (15 May 2012 – 29 July 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 number per square meter], eelgrass aboveground and belowground biomass [in grams per square meter]) from beds at Tomales Bay were compared to Elkhorn Slough (n = 4) 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).

1.5.3 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) (Figure 1.1*C*). We measured response parameters after 30 days 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 (Palacios and Zimmerman 2007) (*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 hr 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 (Figure 1.S4), and to include an actual sea otter predation treatment. 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: 1) simulated low otter predation (closed cage containing two large crabs), 2) simulated high otter predation (closed cage without crabs), 3) actual sea otter predation in the enclosure (cage open to otter and crab predation) and 4) actual sea otter predation without an enclosure (to serve as control for cage effects) (Figure 1.S5; *SI Methods*). Eelgrass shoot lengths were standardized and each cage was seeded with 20 large mesograzers. We used Analysis of Variance (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 using a test Tukey's Honestly Significant Difference (HSD) test (*SI Methods*).

1.6 SUPPORTING INFORMATION

1.6.1 METHODS

All analyses in this study had alpha set at 0.10 to avoid Type II errors that falsely fail to reject the null hypothesis (Underwood 1997) given the challenges of large-scale field sampling and experiments with low replication. All statistics were run using SPSS (version 20; IBM, Armonk, New York, U.S.A.).

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 (Hughes et al. 2011). 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: 1970-71 (Smith 1973), 1974-76 (Nybakken et al. 1977), 1977 from the California Central Coast Regional Water Quality Control Board, and 1989-2011 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, Georgia, U.S.A.), and habitat polygons were manually digitized using ArcView GIS 3.3 (ESRI, Redlands, California, U.S.A.). 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 years 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 due to increased quality of imagery and accuracy of groundtruthing.

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: 1971-76 (a decade before otter immigration) and 2005-2009 (two decades after otter immigration). The 1970s data set was from a study by Nybakken et al. (1977) and the Monterey Bay National

Marine Sanctuary's (MBNMS) Sanctuary Integrated Monitoring Network (SIMoN), and the 2005-09 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 using standard recreational traps (0.1587 m^3) composed of either nylon or wire mesh wrapped around a circular metal frame and baited with either fish, mussels, squid, or shrimp. More recent surveys from 2005-2009 used smaller sized traps (0.0621 m^3) than the 1970s and were constructed out of nylon mesh wrapped around a rectangular metal frame and were baited with anchovies. There were differences in the hours spent crab trapping, 2-8 hours for 1971-76 and 24 hours for 2005-09. However, we found that crab traps from the 1970s generally reached saturation in the 2-8 hr sampling period, and traps from 2005-09 rarely became saturated. Therefore, we did not correct for the differences in soak time so as to avoid any erroneous inflation of data from the 1970s that would bias our results. We did standardized 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 utilized existing data on sea otter distribution and abundance available from standardized bi-annual censuses conducted by the U.S. Geological Survey Western

Ecological Research Center and the California Department of Fish and Wildlife (GIS-compliant data from these censuses are available at http://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 10x binoculars and 50x Questar spotting scopes (Questar Corp, New Hope, PA) 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, using the most recent five years of census count data (2007-2012), over which period sea otter numbers have been approximately stable, we fit a 2-dimensional kernel density smoother using the Spatial Analyst toolbox in ArcGIS 10.1 (ESRI, Redlands, California, USA), and using a 2.5 km smoothing window. The resulting surface provided localized estimates of average otter density (otters $*km^{-2}$) 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 (Figure 1.S2).

To measure crab predation by sea otters, observational data were recorded from feeding otters using high powered (50-80x) telescopes (Questar Corporation). Otters were selected haphazardly for data collection (i.e. without regard for location, status or prey type), and approximately 10,200 feeding dives (occurring in 248

independent feeding bouts) were recorded between 1999 and 2012. For each dive, observers recorded sub-surface dive duration, inter-dive surface interval, success of the dive (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 using a Monte Carlo simulation-based algorithm described elsewhere (Tinker et al. 2012) 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 approximately 43% (± 2.1%) of the biomass consumed by otters in the slough (Figure 1.S1). A typical sea otter consumed 4.69g (+/- 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 (mm) to corresponding estimates of mean edible biomass (g) and total biomass per crab (edible biomass = 0.0077*carapace diameter $^{0.2265}$, $R^2 = 0.97$, and edible biomass = 65% of total mass; see Oftedal et al. 2007). 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 (Costa and Kooyman 1982).

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 over 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 in order to estimate mean crab predation rates in 1 ha areas of each bed from 2007-2012.

We used a cross-validation technique to corroborate estimates of sea otter density and crab predation rates in Elkhorn Slough eelgrass beds. 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 (see *Spatial Comparison* Methods, Figure 1. 2) to the more long-term estimates of sea otter density and crab predation in each bed from 2007-2012 (see above paragraphs on estimation of otter predation rates, Figure 1.1*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

Crab survey methods

Traps used were shrimp pots (61 x 61 x 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 2-3 days. 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 five 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, Nebraska, U.S.A.) to 50% ambient light. In both the mesocosms and Elkhorn Slough eelgrass populations PAR at the canopy was ~50% ambient during mid-day 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). Prior to 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 pre-experimental 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 using cotton pads. During the experiment, algal epiphytes (primarily diatoms) recruited to eelgrass leaves from propagules already occurring in the seawater system. 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% [± 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 *Pugettia 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 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} [± 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 (Figure 1.S4) (http://www.mbari.org/lobo/loboviz.htm). 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 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, 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 x 50 x 50 cm cages (Figure 1.S5). For +Crabs -Otters and -Crabs -Otters cages chicken wire mesh (2.5 x 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. Lastly, 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 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 accept 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 v. Open Controls. The mean PAR in cage treatments was 329.1 µmol photons $*m^{-2}$ $*s^{-1}$, and was 37% the subsurface PAR, both of these values are well within saturating light levels for *Zostera* spp. (Zimmerman et al. 1995, Zimmerman 2006).

Prior to 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 pre-experimental differences ($P > 0.10$) in shoot densities. A 1 m x 1 m buffer zone was created by cutting all of the shoots surrounding the experimental 50 x 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* sp. are swimmers that readily accessed all treatments (B. Hughes, pers. obs.), so we did not seed them into the treatments. Lastly, we added one *Cancer* spp. (80-100 mm) crab and one *Pugettia producta* (60-80 mm) crab to each +Crabs –Otters cage. The crab size was selected based on size selection of otters (Figures 1.1*C* and 1.2*B*) and size-related grazer predation rates as determined by the mesocosm experiment (Figure 1.3*A*). The sizes for each crab species were in the high range for Elkhorn Slough. *Cancer* crabs are benthic carnivores, while *Pugettia* are canopy-dwelling omnivores primarily feeding on algae yet switching to invertebrates in the absence of algae (Ricketts and Calvin 1992; Morris et al. 1980). Mesocosm

experiments determined that both species of crabs eat sea slugs in seagrass systems and can significantly reduce their densities (Figure 1.3*A*). Foraging surveys conducted in the eelgrass beds during the experimental period determined the crabs constitute a high proportion of total prey consumed (~45%), 60% *Cancer* and 40% *Pugettia* respectively.

The experiment lasted for one month. During that period we used self contained underwater breathing apparatus (SCUBA) to inspect cages for crabs, sea slugs, and structural integrity, as well as scrubbing cages to clean off of 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 one month 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 due to a mortality event of the original *Cancer* crab. After one month we counted all shoots and harvested five shoots from all of 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 hr 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 7cm]) (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 Analysis of Variance (ANOVA) (SPSS v. 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 using a Kolmogorov-Smirnov test. To conserve degrees of freedom we first compared control treatments (+Crabs +Otters and Open Control) 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 using a Tukey's Honestly Significant Difference (HSD) test.

1.6.2 FIGURES

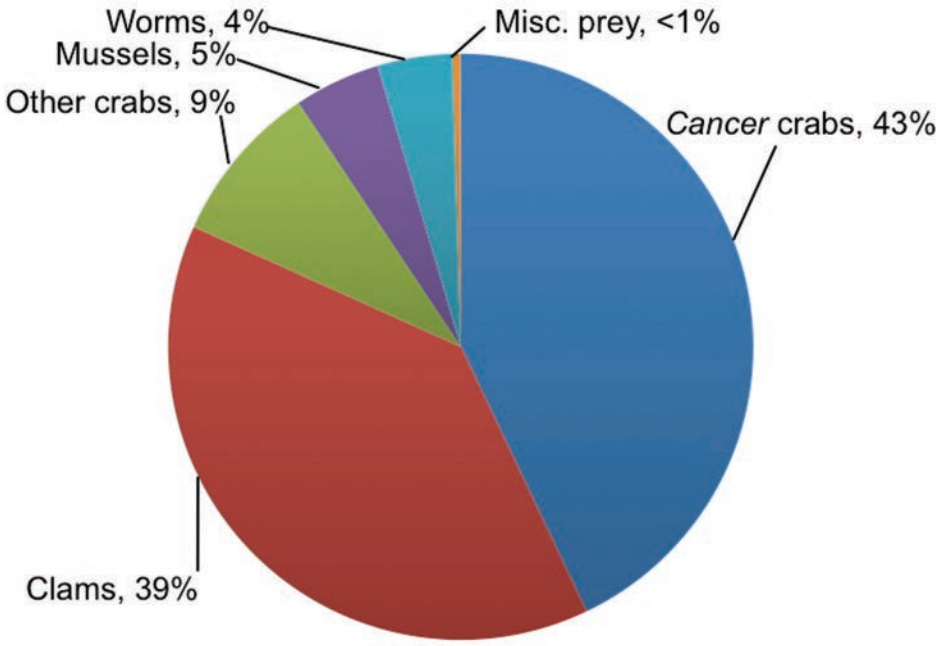

Figure 1.S1.
Predation by sea otters by prey type from approximately 10,200 feeding dives (occurring in 248 independent feeding bouts) recorded between 1999 and 2012 in Elkhorn Slough.

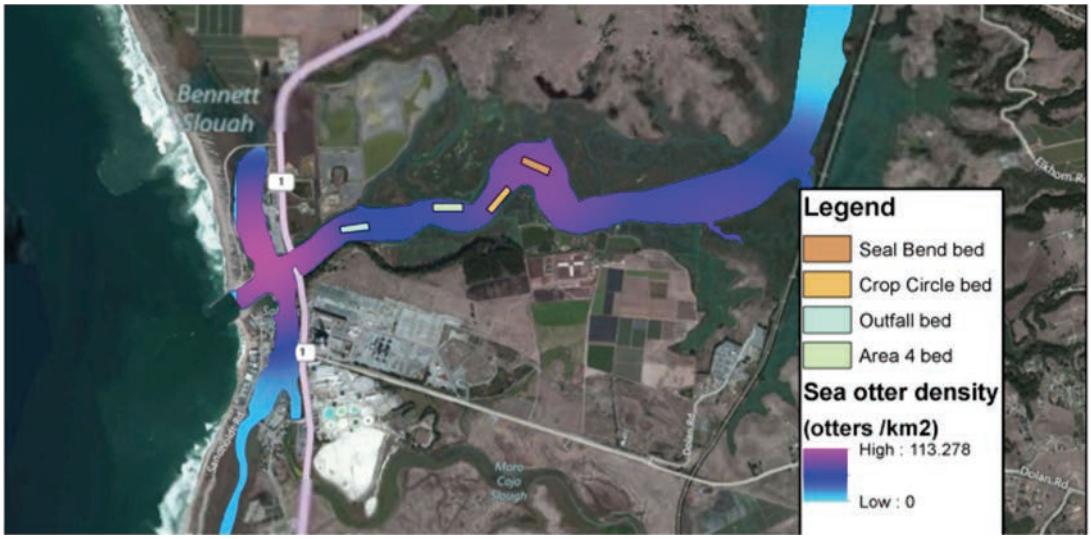

Figure 1.S2.
Survey locations in Elkhorn Slough (n = 4). Rectangular areas are 1 ha plots that encompassed eelgrass community transects and crab surveys for 2012, as well as otter crab predation estimates for 2007-2012. Otter densities were determined using bi-annual surveys (2007-2012) and calculated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

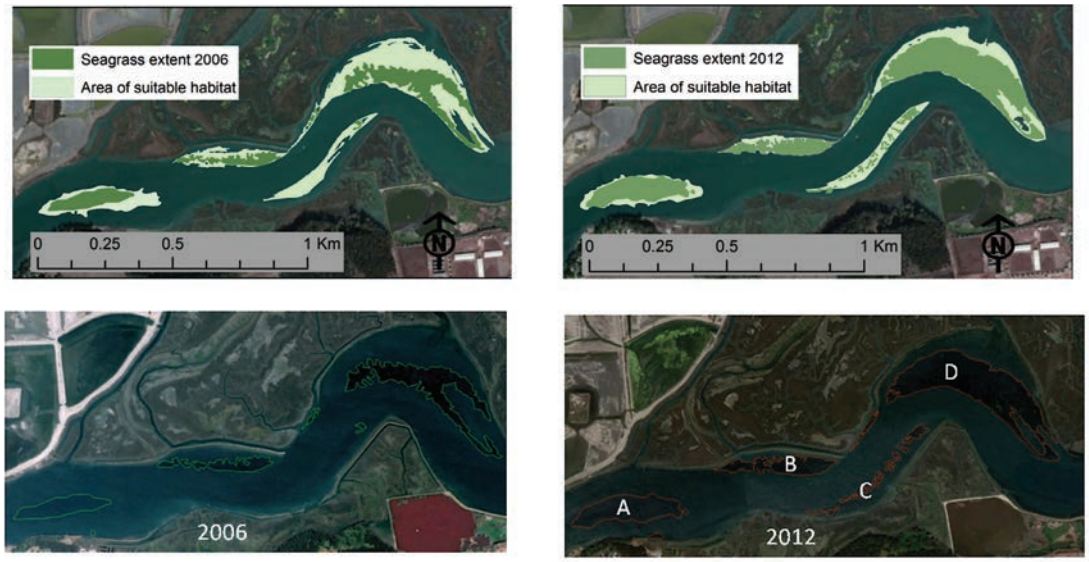

Figure 1.S3.
Object based image analysis of the extent of eelgrass beds in 2006 and 2012. Eelgrass bed expansion was compared over time across four sites: (A) Outfall, (B) Area 4, (C) Crop Circles, (D) Seal Bend. High resolution (1 m) digital elevation models (DEMs) of the Elkhorn Slough main channel from 2005 and 2011 were used to estimate the changes in the area (ha) 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 LiDAR 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 (Figure 1.S2) (Table 1.S1*D*).

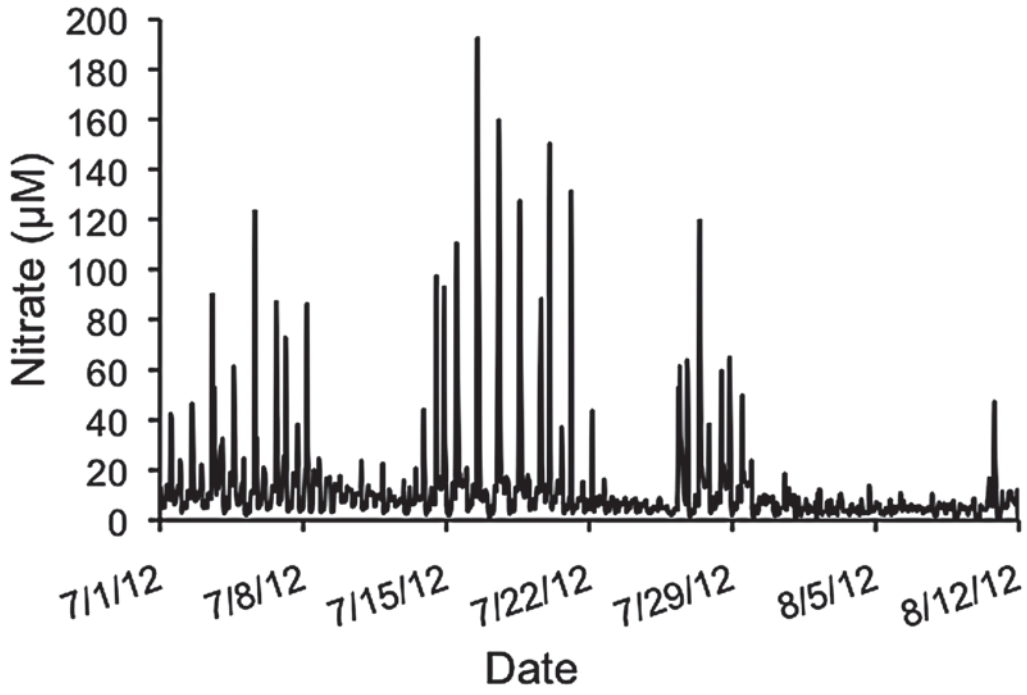

Figure 1.S4.

Nitrate data showing elevated concentrations during the 2012 survey and field experimental period in Elkhorn Slough. Hourly data was collected in situ using an ISUS (In Situ Ultraviolet Spectrophotometer) nitrate sensor attached to the Monterey Bay Aquarium Research Institute's Land/Ocean Biogeochemical Observatory sensor mooring (Jannasch et al. 2008).

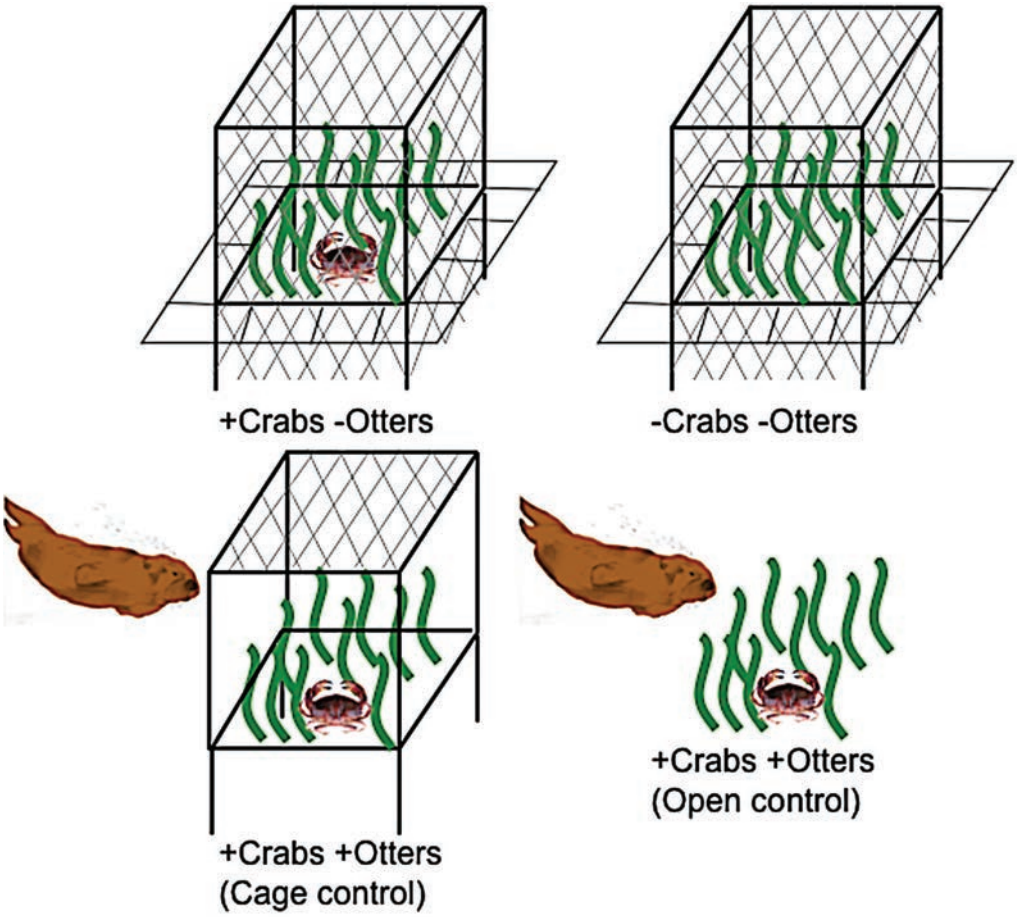

Figure 1.S5.

Cage experimental design (50 cm x 50 cm x 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/cage).

1.6.3 TABLES

Table 1.S1. Historical analysis of Elkhorn Slough for (*A*) exponential regression of mean annual nitrate concentration (µmol *L^{-1}) as a function of time (year) (n = 28) (Figure 1.1*A*), (*B*) Land derived nitrogen loading rates by year (Figure 1.1*B*), (*C*) linear regression analysis of annual eelgrass cover as a function of the average sea otter density (n = 10), (*D*) Independent samples t-test comparing two periods: (1) before sea otter migration (1971-1976) and (2) post sea otter migration (2005-2009) for crab biomass (g FW *$trap^{-1}$), and *C. antennarius* and *C. productus* carapace widths (mm) (Figure 1. 1*C*; see *Methods* for sample size description), and (*E*) linear regression analysis of eelgrass bed expansion (% increase 2006-2012 as a function of available habitat) correlated with estimated sea otter predation rates on crabs in standardized 1 ha plots in each bed (n = 4) (Figure 1.1*D*). Significant variables are in bold.

(A) Nitrate v. time

Dependent variable	R^2	ß	*df*	*F*	*P*
Nitrate	0.902	0.099	1	229.1	**<0.0005**

(B) Land-based nutrient loading rates for Elkhorn Slough

Year	*Kg (nitrogen) *ha^{-1} *$year^{-1}$*
2004	342
2005	N/A
2006	315
2007	354
2008	347
2009	462
2010	470
2011	493
2012	471

(C) Sea otter density v. eelgrass cover

Dependent variable	R^2	ß	*df*	*F*	*P*
Eelgrass cover	0.519	0.069	1	8.623	**0.019**

(D) Crab biomass and size before and after sea otter migration

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
Crab biomass	-1176.759	218.664	-5.382	41	**<0.0005**
C. antennarius	-35.521	4.690	-7.360	23	**<0.0005**
C. productus	-35.011	6.292	-5.564	24	**<0.0005**

(E) Sea otter crab predation v. eelgrass bed expansion

Dependent variable	R^2	ß	*df*	*F*	*P*
Eelgrass bed expansion	0.958	0.001	1	45.550	**0.021**

Table 1.S2.
Independent samples t-test comparing Tomales Bay to Elkhorn Slough (Figure 1.2*B-E*) eelgrass beds (n = 4) for crab biomass (g FW CPUE), *C. antennarius* and *C. productus* carapace widths (mm), grazer biomass (g DW) *shoot $(cm)^{-1}$, large mesograzer (> 2 cm) density $*m^{-2}$, algal epiphyte biomass (g DW) *shoot $(cm)^{-1}$, shoot density $*m^{-2}$, aboveground eelgrass mass (g DW) $*m^{-2}$, and belowground eelgrass mass (g DW) $*m^{-2}$. Significant differences for dependent variables are in bold.

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
Crab biomass[1]	-0.730	0.268	-2.728	6	**0.034**
C. antennarius[1,2]	-0.083	0.029	-2.878	3.6	**0.034**
C. productus[1]	-0.219	0.058	-3.773	6	**0.009**
Grazer mass	0.343	0.189	1.867	6	0.164
Large grazer density	50.031	32.885	1.521	6	0.179
Algal epiphyte mass	0.855	0.832	1.028	6	0.379
Shoot density	7.219	7.679	0.940	6	0.383
Aboveground mass	151.913	55.974	2.714	6	**0.035**
Belowground mass	18.864	11.860	1.591	6	0.163

[1]Log-transformed data, [2]Used Welch's t-test of unequal variances

Table 1.S3.

Regression analysis testing a sea otter density gradient at Elkhorn Slough (Figure 1.2*B-E*) eelgrass beds (n = 4) on crab biomass (g FW CPUE), *C. antennarius* and *C. productus* carapace widths (mm), grazer biomass (g DW) *shoot (cm)$^{-1}$, large mesograzer (> 2 cm) density *m^{-2}, algal epiphyte biomass (g DW) *shoot (cm)$^{-1}$, shoot density *m^{-2}, aboveground eelgrass (g DW) *m^{-2} and belowground eelgrass biomass (g DW) *m^{-2}. Significant otter density effects are in bold.

Dependent variable	R^2	ß	*df*	*F*	*P*
Crab biomass[1]	0.916	-0.304	1	21.944	**0.043**
C. antennarius[1]	0.921	-0.030	1	23.216	**0.040**
C. productus[1]	0.882	-0.058	1	14.933	**0.061**
Grazer mass	0.537	0.183	1	2.318	0.267
Large grazer density	0.919	40.345	1	22.617	**0.041**
Algal epiphyte mass[2]	0.768	0.842^c	1	17.515	**0.025**
Shoot density	0.993	8.463	1	296.823	**0.003**
Aboveground mass	0.977	66.657	1	84.549	**0.012**
Belowground mass	0.974	14.547	1	74.853	**0.013**

[1]Log-transformed data

[2]Data fit using negative exponential regression, y = 1*(x^c)

Table 1.S4.
Results from a 30 d mesocosm experiment (Figure 1.3*A*-*C*) using independent samples t-tests testing for the simulated effects of low (large crabs) (n = 7) and high (small crabs) (n = 8) sea otter predation on the following eelgrass community properties: net change in sea slug biomass (g FW)*mesocosm^{-1}, large sea slug (> 2 cm) mortality *mesocosm^{-1}, algal epiphyte biomass (g DW) *shoot (cm)$^{-1}$, individual eelgrass plant growth (g FW *day^{-1}), and eelgrass rhizome elongation (mm *plant^{-1} *day^{-1}). Significant differences for dependent variables are in bold.

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
Sea slug mass	-11.772	4.978	-2.365	13	**0.034**
Large sea slug mortality	-66.964	19.396	-3.452	13	**0.004**
Algal epiphyte mass	0.556	0.227	2.448	12	**0.031**
Eelgrass growth	-0.121	0.048	-2.492	13	**0.027**
Rhizome growth	-0.473	0.208	-2.277	13	**0.040**

Table 1.S5.
Results from a field caging experiment (Figure 1.3*D-F*) using a randomized blocked ANOVA testing for (i) differences among control treatments (n = 8), (ii) full design testing for the effects of treatment (fixed) and block (random) on (*A*) grazer mass (g DW *cm shoot^{-1}), (*B*) large mesograzer (> 2 cm) density (m^{-2}), (*C*) algal epiphyte mass (g DW *cm shoot^{-1}), (*D*) shoot density (shoots *m^{-2}), and (*E*) aboveground and (*F*) belowground (g DW *m^{-2}) biomass. (iii) Tukey's HSD multiple comparison testing for differences of individual treatments if there was a significant treatment effect from the randomized blocked ANOVA. Significant factors are in bold. Note: sample sizes varied for treatments depending on outcomes of (i), if no significant differences occurred among controls then the two control types (Cage and Open Controls) were grouped. Crab treatments had a lower sample size (n = 6) for epiphytes, shoot density, and eelgrass biomass due to crab damage to eelgrass shoots in two of the cages.

(A) Grazer mass

(i) Randomized blocked ANOVA test between control treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.006	1	0.006	2.475	0.160
	Error	0.018	7	0.003		
Block	Hypothesis	0.309	7	0.044	16.918	**0.001**
	Error	0.018	7	0.003		

(ii) Randomized blocked ANOVA test among all treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.078	2	0.039	12.312	**<0.0005**
	Error	0.069	22	0.003		
Block	Hypothesis	0.573	7	0.082	25.992	**<0.0005**
	Error	0.010	22	0.000		

(iii) Tukey's HSD multiple comparison test among treatments

Treatment1	*Treatment2*	*Mean Difference (1-2)*	*Standard Error*	*P*
Control	**Crab Cage**	0.107	0.024	**0.001**
No Crab Cage	**Crab Cage**	0.124	0.028	**0.001**
Control	No Crab Cage	-0.018	0.024	0.746

Table 1.S5. (continued)

(B) Large mesograzer (> 2 cm) density

(i) Randomized blocked ANOVA test between control treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	255.520	1	255.520	0.201	0.668
	Error	8907.073	7	1272.439		
Block	Hypothesis	11431.133	7	1633.019	1.283	0.375
	Error	8907.073	7	1272.439		

(ii) Randomized blocked ANOVA test among all treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	196579.263	2	98289.631	5.677	**0.010**
	Error	380879.535	22	17312.706		
Block	Hypothesis	215518.524	7	30788.361	1.778	0.143
	Error	380879.535	22	17312.706		

(iii) Tukey's HSD multiple comparison test among treatments

(1) Treatment1	*(2) Treatment2*	*Mean Difference (1-2)*	*Standard Error*	*P*
Control	**Crab Cage**	148.183	56.975	**0.042**
No Crab Cage	**Crab Cage**	213.875	65.789	**0.010**
Control	No Crab Cage	-65.693	56.975	0.493

Table 1.S5. (continued)

(C) Algal epiphyte mass

(i) Randomized blocked ANOVA test between control treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.000	1	0.000	0.022	0.885
	Error	0.107	7	0.015		
Block	Hypothesis	0.781	7	0.112	7.266	**0.009**
	Error	0.107	7	0.015		

(ii) Randomized blocked ANOVA test among all treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.220	2	0.110	3.656	**0.044**
	Error	0.602	20	0.030		
Block	Hypothesis	1.285	7	0.184	6.098	**0.009**
	Error	0.602	20	0.030		

(iii) Tukey's HSD multiple comparison test among treatments

(1) Treatment1	*(2) Treatment2*	*Mean Difference (1-2)*	*Standard Error*	*P*
Control	**Crab Cage**	-0.255	0.083	**0.016**
No Crab Cage	**Crab Cage**	-0.288	0.094	**0.016**
Control	No Crab Cage	0.033	0.075	0.898

Table 1.S5. (continued)

(D) Shoot density

(i) Randomized blocked ANOVA test between control treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	6.8	1	6.8	0.074	0.794
	Error	646.6	7	92.4		
Block	Hypothesis	4599.4	7	657.1	7.113	**0.010**
	Error	646.6	7	92.4		

(ii) Randomized blocked ANOVA test among all treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	6547.2	2	3273.6	13.609	**<0.0005**
	Error	4811.0	20	240.5		
Block	Hypothesis	6929.9	7	990.0	4.116	**0.006**
	Error	4811.0	20	240.5		

(iii) Tukey's HSD multiple comparison test among treatments

(1) Treatment1	*(2) Treatment2*	*Mean Difference (1-2)*	*Standard Error*	*P*
Control	**Crab Cage**	33.35	7.425	**0.001**
No Crab Cage	**Crab Cage**	29.00	8.376	**0.007**
Control	No Crab Cage	4.35	6.716	0.525

Table 1.S5. (continued)

(E) Aboveground biomass

(i) Randomized blocked ANOVA test between control treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	1202.2	1	1202.2	2.333	0.170
	Error	3607.1	7	515.3		
Block	Hypothesis	8531.9	7	1218.9	2.365	0.139
	Error	3607.1	7	515.3		

(ii) Randomized blocked ANOVA test among all treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	7835.8	2	3917.9	7.693	**0.003**
	Error	10186.1	20	509.3		
Block	Hypothesis	12099.8	7	1728.5	3.394	**0.015**
	Error	10186.1	20	509.3		

(iii) Tukey's HSD multiple comparison test among treatments

(1) Treatment1	*(2) Treatment2*	*Mean Difference (1-2)*	*Standard Error*	*P*
Control	**Crab Cage**	37.67	10.804	**0.006**
Crab Cage	**No Crab Cage**	31.33	12.188	**0.046**
Control	No Crab Cage	6.34	9.772	0.795

Table 1.S5. (continued)

(F) Belowground biomass

(i) Randomized blocked ANOVA test between control treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	16.4	1	16.383	0.664	0.442
	Error	172.8	7	24.7		
Block	Hypothesis	139.0	7	19.9	0.805	0.609
	Error	172.8	7	24.7		

(ii) Randomized blocked ANOVA test among all treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	437.6	2	218.8	6.512	**0.007**
	Error	672.0	20	33.6		
Block	Hypothesis	329.9	7	47.1	1.403	0.258
	Error	672.0	20	33.6		

(iii) LSD multiple comparison test among treatments

(1) Treatment1	*(2) Treatment2*	*Mean Difference (1-2)*	*Standard Error*	*P*
Control	**Crab Cage**	8.332	2.775	**0.018**
No Crab Cage	**Crab Cage**	8.632	3.130	**0.031**
Control	No Crab Cage	-0.300	2.510	0.906

2. Chapter 2 - A trophic cascade at the seagrass-macroalgal ecotone enhances seagrass resilience

Citation: Hughes, B.B., K.K. Hammerstrom, N.E. Grant, U. Hoshijima, R. Eby, K. Wasson. 2014. A trophic cascade at the seagrass-macroalgal ecotone enhances seagrass resilience. *In prep. Ecology.*

2.1 ABSTRACT

Many coastal ecosystems worldwide have declined in biodiversity or function, with a variety of human alterations identified as likely causes of the declines. For seagrasses, a century-old paradigm has targeted macroalgal blooms stimulated by anthropogenic nutrient loading as one of the primary drivers of seagrass decline. In this study we demonstrate how a recovering top predator, the sea otter (*Enhydra lutris*), can shift species interactions in favor of seagrass (*Zostera marin*a) expansion in Elkhorn Slough, CA an estuary with extreme nutrient loading and macroalgal blooms. Using a series of field experiments at the seagrass bed interior and edge we show how sea otters, through a trophic cascade, can enhance seagrass in the interior and at the seagrass-macroalgal ecotone. However, the pathways by which seagrass was enhanced varied from the interior and the ecotone. At the interior of the bed sea otter consumption of crabs promoted invertebrate grazers of algal epiphytes growing on seagrass, causing enhanced seagrass biomass and preventing declines in shoots. At the seagrass-macroalgal ecotone sea otter consumption of crabs lowered crab consumption of the bloom-forming alga *Ulva lactuca*, enhancing the epiphyte-reducing grazer assemblage associated with *Ulva*, thus promoting seagrass expansion

and resilience at the ecotone. These results emphasize the importance of investigating species interactions at edges of vegetated habitats, along with the interior, when determining their resilience, and highlight the potential for top predator recoveries to enhance ecosystem resilience.

2.2 INTRODUCTION

Determining the drivers and mechanisms of collapse and resilience in ecosystems is a fundamental goal of ecology and conservation (Hughes 1994; Jackson *et al.* 2001; Lotze *et al.* 2006). Due to widespread human pressure and the subsequent collapse of many global systems there is a heightened urgency to understand the factors driving ecosystem declines (Lotze *et al.* 2006; Worm *et al.* 2006; Waycott *et al.* 2009; Silliman *et al.* 2012). One factor driving ecosystem collapse is a change in limiting resources that alters the competitive balance between two dominant species that are the foundation of different ecosystem states (Valiela *et al.* 1997). At times the two conflicting species phases can form distinct ecotones (Konar & Estes 2003), where dynamics in species interactions can be critical to understanding resilience and shifts in dominance. Ecotones, the transition zones between adjacent ecological systems, may be particularly sensitive to environmental changes (Risser 1995), and are critical for understanding landscape change (Peters *et al.* 2006).

In marine systems, such as coral reefs (Hughes 1994; McCook 1999) and seagrasses (Valiela *et al.* 1997; Burkholder *et al.* 2007), the delivery of anthropogenic

nutrients (bottom-up effects) can shift competitive balances in favor of macroalgal phases (McGlathery 2001; Thomsen *et al.* 2012). For seagrasses, a century-old paradigm has been that eutrophication resulting from anthropogenic nutrient loading leads to phase shifts from seagrass to ephemeral macroalgae (Letts & Adeney 1908; Valiela *et al.* 1997; Burkholder *et al.* 2007). There are two pathways that can cause the shift in dominance: 1) shading effects of epiphytic algae that decrease the photosynthetic output of seagrass leaves (Zimmerman 2010) and thus indirectly favor macroalgae over eelgrass, and 2) direct smothering of existing and newly formed eelgrass shoots by macroalgae (Nelson & Lee 2001). However, these two pathways are not mutually exclusive, and could be acting synergistically to decrease resiliency in seagrass.

Another factor resulting in ecosystem collapse comes from the alteration of food webs through trophic downgrading – the loss of top predators (Estes *et al.* 2011). Conversely, it has been demonstrated that trophic upgrading can alter bottom-up effects resulting in shifts in species dominance (Croll *et al.* 2005). For seagrasses, the last few decades of research has generated theory (Valentine & Duffy 2010) on how recovery of top predators can enhance resilience of seagrass beds under threat from eutrophication. Recently, results from experimental and long-term monitoring studies have demonstrated that resilience 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).

Along the central coast of California, sea otters (*Enhydra lutris*) are slowly recovering from near extinction (Lafferty & Tinker 2014), inhabiting coastal ecosystems where they have been demonstrated to generate a trophic cascade that benefits eelgrass (*Zostera marina*) through predatory control of crabs, enhancing the crabs' mesograzer prey that reduces shade-causing algal epiphytes (Hughes *et al.* 2013). In Elkhorn Slough, sea otter densities are the highest recorded for California and eelgrass has been rapidly expanding after nearly going locally extinct from intense eutrophication driven by anthropogenic nutrient loading from a highly agricultural watershed (Hughes *et al.* 2013). In the lower half of the estuary, eelgrass has been expanding into areas dominated by macroalgae, resulting in a shift in dominance back to its historical, pre-nutrient enriched state.

Using a series of field experiments at the eelgrass bed interior and edge (the seagrass-macroalgal ecotone) we tested the trophic effects of sea otters on the interaction between eelgrass, algal epiphytes, and the bloom-forming macroalgal species *Ulva lactuca* (Figure 2.1). Specifically, we hypothesized that sea otters could generate a trophic cascade that enhances algal-reducing grazers at the seagrass-macroalgal ecotone thus leading to enhanced eelgrass biomass and shoot production (indicators of bed stability and resilience) and decreased algal dominance. Like many seagrass systems (Duarte *et al.* 2010) eelgrass in Elkhorn Slough has experienced resilience through radial bed contraction and expansion through changes in rhizomatic vegetative growth and new shoot formation. Therefore, species interactions at the ecotone, where dominant species overlap at the edges of their

distribution, could be essential for developing a mechanistic understating of regime shifts. Furthermore, demonstrations of ecosystem recovery, despite continued intense anthropogenic stress, are rare in nature, so studies examining shifts back to a historic state supporting valued ecosystem services are critical for informing management and restoration.

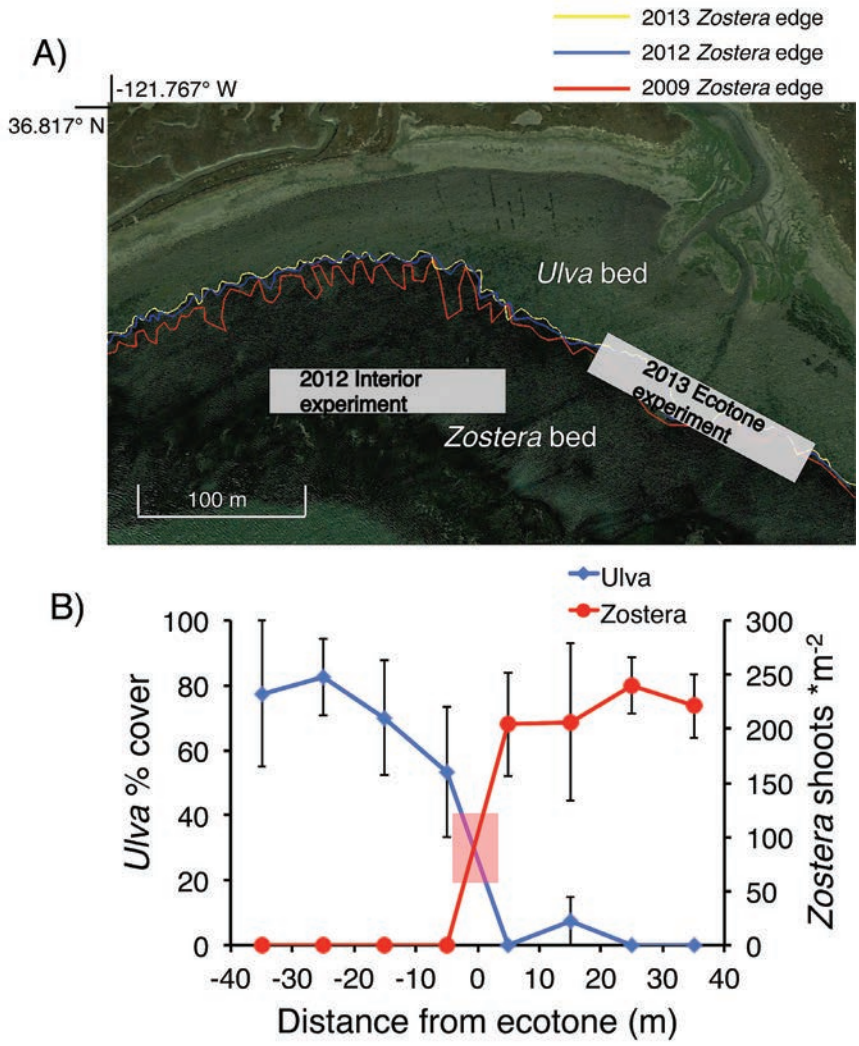

Figure 2.1. Location of cage experiments testing for sea otter effects to eelgrass stability and resilience. (A) Aerial view of the seagrass-macroalgal ecotone showing the northward expansion of eelgrass into the *Ulva* zone from 2009-2013. Colored lines indicate the ecotone boundary for each year. The white boxes indicate where the cage experiments occurred. (B) Profile of the seagrass-macroalgal ecotone based on 70 m transect surveys (n = 5) from 2011 sampling across the seagrass-macroalgal ecotone. The red box demonstrates where the experimental cages were placed along the ecotone. Error bars are ±1 SE.

2.3 MATERIALS AND METHODS

Elkhorn Slough is characterized by extreme nutrient loading (Hughes *et al.* 2011, 2013), 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 (Figure 2.S1), co-occurring with periods of peak eelgrass productivity (Olesen & Sand-Jensen 1994; Zimmerman *et al.* 1995). Despite intense eutrophication, eelgrass has experienced a rapid recovery since the early 1980s, a process that is 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 2 m y^{-1} (Figure 2.1A), and are displacing areas of high macroalgal cover (Figure 2.1B). In Elkhorn Slough eelgrass bed recovery has occurred through new patch formation (recruitment of seedlings) and through expansion of newly formed and existing beds. The seagrass-macroalgal ecotone 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* is capable of higher desiccation stress (Gao *et al.* 2011) and therefore extends higher in the intertidal then eelgrass (Figures 2.1A-B). Both eelgrass and *Ulva* are limited by light towards the lower end of their elevation, yet these light requirements differ (0.5-4% surface irradiance *Ulva*; 11% surface irradiance eelgrass) (Sand-Jensen 1988; Duarte 1991; Palacios & Zimmerman

2007), and therefore these differences in light requirements are not likely to be setting the seagrass-macroalgal ecotone boundary since eelgrass occurs lower in tidal elevation in Elkhorn Slough.

2.3.1 Sea otter effects at the seagrass interior and seagrass-macroalgal ecotone. To test for the effects of sea otters on eelgrass stability and resilience we established predator exclusion-inclusion cage experiments in subsequent years in the interior (2012, Hughes et al. 2013) and at the seagrass-macroalgal ecotone (2013) of a rapidly expanding eelgrass bed (Figure 2.1A) with high sea otter densities. We estimated sea otter densities in this area one 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 minutes during the three to four hour surveys (n = 8, 2012; n = 9, 2013). Previous estimates of crab consumption in the experimental area determined that sea otters were capable of consuming ~8000 crabs ha^{-1} $year^{-1}$ (Hughes *et al.* 2013), essentially eliminating predatory crab effects on small invertebrates that graze algal epiphytes from the eelgrass.

We established the caging experiments by using a randomized block design that consisted of eight blocks and four treatments: (i) cages including crab and excluding sea otters and other mesopredators (e.g., small fish and other unaccounted for predators) (−otters +crab –mesopredator), (ii) cages excluding sea otters, crabs, and mesopredators (−otters –crab –fish), (iii) a 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 +crabs +mesopredator), and (iv) a cage-free control (open control). The −otters +crab –mesopredator cages simulated an otter-free environment, the −otters –crab –mesopredator cages simulated a high predatory otter environment, and the +crabs +otters +mesopredator and open control allowed for natural predation by sea otters, crabs and other mesopredators, as well as access by drifting *Ulva* to occur. The interior portion of the eelgrass bed had no algae during the 2012 experiment and cover was low in 2011 surveys (Figure 2.1B), indicating that eelgrass shoot densities were likely saturated to the point where macroalgae could not persist. Prior to the 2013 seagrass-macroalgae ecotone experiment we sampled *Ulva* biomass in 50 cm x 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 grazer 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 grazers and epiphytes by removing most of their biomass. We created a 1 m x 1 m buffer zone by cutting all the shoots surrounding the 50 cm x 50 cm experimental area down to 20 cm to prevent shading by taller bordering shoots. 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 recruitment of algal epiphytes and mesograzers, yet restricting predator and *Ulva* movement. For the seagrass-macroalgal ecotone experiment we placed cages directly over the ecotone where ~50% of the caged area was composed of eelgrass shoots and the other 50%

was composed of *Ulva* (Figure 2.S2). We seeded all experimental plots with 20 large (> 2 cm) epiphyte grazers (Taylor's seahare, *Phyllaplysia taylori*) to ensure grazers were present during the experiment. Last, we placed one large (> 80 mm carapace width – CW) *Cancer* sp. crab and one large (> 60 mm CW) *Pugettia producta* crab in the +crab –otter –mesopredator cages for the 2012 interior experiment, we only placed one large *Cancer* sp. crab in the +crab –otter –mesopredator cages for the 2013 ecotone experiment as previous crab trapping and SCUBA surveys found few *Pugettia* crabs at the seagrass-macroalgal ecotone.

The experiments 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 grazer and epiphyte assemblages. We counted all mesograzers (> 0.5 mm) on the *Ulva* and on the eelgrass in each plot, and separated them into two size classes (small < 2 cm) and large (> 2 cm). All algal epiphytes were scraped from five representative eelgrass shoots from each plot, dried at 60℃ for 24 h 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 v. 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 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 grazer 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 by using a Kolmogorov–Smirnov test, and log transformed when appropriate. To conserve 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. All ANOVAs and final Tukey HSD tests in this study had α set at 0.10 to avoid type II errors that falsely fail to reject the null hypothesis (Underwood 1997) given the challenges of multi-trophic experiments with low replication.

2.3.2 Modeling the sea otter trophic cascade at the seagrass interior and seagrass-macroalgal ecotone. To further develop a mechanistic understanding on

how sea otters can affect trophic dynamics and eelgrass resilience we used Structural Equation Modeling (SEM) (SPSS Amos software, version 22), which is a framework to test hypothesized relationships among variables and accounts for their shared and unique contributions (Graham 2003). SEM has become a standard analytical tool in determining causal links in simplified food-webs, such as eelgrass communities (Alsterberg *et al.* 2013; Whalen *et al.* 2013) 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 field experiment, along with results from previous eelgrass-consumer studies (e.g., Moksnes *et al.* 2008; Baden *et al.* 2010; Hughes *et al.* 2013; Whalen *et al.* 2013) we developed *a priori* hypotheses about the significant pathways connecting sea otter trophic effects to new eelgrass shoot formation. We used results from our predator exclusion-inclusion experiments to populate the SEM. The factors included were the exogenous binary variables of sea otters (0s for exclusion plots, and 1s for otter mimics and open plots) and mesopredators (small predatory fish and other unaccounted predators, 0s for cage enclosures, and 1s 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 (Oftedal *et al.* 2007), and for the seagrass-macroalgal ecotone experiment we separated out the densities of grazers 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

by using a Kolmogorov–Smirnov test, and log transformed when appropriate. We first tested our hypothetical SEM for model fit using a χ^2 test which tests for differences between observed and estimated covariance matrices, χ^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). If the model did not fit we removed non-significant pathways, and we examined residual covariances to determine if additional pathways were warranted.

2.4 RESULTS

2.4.1 Sea otter effects at the seagrass interior and seagrass-macroalgal ecotone.

Sea otter density was high (2012 mean 3.5 otters ha^{-1} ±1.8 SD; 2013 mean = 2.4 otters ha^{-1} ±0.9 SD) during the experimental period, where we observed otters accessing our experimental cage areas. Additionally, nutrient concentrations were high during the experimental period, and often exceeded 100 μM NO_3 (Figure 2.S3), a value that far greater than background concentrations from the adjacent nearshore which rarely exceeds 30 μM NO_3 (Chapin *et al.* 2004), and is sufficient to stimulate algal epiphyte growth on the eelgrass leaves. For the interior experiment there were no significant pre-experimental differences among treatment and blocks for shoot densities ($P > 0.10$). For the seagrass-macroalgal ecotone experiment 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 ($P > 0.10$), so we standardized the data by using the percentage change in shoots as the response variable for the post-experimental analysis.

Our cage experiments at the interior and seagrass-macroalgal ecotone supported our hypothesis that sea otters can generate a trophic cascade that enhances grazer densities. After one month, grazer densities were significantly greater in both the interior (260%) and ecotone (80%) (Figure 2.2A-B) in the cages with simulated and actual sea otter predation (Table S2.1) compared to the low otter mimic treatment. However, on average 35% of grazers were found within *Ulva* at the ecotone (Figure 2.3B), indicating that *Ulva* could potentially enhance the algal epiphyte grazer assemblage for eelgrass. The grazer assemblage in the interior differed from the ecotone, where the interior assemblage was dominated primarily by two to three species of relatively larger mesograzers: *Phyllaplysia taylori* and *Idotea resecata* and *I. wosnenskii*. Thee large size classes (> 2 cm) comprised on average 48% of the grazer densities in the interior, as opposed to only 1% at the ecotone. Large grazers were present at the ecotone, mainly occurring on the eelgrass but in much lower relative quantities than the interior. This difference in size classes is likely due to the mixture of habitats where the *Ulva* assemblage was dominated primarily by several species of smaller gammarid amphipods, along with *Idotea* spp. and polychaete worms. Furthermore, grazers on the eelgrass at the ecotone were much more exposed to predation by fish due to lower shoot densities and a shorter macroalgal canopy (mean = 14.1 cm ± 5.4 SD) compared to the eelgrass canopy at the end of the experiment (mean = 60.6 cm ± 11.1 SD).

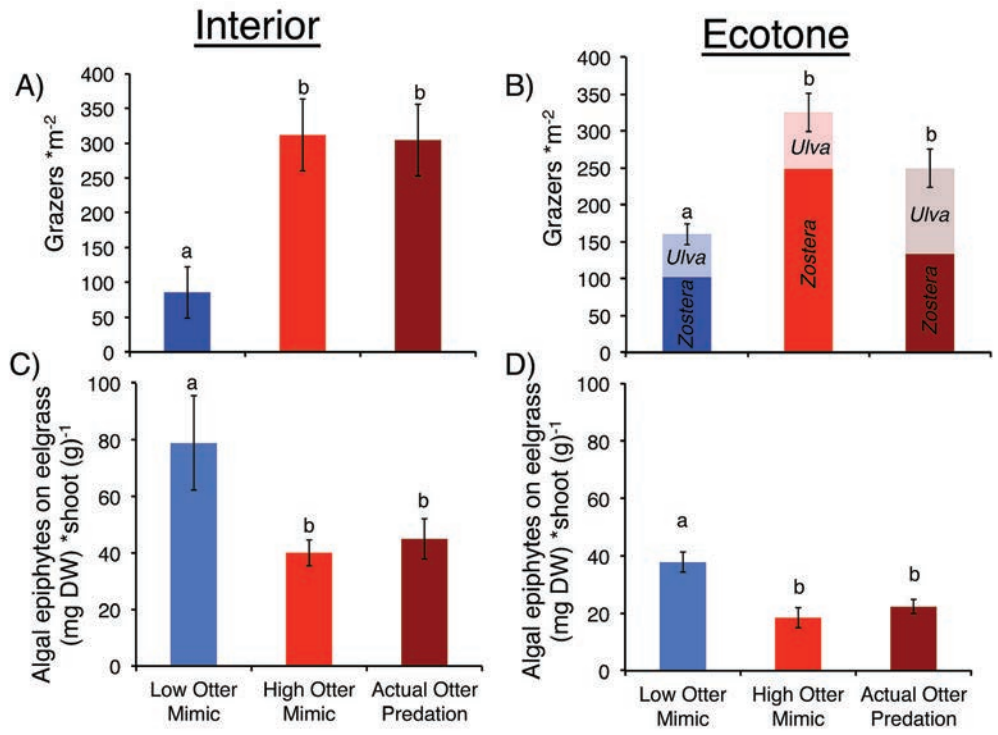

Figure 2.2. Results from 30 day field cage experiments at the seagrass interior and seagrass-macroalgal ecotone (Figure 2.1A) testing for the effects of simulated low sea otter predation (i.e., cages including crab and excluding sea otters; n = 8), simulated high sea otter predation (i.e., cages excluding 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 seagrass and (ii) cage-free plots; n =16) on (A-B) mesograzer density, and (B-C) algal epiphyte load on the eelgrass. Differences in lettering indicate significant differences based on randomized blocked ANOVA and Tukey HSD tests (Table 2.S1). DW = dry weight. Error bars are ±1 SE.

Our results also supported the hypothesis that the sea otter trophic cascade, through enhanced grazer densities, leads to reduced algal epiphyte loads on the eelgrass. Algal epiphyte loads were significantly lower in both the interior (46%) and ecotone (46%) (Figure 2.2C-D) in treatments with actual and simulated sea otter predation compared to the low otter mimic (Table 2.S1). We note that although the reductions in algal epiphyte loads on eelgrass were similar between the interior and ecotone, the ecotone plots had on average a 48% lower epiphyte load than the interior. There could be several reasons for this difference including differences in nutrient and light dynamics between the two locations and years, differences in the grazer assemblage and grazing rates, and the ephemeral nature of algal epiphytes, which were primarily diatoms during the two experiments and are known to be short lived (Orth and Van Montfrans 1984).

Finally, our results supported the hypothesis that the sea otter trophic cascade promotes eelgrass resilience through enhanced biomass at the seagrass interior and ecotone. For eelgrass biomass, both the interior and ecotone were enhanced (82% and 70%, respectively) by the simulated and actual presence of sea otters (Figure 2.3A-B) (Table 2.S1), and the final biomasses were nearly identical. However, one other aspect of eelgrass resilience, change in shoot densities, was enhanced by sea otters but differed in context between the interior and ecotone. At the interior there was a significantly greater loss of shoots in the absence of sea otters (43%), where as plots with simulated and actual sea otter predation only lost 7% of shoots, indicating that sea otters were potentially maintaining the interior portion of the bed (Figure 2.3C)

(Table 2.S1). However, at the ecotone 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%) (Figure 2.3D), indicating that sea otters are promoting bed expansion at the ecotone.

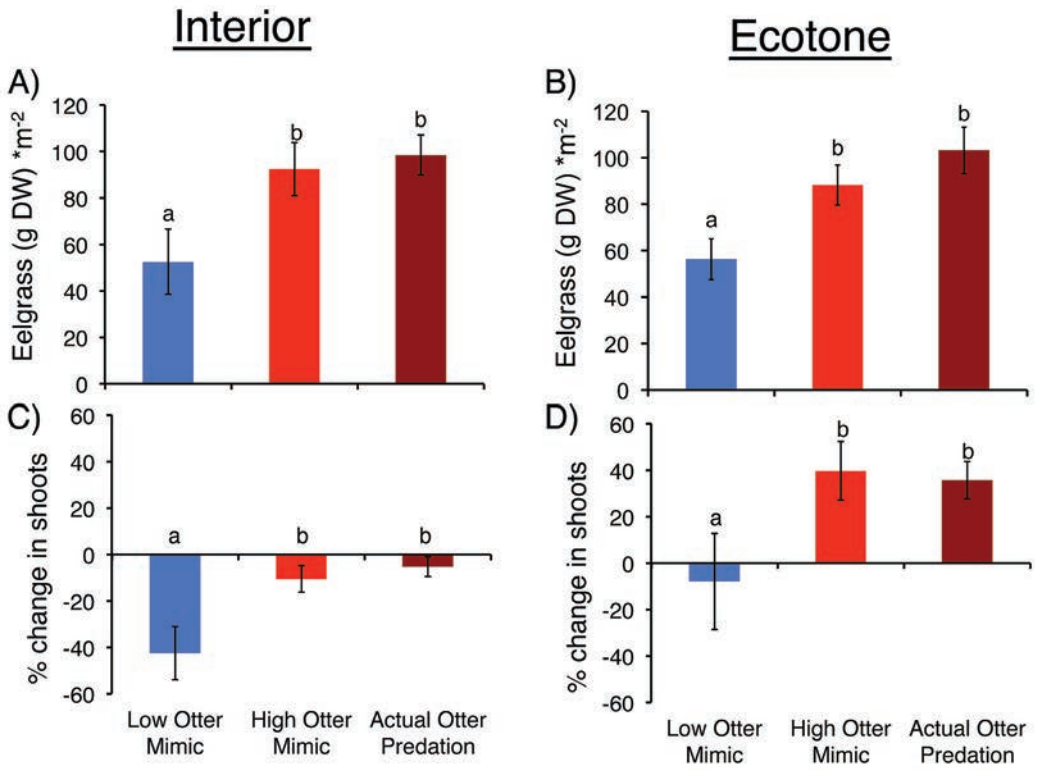

Figure 2.3. Results from 30 day field cage experiments at the seagrass interior and seagrass-macroalgal ecotone (Figure 2.1) testing for the effects of simulated low sea otter predation (i.e., cages including crab and excluding sea otters and mesopredators; n = 8), simulated high sea otter predation (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 seagrass and (ii) cage-free plots; n =16) on (A-B) eelgrass biomass, and (B-C) the % change in shoots. Differences in lettering indicate significant differences based on randomized blocked ANOVA and Tukey HSD tests (Table 2.S1). DW = dry weight. Error bars are ±1 SE.

We had predicted that sea otters would macroalgal dominance in favor of eelgrass at the seagrass-macroalgal ecotone. While the evidence did show benefits to eelgrass, to our surprise, real and simulated otter predation also benefitted macroalgae. There was a significantly greater *Ulva* biomass (Figure 2.4) 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 (Table 2.S1). The ability of eelgrass to expand in otter treatments despite this increase in macroalgae was unexpected, given the known negative effects of macroalgal mats on seagrass demonstrated in other studies worldwide (Valiela *et al.* 1997; Nelson & Lee 2001; Burkholder *et al.* 2007). We observed that *Ulva* in general did not form mats that smothered the older eelgrass shoots, but were instead interspersed between older and taller shoots at the ecotone. On average, *Ulva* biomass exceeded eelgrass-standing biomass in the treatments with actual sea otter predation, indicating either a benign or positive effect of *Ulva* on eelgrass resilience in the otter treatments.

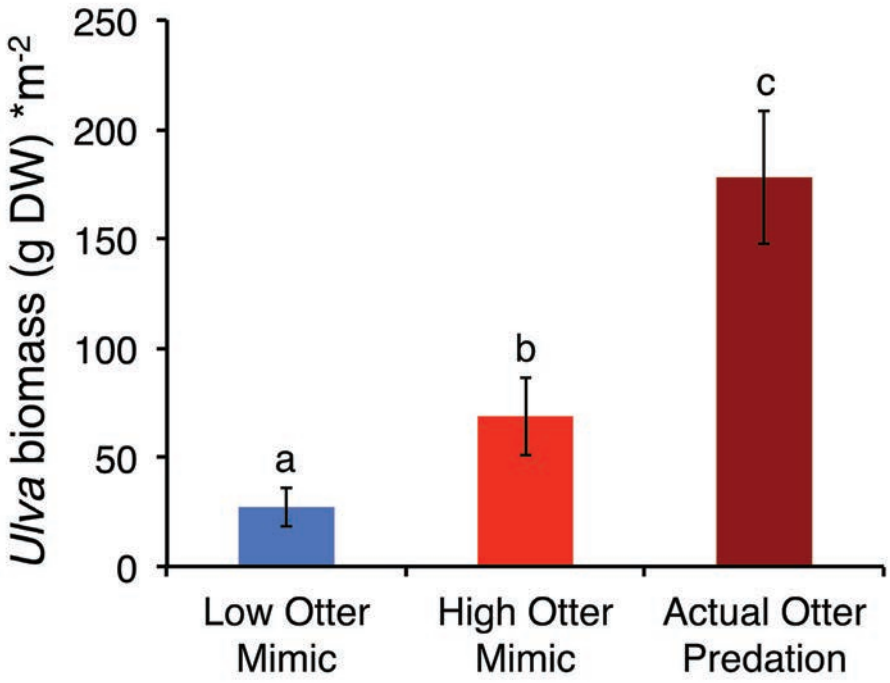

Figure 2.4. Results from a 30 day field cage experiment at the seagrass-macroalgal ecotone (Figure 2.1) testing for the effects of simulated low sea otter predation (i.e., cages including crab and excluding sea otters and mesopredators; n = 8), simulated high sea otter predation (i.e., cages excluding 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 mesopreadtors yet included the top of the cage to test for shading effects on the seagrass 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 (Table 2.S1). DW = dry weight. Error bars are ±1 SE.

2.4.2 Modeling the sea otter trophic cascade at the seagrass interior and the seagrass-macroalgal ecotone. Using SEM, we developed a mechanistic model of how a sea-otter driven trophic cascade at the seagrass interior and seagrass-macroalgal ecotone could drive eelgrass resilience given the surprising results of otters enhancing *Ulva* and of benign or positive effects of *Ulva* on eelgrass biomass and shoot formation (Figures 2.3 and 2.4). We developed *a priori* hypotheses based on results from the interior experiment (Hughes et al. 2013) and our ecotone experiment, as well as previous results from the literature describing consumer dynamics in seagrass-macroalgal systems (Baden *et al.* 2010; Whalen *et al.* 2013) (Figure 2.S4A-B) to generate three SEMs: the seagrass interior (without *Ulva*), the seagrass-macroalgal ecotone (without *Ulva*), and the ecotone (with *Ulva*). First, we developed SEMs for both the interior and ecotone (without *Ulva*) and hypothesized (Figure 2.S4A) that sea otter predation on crabs generated a trophic cascade that enhanced grazer densities on the eelgrass leading to decreased algal epiphyte loading and enhanced eelgrass resilience through enhanced biomass and shoot densities. We also built in linkages going from eelgrass to grazers to hypothesize that positive feedbacks from enhanced eelgrass could enhance grazer densities.

Next, we developed an additional SEM for the ecotone where we hypothesized (Figure 2.S4B) that if *Ulva* had benign or positive effects on eelgrass resilience (a function of increased biomass and change in shoot density) then it was most likely mediated though consumer pathways. Specifically, we hypothesized that sea otters reduced crabs, which can consume *Ulva*, thus leading to increased *Ulva* in

the presence of otters. Increased *Ulva* abundance could enhance grazer densities, which consume algal epiphytes growing on nearby eelgrass leaves, thus leading to increased eelgrass biomass and shoot formation. Additionally, we hypothesized that *Ulva* could directly facilitate eelgrass likely through amelioration of desiccation stress. Furthermore we hypothesized that sea otters could simultaneously, through the reduction of crabs, generate a trophic cascade leading to enhanced grazer densities on the eelgrass, similar to the one generated in the interior of the bed away from the algal ecotone (Hughes *et al.* 2013). For all three SEMs we accounted for the potential effects of other potential mesopredators (fish and other predators not accounted for in open controls) by including them as grazer-reducing pathways in the models. We included the three predators (sea otters, crabs, and mesopredators) in the SEM by assigning covariance structure between them based on hypothesized relationships and experimental design, where sea otters negatively co-varied with crabs and positively co-varied with other mesopredators, and crabs negatively co-varies with other mesopredators. For the ecotone SEM with *Ulva* included we also included covariance structure between other mesopredators and *Ulva* biomass since open cages and plots were accessible to both mesopredators and floating *Ulva* mats.

Our SEMs helped explain the various pathways by which sea otters can promote eelgrass stability and resilience at both the seagrass interior and the seagrass-macroalgal ecotone. First, our final SEM model (without *Ulva*) supported our hypothesized pathways (Figure 2.S4A) at the seagrass interior (Figure 2.5A) (Table 2.S2A-B) but not at the eelgrass macroalgal ecotone (Figure 2.5B) (Table 2.S2C-D).

At the seagrass interior the reduction in crabs enhanced the epiphyte grazer assemblage leading to a reduction in algal epiphytes that enhanced both eelgrass biomass and maintained shoot densities (Figure 2.3C). However, at the seagrass-macroalgal ecotone there was poor model fit ($P < 0.05$), and there was not a trophic pathway leading to enhanced eelgrass biomass and shoot formation, indicating that alternative pathways could have affected eelgrass resilience.

Our final SEM for the seagrass-macroalgal ecotone including *Ulva* (Figure 2.5C) supported our model of hypothesized pathways (Figure 2.S4B), whereby sea otters positively affected eelgrass resilience at the seagrass-macroalgal ecotone (Table 2.S2). There was one path by which sea otter predation on crabs affected eelgrass biomass and shoot formation. Sea otter consumption of crabs enhanced *Ulva* biomass and its associated grazer assemblage at the ecotone, and in turn contributed to the reduction of epiphyte loads on eelgrass leading to greater eelgrass biomass and shoot production. Like the interior of the eelgrass bed (Figure 2.5A; Hughes et al. 2013) sea otter consumption of crabs at the seagrass-macroalgal ecotone enhanced epiphyte grazers, however, this did not correspond to lower algal epiphyte loads and increased eelgrass biomass and shoot formation. Instead our model indicated that increased shoot densities resulted in greater grazer densities in the eelgrass.

There were five other pathways in our original hypothesis that were non-significant in the model output and thus removed from the final model. First, We found that crabs and other mesopredators did not directly impact *Ulva* grazers, but instead crabs reduced *Ulva* grazers through *Ulva* consumption. Second, *Ulva* mass

did not significantly enhance the eelgrass grazer assemblage, and therefore grazers associated with *Ulva* were likely to consume algal epiphytes on eelgrass, but not remaining on the eelgrass itself. The larger-sized grazer assemblage associated with the eelgrass could explain why smaller *Ulva* grazers 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, as indicated by the negative covariance between eelgrass and *Ulva* grazers. Finally, the paths leading to direct facilitation of eelgrass by *Ulva* were non-significant and removed from the final SEM.

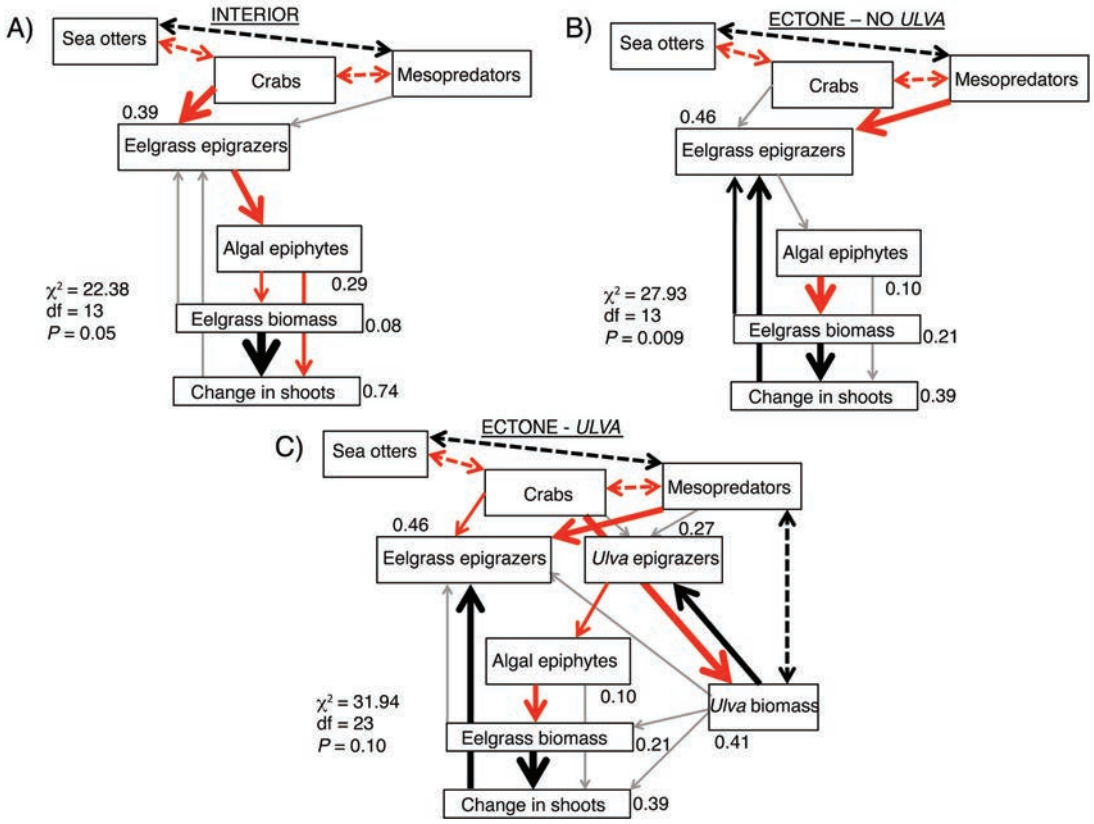

Figure 2.5. Final SEM testing for the effects of sea otters on eelgrass resilience as a function of biomass and change in shoots. The non-significant ($P > 0.05$) χ^2 test indicated good model fit. Arrow widths are proportional to standardized regression weights. Numbers next to endogenous variables indicate R^2. Red arrows indicate negative correlations, black lines indicate positive correlations, and grey arrows were non-significant paths removed from the final model. Dashed double-headed arrows were covariance between endogenous variable error terms and exogenous variables, some were included as additional paths in the final model based on residual covariance analysis. See Table 2.S2 for coefficient values, standard errors, and standardized path coefficients for hypothesized and final SEMs.

2.5 DISCUSSION

Recovery of degraded ecosystems without direct restoration intervention is relatively rare. Determining the mechanisms that enhance such recovery and 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 the resilience and recovery of seagrass beds under threat from overgrowth of epiphytic algae and macroalgal blooms, both a consequence of increasing anthropogenic nutrient loading. Despite the widespread trophic downgrading of global ecosystems (Estes *et al.* 2011), there is hope for systems where the conservation or restoration of top predators can result in a trophic upgrade that benefits the ecosystem.

Our results support a new emerging paradigm in seagrass ecology that macroalgal blooms do not always result in declines to seagrass. Surprisingly, the sea otter-driven trophic cascade enhanced both competing vegetation types, seagrass and macroalgae, yet this did not result in increased competition between the two. Recent evidence has emerged that macroalgal blooms can, at times, have benign or positive effects on seagrass performance (Hessing-Lewis *et al.* 2011; Thomsen *et al.* 2012), contrary to the long-standing paradigm (Letts & Adeney 1908; Valiela *et al.* 1997; Burkholder *et al.* 2007). Despite competitive interactions that may exist between macroalgae and seagrass, these benign/positive effects of macroalgae on seagrass occur when there is enhanced epiphyte grazers in seagrass due to trophic control

(Baden *et al.* 2010). Furthermore, drift macroalgae has been shown to indirectly facilitate seagrasses through the delivery of important mesograzers 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, the variation in relative abundance of mesopredators and top predators, greatly influences mesograzer 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). Therefore, trophic structure has the potential to dictate the direction and interaction of regime shifts between macroalgae and seagrass in anthropogenically nutrient loaded systems.

Both direct and indirect facilitation could be a key driver of context-dependency in seagrass and macroalgal associations (Bruno *et al.* 2003). Results from our study indicate positive associations with eelgrass and *Ulva* in the presence of sea otters. There could be alternative pathways that were not explained through our trophic cascade nor detected by our SEM. First, the eelgrass canopy in our experimental plots at the ecotone was much taller at the end of the experiment than the *Ulva* canopy. Only new shoots can be shaded by *Ulva*, and are eventually released from competition once they exceed the *Ulva* canopy, a result that has been demonstrated in other seagrass-macroalgal systems (Thomsen *et al.* 2012). Furthermore, aerial exposure times at the seagrass-macroalgal ecotone in Elkhorn Slough can approach 300 hours per year, which exposes eelgrass to desiccation stress and sets its upper limits (Boese *et al.* 2003). Most of the eelgrass beds in the system are primarily located in the lower intertidal -1.0 m (MLLW) to subtidal, and thus not

affected by desiccation. However, the upward expansion of eelgrass and the seagrass-macroalgal ecotone could be enhanced by *Ulva* facilitation through decreased desiccation stress in the upper intertidal. Thus, *Ulva* could play a facilitating role to eelgrass, however facilitation may only occur in warmer, summer conditions when desiccation stress and epiphyte loading are greatest.

Here we have demonstrated that although trophic cascades at the exterior of an ecosystem can produce similar results as the interior (Hughes *et al.* 2013), the mechanism driving resilience can greatly differ. The experiment in the seagrass interior showed how sea otters can play an important role in maintaining the health of eelgrass by preventing loss of eelgrass biomass and shoots. Whereas in our experiment at the seagrass-macroalgal ecotone we demonstrated that sea otters can enhance eelgrass resilience by promoting *Ulva* and its grazer assemblage that are important for removing algal growth on the eelgrass, thus leading to eelgrass expansion. This trophic mechanism could be especially important for eelgrass seedling recruitment and new patch formation, which is another mode of eelgrass expansion that has occurred in the presence of sea otters (Brent Hughes, *pers. obs.*). Furthermore, this example provides insight into how trophic cascades drive resilience, and suggests that research on resilience in patch-forming foundation species, such as kelps (Konar & Estes 2003), tree forests, and grasslands should consider both the dynamics in the interior and exterior.

The recovery and effects of sea otters along the northeast Pacific (Estes *et al.* 1998; Lafferty & Tinker 2014; Ripple *et al.* 2014b) serves as a model for determining

the role of top predators in coastal ecosystems. Shifting baselines and the loss of top predators have generated uncertainty on the causes of instability of coastal ecosystems (Jackson *et al.* 2001; Estes *et al.* 2011). Studies like the one presented here provide important insight into how restored food webs influence resilience in anthropogenically degraded ecosystems. For seagrasses, the loss of top predators, such as seals, crocodiles, sharks, and other large predatory fish have been determined to cause the loss and function of seagrasses when combined with the harmful effects of eutrophication (Jackson *et al.* 2001; Moksnes *et al.* 2008). Therefore, future investigations into the role of top predators in coastal ecosystems should target locations where top predators are protected such as in marine protected areas (Heithaus *et al.* 2012) and compare these with nearby systems still affected by human exploitation.

2.6 SUPPORTING INFORMATION

2.6.1 FIGURES

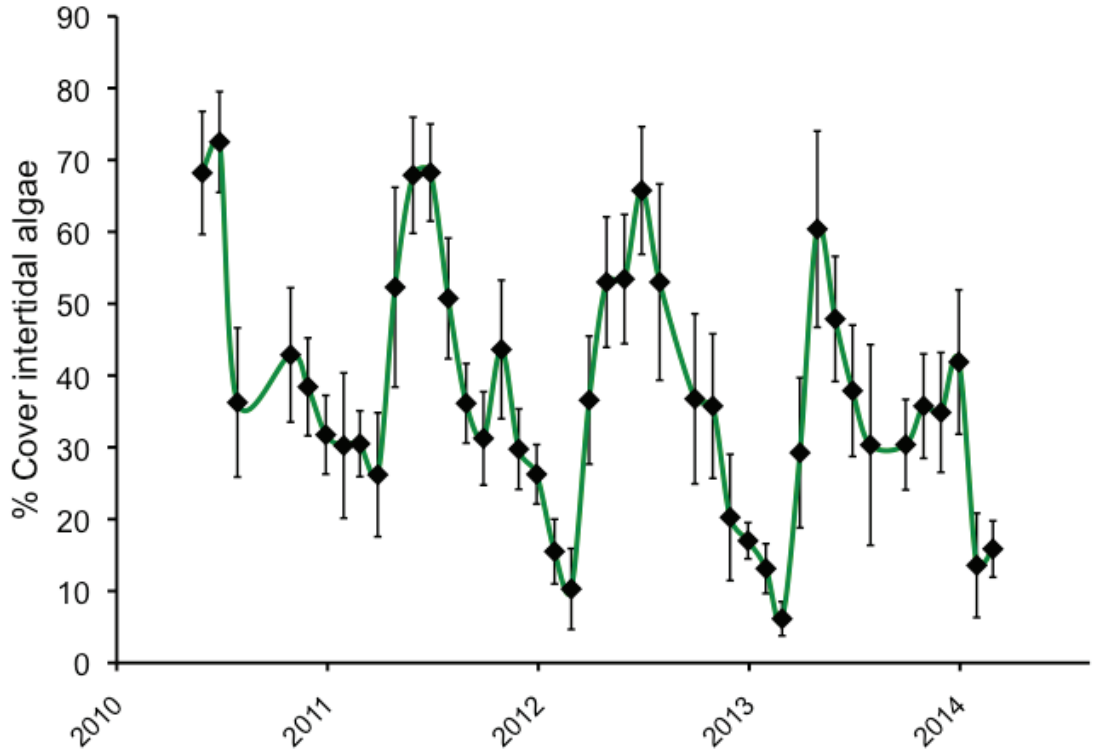

Figure 2.S1. Annual and seasonal variation of intertidal algal cover in 1 ha plots in Elkhorn Slough (n = 8). Plots are surveyed using a shore-based random point contact survey (Nedwell *et al.* 2002). Error bars are ±1 SE.

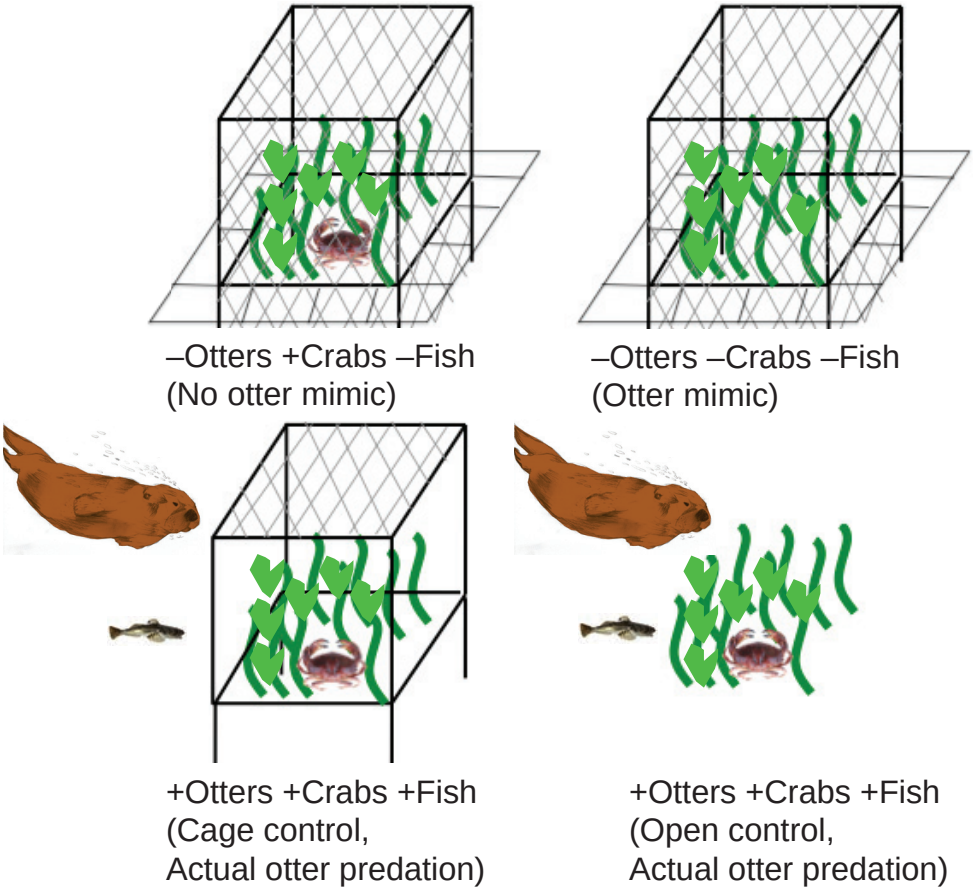

Figure 2.S2. Cage experimental design (50 cm x 50 cm x 50 cm), testing for the trophic cascade effects of crabs (+Crab –Otters –Mesopredators [i.e., fish]), the simulated trophic cascade effects of sea otters (-Crabs –Otters –Mesopredators), and the direct effects of sea otters and mesopredators (Partial Cage Control and Open Control) on grazers, algal epiphytes, eelgrass biomass and shoot formation, and *Ulva* biomass.

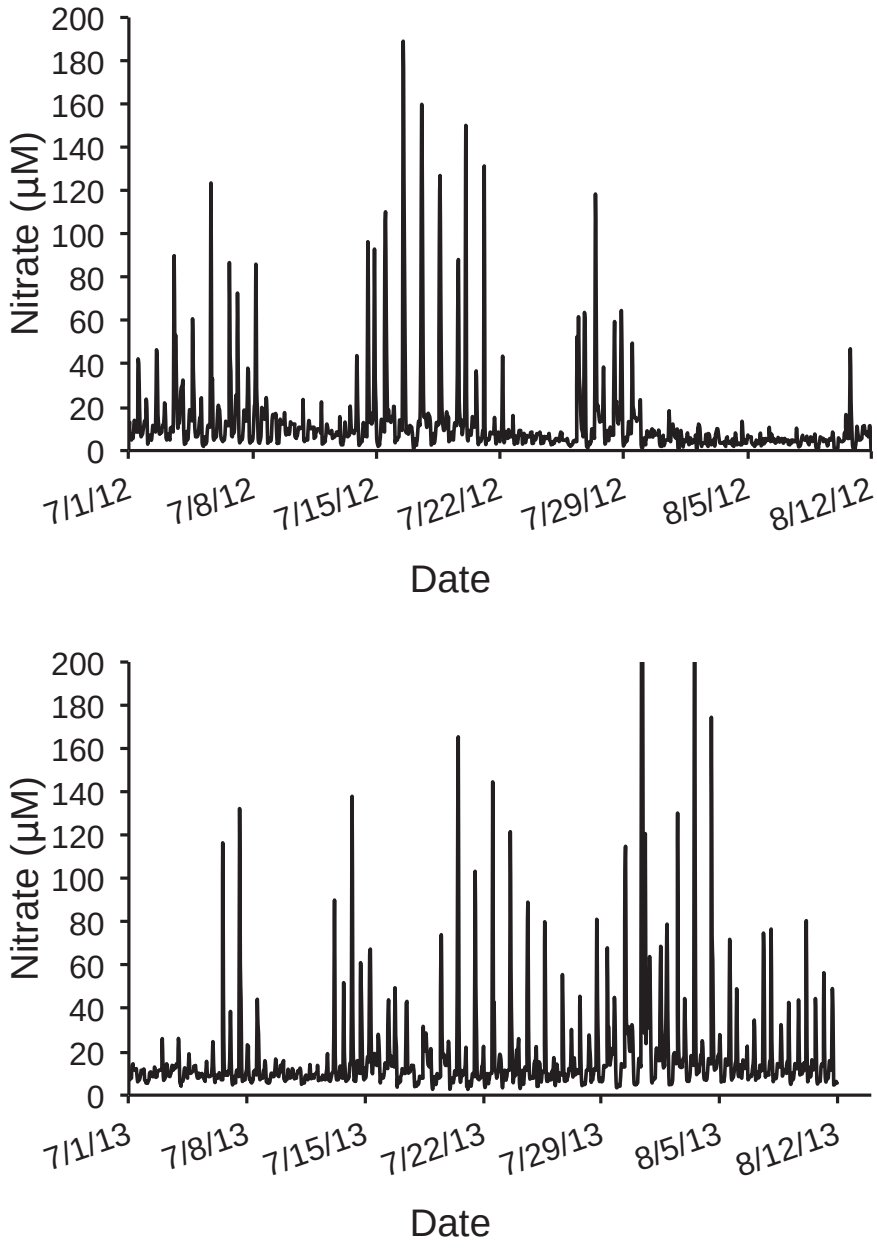

Figure 2.S3. Nitrate data showing elevated concentrations during the 2012 and 2013 experimental periods in Elkhorn Slough. Hourly data was collected in situ using an ISUS (In Situ Ultraviolet Spectrophotometer) nitrate sensor attached to the Monterey Bay Aquarium Research Institute's Land/Ocean Biogeochemical Observatory sensor mooring (Jannasch *et al.* 2008).

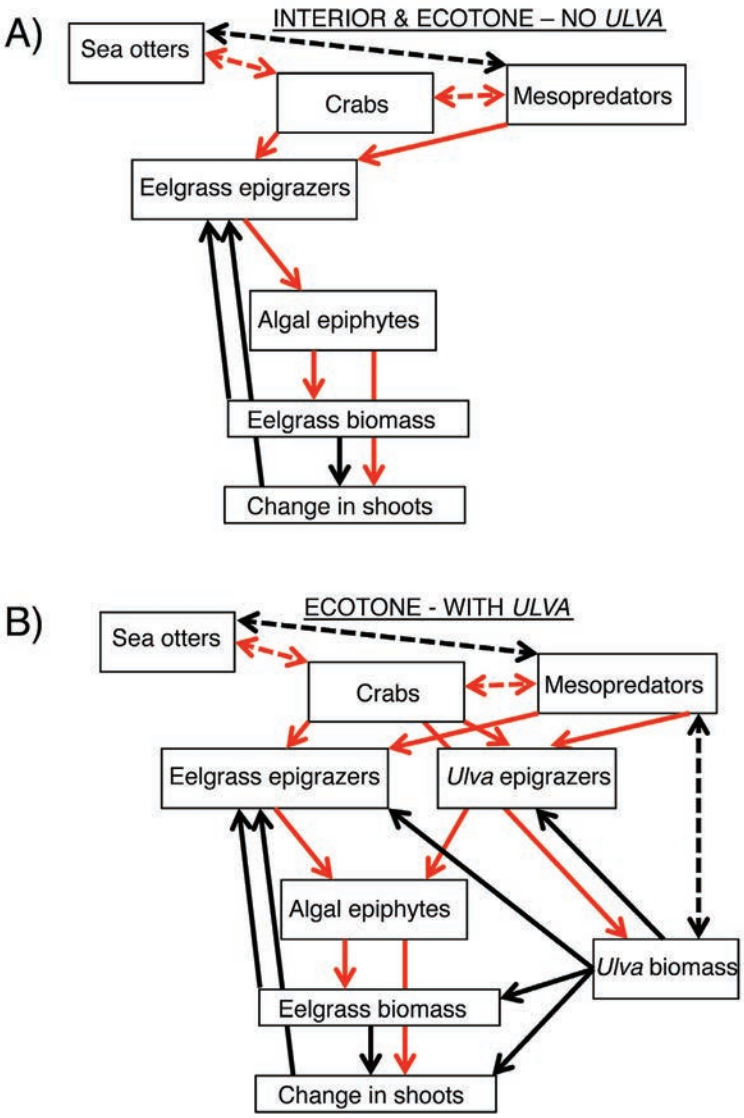

Figure 2.S4. Hypothesized SEMs testing for the effects of sea otters on eelgrass resilience as a function of biomass and change in shoot densities at the (A) seagrass interior and seagrass-macroalgal ecotone without *Ulva*, and (B) seagrass-macroalgal ecotone with *Ulva* included. To produce the final model we removed non-significant pathways, and included covariance structure (dashed double-headed arrows) as indicated by residual covariance analysis. Red arrows indicated negative correlations, black lines indicated positive correlations. See Table 2.S2 for coefficient values, standard errors, and standardized path coefficients for hypothesized and final SEM.

2.6.2 TABLES

Table 2.S1. Results from field caging experiments at the seagrass interior and seagrass-macroalgal ecotone (Figures 2.1-2.4) using a randomized blocked ANOVA. (i) Full design testing for the effects of treatment (fixed) and block (random) on (A) grazer density (grazers m^{-2}), (B) algal epiphyte mass (mg DW *g $shoot^{-1}$), (C) eelgrass biomass (g DW m^{-2}), (D) % change in shoots, and (E) *Ulva* biomass (g DW m^{-2}). (ii) Tukey's HSD multiple comparison testing for differences in control treatments. (iii) Reduced randomized blocked ANOVA, grouping Cage and Open Controls, if $P > 0.25$ from (ii). (iv) Final Tukey's HSD multiple comparison (with combined controls) testing for differences of individual treatments if there was a significant treatment effect from the randomized blocked ANOVA. Significant factors are in bold. Note: sample sizes varied for treatments depending on outcomes of (i), if no significant differences occurred among controls then the two control types (Cage and Open Controls) were grouped ($n = 16$), if differences occurred then $n = 8$ for all treatments. Crab treatments for the interior experiment had a lower sample size ($n = 6$) for epiphytes, shoot density, and eelgrass biomass due to crab damage to eelgrass shoots in two of the cages. *indicates analysis run on log-transformed data.

(A) Grazer density*

(i) Full randomized blocked ANOVA test between all treatments

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	5.938	3	1.979	5.839	**0.005**
	Error	7.118	21	0.339		
Block	Hypothesis	1.810	7	0.259	0.763	0.624
	Error	2.435	21	0.116		

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	2.157	3	0.719	6.202	**0.003**
	Error	2.435	21	0.116		
Block	Hypothesis	0.877	7	0.125	1.081	0.410
	Error	2.435	21	0.116		

Table 2.S1. (continued)

(A) Grazer density* (continued)

(ii) Tukey's HSD multiple comparison test among control treatments

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	0.059	0.291	0.997

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	-0.013	0.170	1.000

(iii) Reduced randomized blocked ANOVA test

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	5.924	2	2.962	9.136	**0.001**
	Error	7.132	22	0.324		
Block	Hypothesis	1.810	7	0.259	0.798	0.598
	Error	7.132	22	0.324		

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	2.156	2	1.078	9.740	**0.001**
	Error	2.435	22	0.111		
Block	Hypothesis	0.877	7	0.125	1.132	0.379
	Error	2.435	22	0.111		

(iv) Tukey's HSD multiple comparison test among treatments with controls combined

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	0.447	0.144	**0.014**
No Crab Cage	**Crab Cage**	0.724	0.166	**0.001**
Control	No Crab Cage	-0.277	0.144	0.156

Table 2.S1. (continued)

(A) Grazer density* (continued)

(iv) Tukey's HSD multiple comparison test among treatments with controls combined

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	0.952	0.247	**0.002**
No Crab Cage	**Crab Cage**	1.060	0.285	**0.003**
Control	No Crab Cage	-0.108	0.247	0.900

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	0.447	0.144	**0.014**
No Crab Cage	**Crab Cage**	0.724	0.166	**0.001**
Control	No Crab Cage	-0.277	0.144	0.156

Table 2.S1. (continued)

(B) Algal epiphytes

(i) Full randomized blocked ANOVA test between all treatments

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	4512.485	3	1504.162	3.751	**0.029**
	Error	7619.320	19	401.017		
Block	Hypothesis	14147.35	7	2021.050	5.040	**0.002**
	Error	8030.742	21	382.416		

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	1798.858	3	599.619	6.479	**0.003**
	Error	1943.397	21	92.543		
Block	Hypothesis	939.562	7	134.223	1.450	0.238
	Error	1943.397	21	92.543		

(ii) Tukey's HSD multiple comparison test among control treatments

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	3.57	10.013	0.984

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	2.348	4.810	0.961

(iii) Reduced randomized blocked ANOVA test

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	4461.506	2	2230.753	5.81	**0.010**
	Error	7670.299	20	383.515		
Block	Hypothesis	14147.350	7	2021.050	5.270	**0.002**
	Error	7670.299	20	383.515		

Table 2.S1. (continued)

(B) Algal epiphytes (continued)

(iii) Reduced randomized blocked ANOVA test

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	1776.798	2	888.399	9.944	**0.001**
	Error	1965.457	22	89.339		
Block	Hypothesis	939.562	7	134.223	1.502	0.218
	Error	1965.457	22	89.339		

(iv) Tukey's HSD multiple comparison test among treatments with controls combined

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	-33.956	9.375	**0.005**
No Crab Cage	**Crab Cage**	-38.842	10.576	**0.004**
Control	No Crab Cage	4.886	8.480	0.834

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	-15.519	4.092	**0.003**
No Crab Cage	**Crab Cage**	-19.404	4.726	**0.001**
Control	No Crab Cage	3.3884	4.092	0.616

Table 2.S1. (continued)

(C) Eelgrass biomass*

(i) Full randomized blocked ANOVA test between all treatments

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.826	3	0.275	6.302	**0.004**
	Error	0.830	19	0.044		
Block	Hypothesis	0.757	7	0.108	2.477	**0.055**
	Error	0.830	19	0.044		

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.482	3	0.161	4.939	**0.009**
	Error	0.683	21	0.033		
Block	Hypothesis	0.242	7	0.035	1.064	0.419
	Error	0.683	21	0.033		

(ii) Tukey's HSD multiple comparison test among control treatments

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	-0.093	0.105	0.811

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	-0.102	0.090	0.672

(iii) Reduced randomized blocked ANOVA test

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.791	2	0.396	9.154	**0.002**
	Error	0.864	20	0.043		
Block	Hypothesis	0.757	7	0.108	2.503	**0.051**
	Error	0.864	20	0.043		

Table 2.S1. (continued)

(C) Eelgrass biomass* (continued)

(iii) Reduced randomized blocked ANOVA test

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	0.440	2	0.220	6.676	**0.005**
	Error	0.725	22	0.033		
Block	Hypothesis	0.242	7	0.035	1.050	0.426
	Error	0.725	22	0.033		

(iv) Tukey's HSD multiple comparison test among treatments with controls combined

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	0.388	0.100	**0.002**
No Crab Cage	**Crab Cage**	0.358	0.112	**0.012**
Control	No Crab Cage	0.030	0.090	0.941

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	0.284	0.079	**0.004**
No Crab Cage	**Crab Cage**	0.228	0.091	**0.050**
Control	No Crab Cage	0.057	0.079	0.754

Table 2.S1. (continued)

(D) Eelgrass shoot change

(i) Full randomized blocked ANOVA test between all treatments

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	6484.799	3	2161.600	8.419	**0.001**
	Error	4878.231	19	256.749		
Block	Hypothesis	5345.15	7	763.593	2.974	**0.028**
	Error	40961.151	21	1950.531		

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	13164.932	3	4388.311	2.25	0.112
	Error	40961.151	21	1950.531		
Block	Hypothesis	6496.784	7	928.112	0.476	0.841
	Error	40961.151	21	1950.531		

(ii) Tukey's HSD multiple comparison test among control treatments

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	-0.250	8.012	0.902

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	-15.647	22.082	0.893

(iii) Reduced randomized blocked ANOVA test

Interior

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	6484.549	2	3242.275	13.292	**<0.0005**
	Error	4878.480	20	243.924		
Block	Hypothesis	5345.150	7	763.593	3.13	**0.021**
	Error	4878.480	20	243.924		

Table 2.S1. (continued)

(D) Eelgrass shoot change (continued)

(iii) Reduced randomized blocked ANOVA test

Ecotone

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	12185.644	2	6092.822	3.196	**0.060**
	Error	41940.44	22	1906.384		
Block	Hypothesis	6496.784	7	928.112	0.487	0.834
	Error	41940.440	22	1906.384		

(iv) Tukey's HSD multiple comparison test among treatments with controls combined

Interior

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	37.245	7.477	**<0.0005**
No Crab Cage	**Crab Cage**	31.887	8.435	**0.003**
Control	No Crab Cage	5.359	6.763	0.712

Ecotone

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	43.604	18.906	**0.076**
No Crab Cage	**Crab Cage**	47.532	21.831	**0.098**
Control	No Crab Cage	-3.928	18.906	0.977

Table 2.S1. (continued)

(D) *Ulva* biomass*

(i) Full randomized blocked ANOVA test between all treatments

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	3.805	3	1.268	12.767	**<0.0005**
	Error	2.086	21	0.099		
Block	Hypothesis	1.374	7	0.196	1.976	0.107
	Error	2.086	21	0.099		

(ii) Tukey's HSD multiple comparison test among control treatments

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Cage Control	Open Control	-0.088	0.158	0.943

(iii) Reduced randomized blocked ANOVA test

Source		*SS*	*df*	*Mean Square*	*F*	*P*
Treatment	Hypothesis	3.774	2	1.887	19.607	**<0.0005**
	Error	2.117	22	0.096		
Block	Hypothesis	1.374	7	0.196	2.040	**0.095**
	Error	2.117	22	0.096		

(iv) Tukey's HSD multiple comparison test among treatments with controls combined

Treatment 1	*Treatment 2*	*Mean Difference (1-2)*	*Std. Error*	*P*
Control	**Crab Cage**	0.834	0.134	**<0.0005**
No Crab Cage	**Crab Cage**	0.449	0.155	**0.022**
Control	**No Crab Cage**	0.385	0.134	**0.024**

Table 2.S2. Model fit parameters (χ^2, df , *P*), unstandardized path coefficients, standard errors, and standardized path coefficients for SEMs testing for sea otter effects on eelgrass resilience as a function of biomass and changes in shoot densities for the: (A) hypothesized seagrass interior SEM, (B) final seagrass interior SEM with model fit, (C) hypothesized seagrass-macroalgal ecotone (without *Ulva* included) SEM, (C) final seagrass-macroalgal ecotone (without *Ulva* included) SEM with poor model fit (D) hypothesized seagrass-macroalgal ecotone (with *Ulva* included) SEM, and (E) final seagrass-macroalgal ecotone (with *Ulva* included) SEM with good model fit. Double-headed arrows indicate covariance among variables. Significant paths ($P < 0.10$) are in bold.

A) Seagrass interior with all hypothetical linkages ($\chi^2 = 20.826$, df = 10, $P = 0.022$)

Path			*Unstandardized coefficient*	*Standard error*	*Standardized coefficient*
Sea otter	↔	**Crabs***	**-0.43**	**0.11**	**-1.00**
Sea otter	↔	**Mesopredators**	**0.13**	**0.05**	**0.58**
Crabs*	↔	**Mesopredators**	**-0.28**	**0.10**	**-0.58**
Crabs*	→	**Eelgrass grazers***	**-0.48**	**0.13**	**-0.69**
Mesopredators	→	Eelgrass grazers*	-0.10	0.24	-0.07
Eelgrass grazers*	→	**Epiphytes**	**-26.40**	**7.91**	**-0.55**
Epiphytes	→	Eelgrass mass	-0.27	0.23	-0.23
Epiphytes	→	**Eelgrass shoots**	**-0.003**	**0.001**	**-0.38**
Eelgrass mass	→	**Eelgrass shoots**	**0.005**	**0.001**	**0.70**
Eelgrass shoots	→	Eelgrass grazers*	-0.80	0.89	-0.28
Eelgrass mass	→	Eelgrass grazers*	0.006	0.005	0.31

*indicates analysis run on log-transformed data.

B) Final reduced seagrass interior SEM with good model fit (χ^2 = 22.38, df = 13, *P* = 0.050)

Path			*Unstandardized coefficient*	*Standard error*	*Standardized coefficient*
Sea otter	↔	**Crabs***	**-0.33**	**0.11**	**-1.00**
Sea otter	↔	**Mesopredators**	**0.13**	**0.05**	**0.58**
Crabs*	↔	**Mesopredators**	**-0.28**	**0.10**	**-0.58**
Crabs*	→	**Eelgrass grazers***	**-0.43**	**0.10**	**-0.63**
Eelgrass grazers*	→	**Epiphytes**	**-24.03**	**6.74**	**-0.54**
Epiphytes	→	**Eelgrass mass**	**-0.35**	**0.20**	**-0.29**
Epiphytes	→	**Eelgrass shoots**	**-0.003**	**0.001**	**-0.34**
Eelgrass mass	→	**Eelgrass shoots**	**0.005**	**0.001**	**0.70**

*indicates analysis run on log-transformed data.

C) Seagrass-macroalgal ecotone (without *Ulva*) SEM with all hypothesized linkages (χ^2 = 23.647, df = 10, P = 0.009)

Path			*Unstandardized coefficient*	*Standard error*	*Standardized coefficient*
Sea otter	↔	**Crabs***	**-0.40**	**0.10**	**-1.00**
Sea otter	↔	**Mesopredators**	**0.12**	**0.05**	**0.58**
Crabs*	↔	**Mesopredators**	**-0.26**	**0.10**	**-0.58**
Crabs*	→	Eelgrass grazers*	-0.05	0.05	-0.16
Mesopredators	→	**Eelgrass grazers***	**-0.33**	**0.09**	**-0.57**
Eelgrass grazers*	→	Epiphytes	14.76	14.62	0.36
Epiphytes	→	**Eelgrass mass**	**-1.83**	**0.68**	**-0.60**
Epiphytes	→	Eelgrass shoots	-0.95	0.61	-0.27
Eelgrass mass	→	**Eelgrass shoots**	**0.65**	**0.19**	**0.56**
Eelgrass shoots	→	**Eelgrass grazers***	**0.003**	**0.001**	**0.47**
Eelgrass mass	→	**Eelgrass grazers***	**0.003**	**0.001**	**0.32**

*indicates analysis run on log-transformed data.

D) Final reduced seagrass-macroalgal ecotone (without *Ulva*) SEM with poor model fit (χ^2 = 27.928, df = 13, *P* = 0.009)

Path			*Unstandardized coefficient*	*Standard error*	*Standardized coefficient*
Sea otter	←→	**Crabs***	**-0.40**	**0.10**	**-1.00**
Sea otter	←→	**Mesopredators**	**0.13**	**0.05**	**0.58**
Crabs*	←→	**Mesopredators**	**-0.26**	**0.10**	**-0.58**
Mesopredators	→	**Eelgrass grazers***	**-0.27**	**0.07**	**-0.43**
Epiphytes	→	**Eelgrass mass**	**-1.39**	**0.49**	**-0.45**
Eelgrass mass	→	**Eelgrass shoots**	**0.73**	**0.16**	**0.63**
Eelgrass shoots	→	**Eelgrass grazers***	**0.003**	**0.001**	**0.44**
Eelgrass mass	→	**Eelgrass grazers***	**0.002**	**0.001**	**0.26**

*indicates analysis run on log-transformed data.

E) Seagrass-macroalgal ecotone (with *Ulva*) SEM with all hypothesized linkages (χ^2 = 30.013, df = 16, *P* = 0.018)

Path			*Unstandardized coefficient*	*Standard error*	*Standardized coefficient*
Sea otter	↔	**Crabs***	**-0.40**	**0.10**	**-1.00**
Sea otter	↔	**Mesopredators**	**0.12**	**0.05**	**0.57**
Crabs*	↔	**Mesopredators**	**-0.26**	**0.10**	**-0.57**
Mesopredators	↔	***Ulva****	**0.07**	**0.03**	**0.37**
Crabs*	→	***Ulva****	**-0.33**	**0.07**	**-0.64**
Crabs*	→	Eelgrass grazers*	-0.07	0.05	-0.24
Mesopredators	→	**Eelgrass grazers***	**-0.32**	**0.09**	**-0.57**
Ulva*	→	***Ulva* grazers***	**0.27**	**0.15**	**0.41**
Mesopredators	→	*Ulva* grazers*	0.07	0.13	0.12
Crabs*	→	*Ulva* grazers*	-0.02	0.07	-0.07
*Ulva**	→	Eelgrass grazers*	-0.01	0.11	-0.02
Eelgrass grazers*	→	Epiphytes	-0.76	9.24	-0.02
Ulva* grazers	→	**Epiphytes**	**-12.59**	**6.5**	**-0.33**
Epiphytes	→	**Eelgrass mass**	**-1.27**	**0.56**	**-0.42**
Epiphytes	→	Eelgrass shoots	-0.67	0.59	-0.19
*Ulva**	→	Eelgrass mass	5.15	12.70	0.07
Eelgrass mass	→	**Eelgrass shoots**	**0.63**	**0.18**	**0.54**
*Ulva**	→	Eelgrass shoots	-0.90	12.76	-0.02
Eelgrass mass	→	Eelgrass grazers*	0.002	0.001	0.24
Eelgrass shoots	→	**Eelgrass grazers***	**0.003**	**0.001**	**0.41**

*indicates analysis run on log-transformed data.

Table 2.S2. (continued)

F) Final seagrass-macroalgal ecotone (with *Ulva*) SEM with good model fit (χ^2 = 31.936, df = 23, *P* = 0.101)

Path			*Unstandardized coefficient*	*Standard error*	*Standardized coefficient*
Sea otter	↔	**Crabs***	**-0.40**	**0.10**	**-1.00**
Sea otter	↔	**Mesopredators**	**0.12**	**0.05**	**0.57**
Crabs*	↔	**Mesopredators**	**-0.26**	**0.10**	**-0.57**
Mesopredators	↔	***Ulva****	**0.07**	**0.03**	**0.37**
Crabs*	→	***Ulva****	**-0.33**	**0.07**	**-0.64**
Crabs*	→	**Eelgrass grazers***	**-0.09**	**0.05**	**-0.29**
Mesopredators	→	**Eelgrass grazers***	**-0.29**	**0.08**	**-0.53**
Ulva*	→	***Ulva* grazers***	**0.34**	**0.10**	**0.52**
Ulva* grazers	→	**Epiphytes**	**-12.41**	**6.57**	**-0.32**
Epiphytes	→	**Eelgrass mass**	**-1.39**	**0.49**	**-0.45**
Eelgrass mass	→	**Eelgrass shoots**	**0.73**	**0.16**	**0.63**
Eelgrass shoots	→	**Eelgrass grazers***	**0.003**	**0.001**	**0.55**
Ulva* grazers	↔	**Eelgrass grazers***	**-0.02**	**0.01**	**-0.27**

*indicates analysis run on log-transformed data.

3. Chapter 3 - Climate impacts on fish diversity and nursery function of a highly threatened estuary

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3.1 ABSTRACT

Coastal ecosystems provide numerous important ecological services, including provision of biodiversity and nursery grounds for many fish species of ecological and economic importance. However, human population expansion has led to increased pollution, ocean warming, hypoxia, and habitat alteration that threaten ecosystem services. In this study we used a 40-y data set of fish abundance, water quality, and climatic factors to assess the threat of hypoxia and the regulating effects of climate on fish diversity and nursery conditions in Elkhorn Slough, a highly eutrophic estuary in central California (U.S.A.), which also serves as a biodiversity hotspot and critical nursery grounds for offshore fisheries in a broader region. We found that hypoxic conditions had strong negative effects on extent of suitable fish habitat, fish diversity, and abundance of the two most common flatfish species. One species, English sole (*Parophrys vetulus*), uses the estuary as its primary nursery grounds and is susceptible to anthropogenic threats. We determined that estuarine hypoxia was associated with significant declines in English sole nursery habitat, as well as the offshore adult population, recruitment, and fishery, indicating that human land-use activities can indirectly affect offshore fisheries. We found that hypoxic

conditions varied spatially and temporally and were alleviated by strengthening of El Niño conditions through both direct and indirect pathways, a result that was consistent in estuaries across the northeast Pacific. These results demonstrate that changes to coastal land-use and climate can fundamentally alter the diversity and functioning of coastal nurseries and their adjacent ocean ecosystems.

3.2 INTRODUCTION

Over a third of Earth's human population is concentrated along coastal margins (Barbier et al. 2008) and much of the planet is dependent on the many functions and services provided by coastal ecosystems. Coastal ecosystems face multiple threats that include habitat loss and modification through urban development, intensification of agriculture and subsequent eutrophication, climate change, and overfishing, all of which decrease ecosystem functioning and diminish the ecologic and economic value of continental shelves around the world (Jackson et al. 2001; Rabalais et al. 2002; Lotze et al. 2006; Halpern et al. 2008; Selman et al 2008). The effect of multiple stressors, such as climate change and hypoxia over spatial and temporal scales relevant to the diversity and function of coastal systems is poorly understood. Furthermore, there are very few predictions on how climate change will interact with other anthropogenic threats to influence ecosystem functioning and services.

Certain critical functions and services of coastal ecosystems, such as estuaries, are potentially affected by anthropogenic threats. These services include supporting

biodiversity (Millennium Ecosystem Assessment 2005) and the provision of nursery habitat for species, where estuaries can contribute disproportionately to offshore fisheries productivity (Beck et al. 2001 and 2003; Nagelkerken et al. 2013; Sheaves et al. 2014). The nursery function, in particular, could be affected by a suite of anthropogenic stressors, manifesting in declines to offshore fisheries production. Multiple indirect factors could be influencing an already complicated path of nursery function to offshore fisheries. Along the California Current factors influencing the coastal nursery function can be climatic effects, such as El Niño and Upwelling (Cloern et al. 2007 and 2010); or they can be anthropogenic factors operating on multiple scales, such as ocean warming on ocean basin scales (Todd et al. 2008; Sherman et al. 2009), or anthropogenic nutrient loading on local to regional scales. The latter of which can cause the depletion of oxygen from the water column, hypoxia, with negative consequences to aquatic life (Rabalais et al. 2002; Diaz and Rosenberg 2008; Vaquer-Sunyer and Duarte 2008; Breitburg et al. 2009).

Using a highly altered, albeit regionally important estuarine ecosystem, we set out to test how anthropogenically induced hypoxia influences vital ecosystem services, such as the provision of biodiversity and nursery habitat, and how climate indirectly drives these ecosystem services through the modulation of hypoxia. By determining the climatic drivers of hypoxia and its association with fish diversity and nursery function we are able to make predictions on how climate change might impact coastal ecosystems. To strengthen our predictions we examined the association of climate - El Niño conditions, and hypoxia in estuaries that span the

northeast Pacific.

3.3 STUDY SYSTEM

Anthropogenic stress threatening a valuable nursery and biodiversity hotspot. Elkhorn Slough is an estuary on the central California coast that is representative of temperate estuaries worldwide facing multiple anthropogenic threats. Although is has a relatively small area, Elkhorn Slough provides a nursery for several species of cultural, ecological and commercial importance, which includes marine mammals (Hughes et al. 2013), sharks (Carlisle and Starr 2009), and commercially important flatfish (Brown 2006). Additionally, it is the only major estuary along 350 km of coastline, making it an important system for supporting regional biodiversity. Finally, Elkhorn Slough has a rare 40-y data set on the estuarine and adjacent offshore fish assemblages, water quality and regional climatic and oceanographic indices, making it possible to test the relationship between anthropogenic stressors, climate and ecosystem services, specifically fish biodiversity and the estuarine nursery function. Systems like Elkhorn Slough, where it is possible to examine climate and anthropogenic effects to ecosystem services over meaningful spatial and temporal scales are essential for making predictions on the effects of anthropogenic stressors in a changing climate.

Despite its importance as a nursery and biodiversity hotspot the estuary is threatened by anthropogenic stressors, most notably enhanced nutrient loading. Nutrient loading in the estuary is some of the highest recorded for temperate estuaries world wide (Caffrey 2002; Hughes et al. 2013), a consequence of a highly

agricultural landscape. This nutrient loading has created eutrophic and hypoxic conditions in the estuary (Caffrey et al. 2010; Hughes et al. 2011; Hughes et al. 2013), which are among the most severe in the United States (Figure 3.S1). Consequently, spatially- and temporally variable hypoxic conditions develop in the estuary (Beck and Bruland 2000, Hughes et al. 2011), a condition which is known to cause declines in fish populations through reduced survival, growth, and reproduction (Diaz 2001; Rabalais et al. 2002; Diaz and Rosenberg 2008; Vaquer-Sunyer and Duarte 2008).

Along with fish diversity indices (measured as species richness), we focused on the two most abundant flatfish species in the estuary, English sole (*Parophrys vetulus*) and speckled sanddabs (*Citharichthys stigmaeus*) (present in 29.3% and 45.8%, respectively, in surveys from 1970-2010, $n = 371$). The juvenile life-history stage for both English sole (19-250 mm, Love 2011; $\mu_{\text{Elkhorn Slough}} = 55.9 \pm 29.7$ SD) and speckled sanddabs (20-90 mm, Love 2011; $\mu_{\text{Elkhorn Slough}} = 73.4 \pm 22.1$ SD) were the most common life-history stage caught in surveys (95.3% and 69.1%, respectively), emphasizing the nursery role of the estuary. Both species are known to use estuaries as nurseries for juvenile life-history stages (Baxter et al. 1999; Brown 2006), for English sole the majority leave estuaries as age-0 (< 1 y, ~120 mm) juveniles (Lassuy 1989) and nearly all leave by age-1 (< 2 y, ~180 mm) (Baxter et al. 1999). English sole is a commercially important fish that uses Elkhorn Slough as one of its primary nursery habitats in the region around Monterey Bay and it has been estimated that ~50% of adults caught in Monterey Bay use Elkhorn Slough as a

nursery (Brown 2006). Elkhorn Slough is also at the southern end of the range for English sole (Emmett et al. 1991), making it potentially more susceptible to both temperature stress and hypoxia.

3.4 RESULTS AND DISCUSSION

3.4.1 Impaired water quality drives the loss of nursery function and fish diversity.

Hypoxia had negative consequences for the two ecosystem services we measured: the provision of fish diversity and nursery function. The negative effects of hypoxia on fish have been demonstrated, yet studies on hypoxic effects are usually on short time scales (hours to < 10 y) or are not spatially and temporally explicit (Vaquer-Sunyer and Duarte 2008; Breitburg et al. 2009). Here we present results from 40 y of monitoring that suggests that the effects of anthropogenic nutrient loading and hypoxia on key ecosystem services are variable through time and space.

Flatfish presence was negatively correlated with hypoxic conditions in Elkhorn Slough. Sequential logistic regression determined that of all potential predictor variables (temperature, upwelling, El Niño Southern Oscillation - ENSO, Pacific Decadal Oscillation - PDO, salinity, and sampling effort), dissolved oxygen (DO) was the only factor that consistently correlated with flatfish presence (Table 3.S1*A*-*B*). The two target flatfish species demonstrated significant declines as a function of decreasing DO based on logistic regression (Figure 3.1*A*-*B*) (Table 3.S1*A*-*B*), suggesting a general negative effect of low DO on fish presence in two habitat types: deep channels (~2 – 10 m depth) and shallow margins (< 2 m depth).

Temperature was also consistently included in the best-fit models using Akaike Information Criterion (AIC) (Table 3.S1*A*-*B*), however the direction of the correlation varied from positive to negative and was often marginally significant (P = 0.05 – 0.10) (Table 3.S1*A*-*B*). Remote drivers (Upwelling, ENSO, and PDO) were also significant predictors in the logistic regression models, however, there were few consistent patterns among species in both deep channel and shallow margin habitats, suggesting that local habitat conditions, such as hypoxia, are more important factors influencing flatfish presence in the estuary. Deep channel surveys indicated that speckled sanddab presence was also positively correlated with El Niño conditions (Table 3.S1*B*). On the other hand, presence of English sole was positively correlated with increased upwelling (Table 3.S1*A*), and this pattern was consistent for both deep channel and shallow margin habitats, indicating upwelling could be driving the recruitment of English sole to the estuary.

We determined the spatial extent of flatfish habitat quality in the estuary by spatially modeling flatfish presence as a function of hypoxia. We targeted hypoxia because it was the strongest and most consistent predictor of flatfish presence based on the logistic regressions described above. The lower section of the main channel of Elkhorn Slough provided the highest quality habitat for flatfish (Figure 3.S2) based on higher levels of DO and the strong positive relationship between DO and fish presence. Flatfish habitat quality (i.e. reduced DO levels) generally worsened outside of the lower main channel of the estuary. Peripheral areas of the estuary where tides were restricted by water control structures were the poorest habitat for flatfish in the

estuary, due to more frequent and intense hypoxic conditions (Hughes et al. 2011). Also, difficulty in fish passage through water control structures could compound the effects of severe hypoxia. Spatial patterns of the predicted probability of flatfish presence identified by habitat modeling (Figure 3.S2) corresponded to areas with low to moderate eutrophication from a previous study (Hughes et al. 2011). These results indicated that hypoxia can have negative effects on fish habitat extent and potentially the nursery function of the estuary.

The logistic regression model and the spatial modeling of predicted probabilities were validated using a 2005 spatially explicit survey of 16 water quality (Figure 3.S2*A*) and fish (Figure 3.S3; Ritter et al. 2008) sampling stations around Elkhorn Slough (Table 3.S2). The logistic regression analysis of flatfish presence indicated that the probability of flatfish occurrence decreases with increased hypoxia ($P = 0.040$). Sites that were hypoxic and behind water control structures were devoid of flatfish during the 2005 surveys. The threshold for absence was ~ 4 mg $*L^{-1}$ DO (Figure 3.S2) in daytime DO samples; DO levels are typically much lower at nighttime and often go anoxic in Elkhorn Slough (Hughes et al. 2011). This value was consistent with the lower threshold from the logistic regressions at long-term fish sampling locations (Figure 3.1*A-B;* Figure 3.S2), and is also consistent with previous studies documenting flatfish density declines occurring below 3 mg $*L^{-1}$ DO (Levings 1980, Eby and Crowder 2002). These results also suggest that negative hypoxic effects to flatfish are compounded by additional stressors, in this case habitat alterations through water control structures.

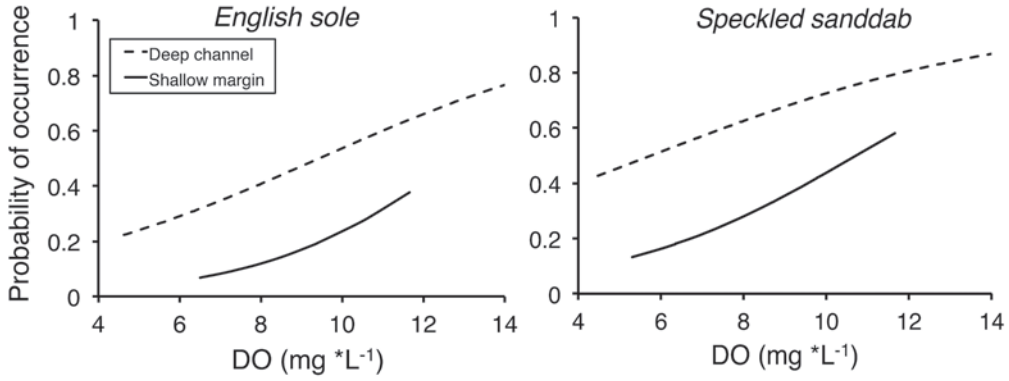

Figure 3.1. Logistic regression analysis of the predicted probability of presences for three species of flatfish as function of DO concentration (mg $*L^{-1}$) for deep channel (solid line) (*n* = 169) and shallow margin (broken line) (*n* = 78) habitats for English sole and speckled sanddab. See Table 3.S1 for logistic regression results.

The nursery and suitable fish habitat in Elkhorn Slough is dynamic in time and space as revealed by a Dissolved Oxygen Anomaly (DOA), which identified periods of hypoxia and normoxia in non-artificially restricted areas of the estuary (Figure 3.2A; Figure 3.S2; Table 3.S3*A*). During periods of normoxia English sole abundance increased by 367% ($P = 0.034$; Figure 3.2*B*), and species richness increased by 49% ($P = 0.029$; Figure 3.2*D*). Although there was no significant difference in normoxic and hypoxic periods for speckled sanddabs ($P = 0.175$) there was a trend of greater abundance during normoxic periods (Figure 3.2*C*).

During periods of normoxia, unrestricted areas in the upper half of the estuary become suitable nursery habitat and supported a higher diversity of fish compared to hypoxic periods (Table S3*B*). Therefore, the ecosystem services of enhanced biodiversity and nursery habitat are only provided for half of the estuary during hypoxic periods, but nearly the entire estuary is available during normoxia. During periods of normoxia there were no significant differences between the upper and lower estuary for species richness ($P = 0.153$), and both English sole ($P = 0.561$) and speckled sanddab ($P = 0.305$) abundances indicating the upper estuary is suitable habitat for fish when conditions are favorable (Figure 3.2*E*-*G*; Table 3.S3*Bi*). However, significant differences between the upper and lower estuary emerged during hypoxic conditions, as significantly fewer species ($P = 0.005$), English sole ($P = 0.018$) and speckled sanddabs ($P = 0.042$) used the upper estuary (Table 3.S3*Bii*). Additionally, there were no significant differences between hypoxic and normoxic conditions in the lower estuary for species richness, nor English sole or speckled

sanddab abundance (all $P > 0.10$; Figure 3.2*E*-*G* and Table 3.S3*Ci*). However, there were significantly lower species richness ($P = 0.002$) and abundance of English sole ($P = 0.068$) (93.3% reduction) and speckled sanddab ($P = 0.054$) (82.0% reduction) in the upper estuary during hypoxic conditions when compared to normoxic conditions (Table 3.S3*Cii*). The estuary-wide decline of fish associated with hypoxia resulted in an average annual loss of ~7,000 speckled sanddabs and ~18,000 English sole, the latter of which could translate to a substantial loss of recruitment to the offshore adult population with consequences to the overall population and fishery, thus decreasing the nursery function of the estuary.

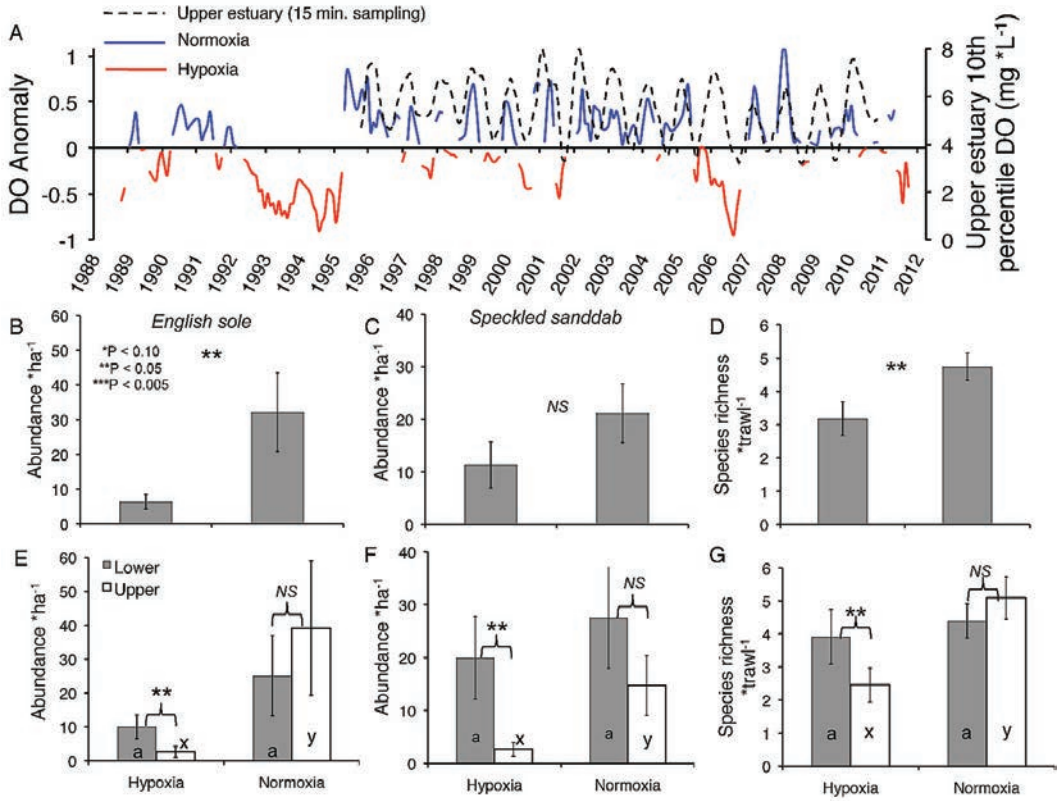

Figure 3.2. (A) Time series of the three-month moving average of the DOA (± 0.38 SD) overlaid with the 10th percentile DO from an upper slough water quality station (Figure 3.S2) with continuous data collection (15 min.) confirming patterns in the DOA. Anomalous patterns in 2006 were due to normal DO conditions at the continuous water quality station, despite hypoxic conditions in the rest of the estuary. (B-D) Results from an independent samples t-tests comparing English sole and speckled sanddab abundance and fish species richness, between hypoxic and normoxic periods using data from deep channel survey data pooled from both the upper and lower estuary (see Table 3.S3*A* for statistical results and sample sizes). (E-G) Results from both paired samples t-tests comparing differences in the lower and upper estuary on fish parameters during hypoxia and normoxia, respectively (see Table 3.S3*Bi-ii* for statistical results and sample sizes); and independent samples t-tests comparing the hypoxic and normoxic periods on fish parameters for both the lower and upper estuary respectively (see Table 3.S3*Ci-ii* for statistical results and sample sizes). Differences in letters indicate significant differences ($P < 0.10$). All error bars represent ± 1 SE.

Periods of hypoxia corresponded to declines in the offshore English sole population (Table 3.S4). The majority of juvenile English sole recruit to the offshore adult population after their first or second year in the nursery (Gunderson et al. 1990), so we predicted that the 80% decline in juvenile English sole abundance observed in Elkhorn Slough during hypoxic years (Figure 3.2B) would translate to a significantly reduced abundance in offshore catch in the following year. As predicted, cross-correlation analysis detected a one year lag effect on the offshore abundance of English Sole based on National Marine Fisheries Service (NMFS) trawl surveys in the Monterey Bay region (Figure 3.S4). Increases in the DOA, which indicated normoxic conditions, were correlated with greater English sole Catch Per Unit Effort (CPUE; kg *trawl^{-1}) in this fishery-independent dataset. Using the one-year lag correlation we detected a significantly greater abundance (almost double) of English sole offshore after normoxic periods compared to hypoxic periods in Elkhorn Slough as determined by the DOA (P = 0.054; Figure 3.3*A* and Table 3.S4*A*). There are two potential mechanisms driving the poor recruitment to the offshore population resulting from hypoxic periods: either hypoxia in the estuary caused increased mortality or they fled the estuary to areas of poorer nursery quality (e.g., nearshore or offshore) where they experienced decreased growth and survival rates. English sole abundance in trawl surveys in the adjacent offshore region of Half Moon Bay, which served as a control region, did not differ significantly (P = 0.308) between periods of normoxia and hypoxia in Elkhorn Slough. To our knowledge, English sole offshore recruits in the Half Moon Bay region are not influenced by an intermittently hypoxic

nursery. For an additional control, we used Rex sole (*Glyptocephalus zachirus*), a functionally similar flatfish species that does not use the Elkhorn Slough estuary as nursery grounds, and compared its abundance in trawl surveys in Monterey Bay and offshore of Half Moon Bay for normoxic and hypoxic periods in Elkhorn Slough. We found no differences in Rex sole abundance between periods of Elkhorn Slough normoxia and hypoxia in either Monterey Bay (P = 0.896) or Half Moon Bay (P = 0.355; Figure 3.3*A-B* and Table 3.S4*B*), further implicating hypoxia in the Elkhorn Slough nursery as a negative factor for adult English sole in Monterey Bay. Additionally, there were significant declines in new sub-adult and young adult recruits of English sole for the offshore Monterey Bay population following hypoxic years (P = 0.045; Figure 3.3*C* and Table 3.S4*C*), yet no significant differences in the adjacent Half Moon Bay population (P = 0.588), suggesting that negative effects of hypoxia on the coastal nursery function can have consequences for the adult population due to limited recruitment.

Finally, there was a significant negative relationship between the number of hypoxic months in Elkhorn Slough per year and annual fisheries landings of English sole caught in the Monterey Bay commercial fishery (P = 0.032, R^2 =0.201; Figure 3.3*D* and Table 3.S4*D*). This suggests that severity in hypoxia in the English sole nursery grounds results in decreased abundance of fish for the Monterey Bay fishery.

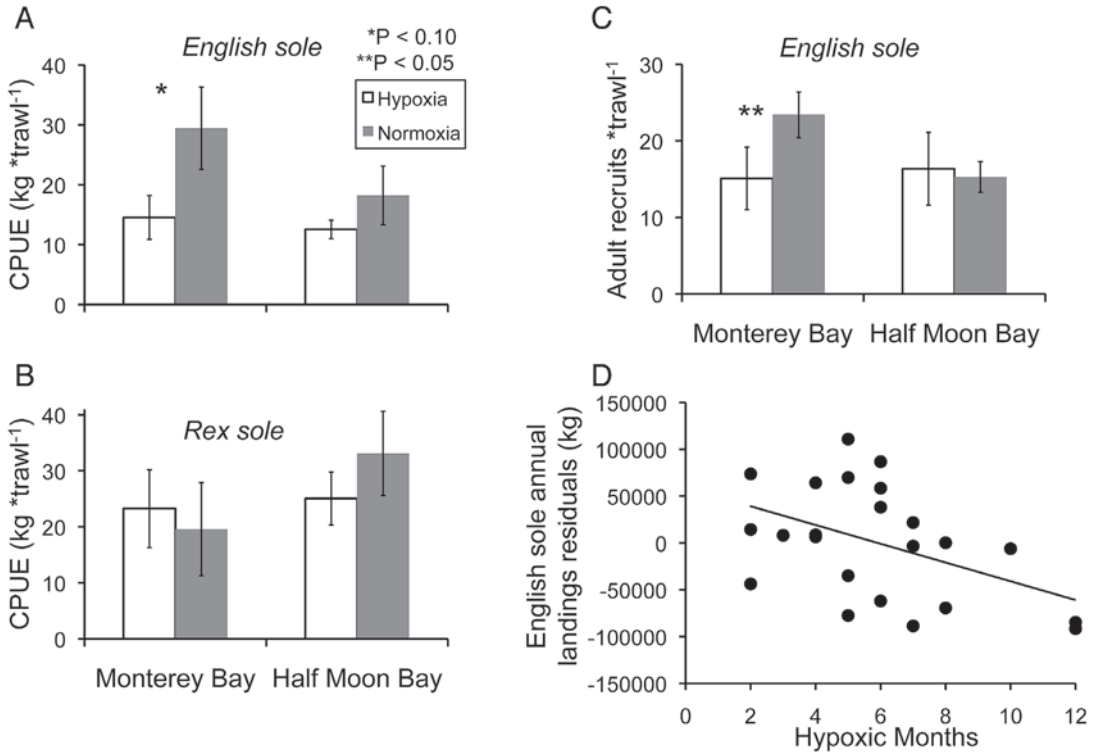

Figure 3.3. Lag effects of hypoxic (n = 10) and normoxic (n = 6) years in Elkhorn Slough on NMFS trawl data for (A) English sole and (B) Rex sole (an analogous species that does not use Elkhorn Slough for habitat) for the Monterey Region and the adjacent Half Moon Bay region (not influenced by Elkhorn Slough); see Table 3.S4 for independent samples t-test results, (C) Differences in adult recruits to the offshore English sole population after hypoxic (n = 9) and normoxic (n = 6) years in Elkhorn Slough, (D) effect of number of hypoxic months on English sole landings with effort (year) removed (n = 23). Error bars represent ± 1 SE.

3.4.2 Moderating influences of climate on coastal eutrophication, hypoxia, and the nursery role. By causing variation in precipitation and runoff, climate can be a powerful moderator of coastal hypoxia stress from anthropogenic nutrient loading (Rabalais et al. 2002 and 2010; Scully 2010). Variation in hypoxic conditions in Elkhorn Slough suggested that external drivers could influence ecosystem responses to anthropogenic threats. The periods from 1988-1990, 1992-1995, 2000-2002, 2005-2006, and 2010-2011 were generally hypoxic regimes in the estuary followed by normoxic regimes from 1990-1991, 1995-1999, 2002-2005, and 2007-2010 (Figure 3.2*A*). This result indicated other processes are modulating the effect of the exponential increase in nutrient loading documented in Elkhorn Slough over the last four decades (Hughes et al. 2013).

Structural equation modeling (SEM) identified mean annual salinity as the key correlate of the annual lower limit of DO in the upper estuary (μ = 4.85 mg *L^{-1}; ±0.51 SD; Figure 3.S2*A*; Figure 3.4). Path analysis revealed that increases in El Niño conditions including increased precipitation and decreased salinity, were associated with reduced hypoxic conditions in the upper estuary. These relationships were most likely due to increased flushing rates in the estuary (Hughes et al. 2011). In the lower estuary (Figure 3.S2*A*) increased upwelling intensity, occurring primarily as spring-time events (Booth et al. 2012), correlated positively with hypoxia (Figure 3.4). However, the annual lower limit in the lower estuary (μ = 5.81; ±0.51 SD) rarely approached the 4 mg *L^{-1} threshold for flatfish indicating that upwelling lowered DO, but probably not enough to cause severe deleterious effects to fish.

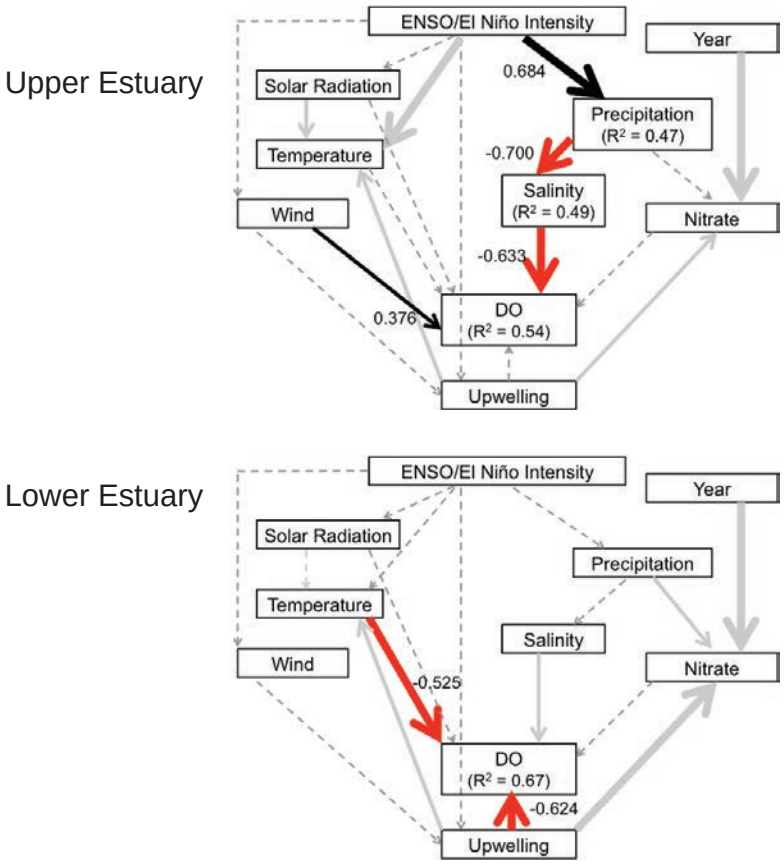

Figure 3.4. Path analysis from results of SEM showing the direct and indirect drivers of DO in upper (n = 15, χ^2 = 5.76, df = 6, P = 0.45) and lower (n = 10, χ^2 = 0.36, df = 1, P = 0.55) Elkhorn Slough, respectively. Sold lines indicate significant correlations ($P < 0.10$), dashed lines indicate insignificant correlations ($P > 0.10$), line widths are proportional to regression coefficients and only paths with significant effects on DO and their corresponding coefficients and R^2 are shown. Black lines indicate positive effects and red lines indicate negative effects on DO, respectively.

The results of the SEM suggest that low salinity as well as lower upwelling indices, factors both driven by El Niño, had a positive effect on the oxygen condition of the estuary. Increases in El Niño intensity result in increased local precipitation along with warmer ocean waters, the latter can relax upwelling intensity (Chavez 1996; Friederich et al. 2002). An increase in the frequency and intensity of El Niño events could mitigate Elkhorn Slough's hypoxic condition and thus support flatfish in two ways: 1) increased precipitation (as indicated by decreases in salinity) increases the flushing of the estuary which has been shown to decrease eutrophication in hypoxic estuaries (Paerl et al. 1998), and 2) relaxation of local upwelling that brings hypoxic water from the deep sea over the continental shelf during intense upwelling years (Chan et al. 1998, Booth et al. 2012), although this effect was not detected in our analysis. It should be noted that despite hypoxia occurring in both the lower and upper estuary, the greatest declines to fish occurred in the upper estuary (Figure 3.2*F-I*) and could be due to a combination of factors, which include more frequent and severe hypoxia (Figure 3.S2), higher temperatures (Hughes et al. 2011), and less refugia due to shallower depths in the upper estuary.

Finally, we hypothesized that if severity in estuarine hypoxia is mediated by large-scale climatic factors, such as El Niño, then patterns in hypoxic conditions should be consistent across the entire northeast Pacific. We found significant negative associations with El Niño and severity of hypoxic conditions in estuaries across the northeast Pacific ($P = 0.018$; $t_{18} = 2.609$; Figure 3.5), indicating that cyclical climatic patterns are indeed strong predictors of hypoxia severity. El Niño conditions

measured from 1997-2010 were associated with improved hypoxic conditions in 5 of the 6 estuaries we investigated. The only estuary, Padilla Bay, WA, that did not indicate positive effects from El Niño conditions is located inland approximately 200 km from the exposed outer coast, and is likely not as exposed to upwelling-driven hypoxia as the other five estuaries with mouths opening directly to the outer coast.

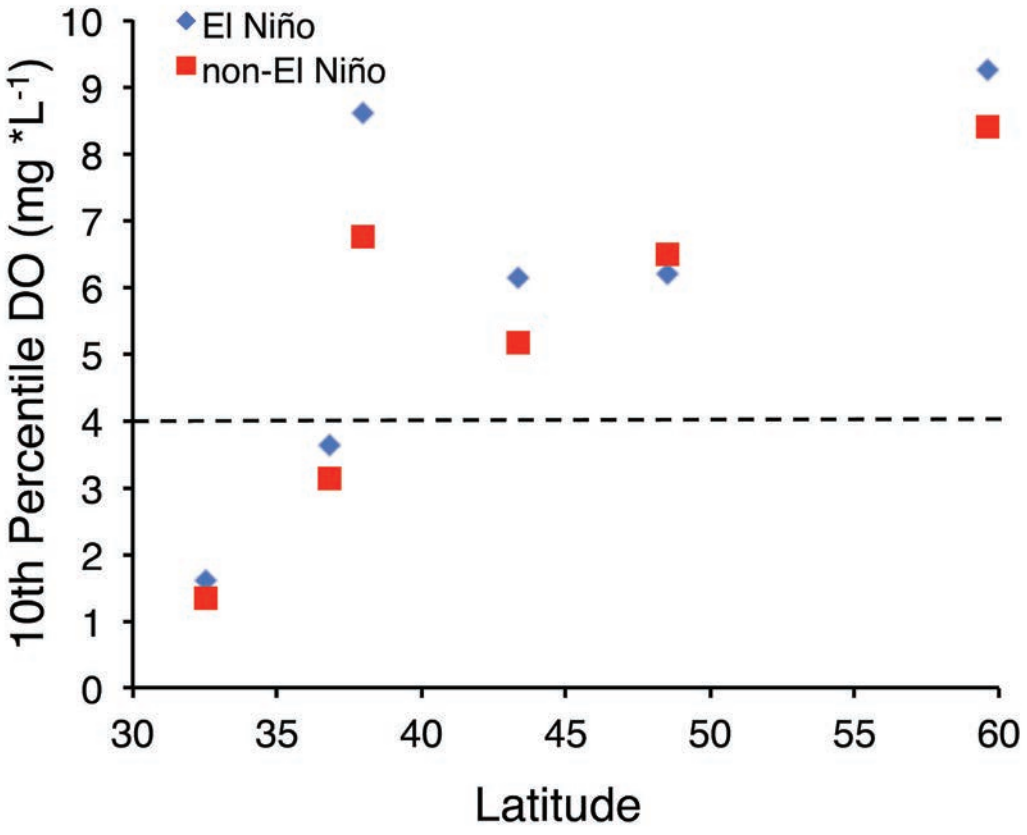

Figure 3.5. Hypoxia in northeast Pacific estuaries of the United States as a function of latitude and El Niño (n = 3) and non-El Niño (n = 3) years. Each point represents the mean among stations at each estuary. The Dotted line represents the threshold for flatfish occurrence as determined by modeling and previous literature results (Levings 1980, Eby and Crowder 2002, Stierhoff et al. 2006).

3.5 CONCLUSION

Climate drives ecosystem resilience to anthropogenic threats. Here we have demonstrated that anthropogenic threats to available fish habitat in one small, albeit important, estuary can have consequences for a larger region and adjacent ecosystems, and that climate interacts with these threats to influence ecosystem services. In this case, El Niño alleviated stress to the system by improving oxygen conditions. Further analysis revealed that El Niño conditions consistently lead to improved conditions to estuaries along the northeast Pacific (Figure 3.5). Elkhorn Slough is representative of other threatened estuaries in the U.S. and worldwide (Figure 3.S1; Hughes et al. 2013, Figure 1.1). Given the well-known importance of estuaries as nurseries (Beck et al. 2001 and 2003; Nagelkerken et al. 2013; Sheaves et al. 2014) and their highly altered state (Jackson et al. 2001; Lotze et al. 2006; Selman et al 2008) suggests that anthropogenic impacts and climate change may impact other estuaries in a similar fashion to Elkhorn Slough. Thus, determining climate and threat scenarios that produce diminishing returns will be essential for informing managers on the consequences of a changing climate to natural resources and ecosystem services. This is only possible through long-term monitoring programs so climate variation can be incorporated into models of threats to ecosystem services.

Determining how climate change will affect coastal ecosystems under threat is a difficult challenge facing researchers and managers (Côté and Darling 2010; Micheli et al. 2012). Our results offer insight as to how climate change may impact the effects of anthropogenic nutrient loading, which is a threat that is likely to persist

given earth's human population growth in coastal environments (Barbier et al. 2008). Here the effect of climate change is dependent on the direction of change, the context of other stressors, and the life history of the species of concern. For example, if El Niño conditions are predicted to intensify with climate change then this could result in sustained ecosystem services through the suppression of upwelling, increased flushing, improved oxygen conditions, and enhanced biodiversity and nursery function of northeast Pacific estuaries. However, if climate change results in moderate El Niño or enhanced La Niña conditions and upwelling in the northeast Pacific intensifies, as predicted (Aud et al. 2006; Sydeman et al. 2014), then it could result in poor oxygen conditions in coastal zones, especially when combined with increased anthropogenic nutrient loading, resulting in an overall decline in ecosystem services. As it currently stands, future ENSO conditions are highly unpredictable and there is little understanding of its long-term variation (Collins et al. 2010; Giese et al. 2011). Therefore there is an urgent need to develop models predicting how climate change might impact ENSO conditions and its influence on ecosystem services.

3.6 METHODS

See *SI Methods* for a description of data sources.

3.6.1 Correlating habitat condition with presence of select flatfish species. To test for the potential effects of hypoxia on flatfish species we examined differences in presence/absence data of the two most abundant flatfish species found in the estuary: English sole and speckled sanddab. Both species are marine immigrants that primarily

use the estuary as nursery grounds during their juvenile stages (Yoklavich et al. 1991; Brown 2006). To determine the key correlates with individual flatfish species we used sequential logistic regression with the GLM package in R version 3.0.2. Logistic regression is derived from the Generalized Linear Model family, and differs from linear regression because it relaxes assumptions on normality of response variables by using binary (i.e. presence/absence) data instead of continuous data. It then models the probability of presence/absence of a species using a linear function of the predictor variables. The sequential predictor variables were selected based on our *a priori* hypothesis that DO was the most important driver of fish in Elkhorn Slough (see methods for sequential regression in Graham 2003), and on F-values from preliminary multiple linear regressions in the following order: DO (mg $*L^{-1}$), temperature (^{o}C), Monterey Bay Upwelling (MBU), El Niño Southern Oscillation (ENSO) index ENSO, Pacific Decadal Oscillation (PDO) index, salinity (ppt), and daily sampling effort (number of trawls or seines). We omitted nitrate and the North Pacific Gyre Oscillation index from the sequential logistic regression because they were not significant predictors ($P < 0.10$) in the preliminary multiple regressions. We did not include sampling location or season as factors in the analysis because flatfish were generally caught at all times of the year throughout the primary sampling stations (Figure 3.S2*A*) in Elkhorn Slough. To confirm the relationship between environmental patterns and presence/absence for each flatfish species we replicated the logistic regression analysis using both deep channel and shallow margin habitat data, respectively.

We determined the key correlates of flatfish presence using the Akaike Information Criterion (AIC), which selects the best model by incorporating all variables, similar to a stepwise multiple regression. Once the significant correlates were identified, we applied a reduced logistic regression by correlating the presence/absence data to the significant individual environmental predictors to determine the direction of the correlation. We used model selection based on AIC weighting (Johnson and Omland 2004) to confirm that the final sequential logistic regression was the most appropriate model. Last, we applied the DO concentration (mg $*L^{-1}$) as the only predictor variable to determine general patterns in the relationship between DO and flatfish presence. All alphas were set at 0.10 to reduce Type II errors that fail to reject the null hypothesis (Underwood 1997) given the challenges of large-scale field sampling.

3.6.2 Modeling the extent of suitable flatfish habitat. To determine the spatial extent of suitable flatfish habitat in Elkhorn Slough we modeled the probability of flatfish presence (using logistic regression curves) as a function of hypoxia extent in the estuary and ENSO conditions. We only incorporated logistic regression curves from deep channel habitats because they had a larger range of probabilities and DO. We did not use fish data from 1970-1988 because of a gap in water quality data from 1976-1988, therefore we opted to use the data set that was most continuously sampled (1988-2012, Elkhorn Slough National Estuarine Research Reserve - ESNERR).

Using a spatial interpolation analysis we mapped out the probability of occurrence for English sole and speckled sanddab using DO values among sites within Elkhorn Slough. We combined Python and Numerical Python (NumPy) scripting with ArcGIS 10.2 (Environmental Systems Research Institute, Redlands, CA) to parse, prepare, and analyze tabular DO data. To calculate the 10th percentile of DO throughout Elkhorn Slough, we converted each monthly sample into raster format and interpolated (25 m x 25 m cell size) using the Spatial Analyst extension of ArcGIS. We used the Spline with Barriers interpolation method, which attempts to fit a surface among all values while minimizing the amount of curvature and while respecting breaks and discontinuities imposed on the surface (Childs 2004). Each resulting raster was converted into a NumPy array, and the NumPy percentile function was used to determine the 10^{th} percentile for each cell through the stack of arrays (n = 252), resulting in a single array that was then converted back into a raster for further analysis (Figure 3.S5). The resulting raster of 10^{th} percentile DO values was used to calculate the probability of occurrence of English sole, speckled sanddabs, using the logistic regression analysis described above. The raster calculator function of ArcGIS was used to apply the algorithm to each 25 m x 25 m cell based on the 10^{th} percentile DO value for that cell.

3.6.3 Model validation of flatfish logistic regressions. Next, we validated our logistic regression, which had good temporal coverage, using a directed flatfish survey within Elkhorn Slough. A 2005 survey by Ritter et al. (2008) thoroughly

sampled the Elkhorn Slough fish assemblage by sampling shallow margin habitats using beach seines at 16 stations strategically located (< 500 m) to the nearest water quality monitoring station (Figures 3.S1-S2). Each station was sampled in the spring and again in the summer coinciding with periods of increased hypoxia (Figure 3.S6) and nursery function. We used each sampling date at each station as a replicate in a logistic regression analysis. The logistic regression analysis was run using presence/absence for flatfish species as the dependent variable and the 10^{th} percentile DO calculated for the entire ESNERR dataset (1989-2011). By using the 10^{th} percentile of DO we were able to compare the relative degree of hypoxia for each sampling station (Hughes et al. 2011). We combined all flatfish species: speckled sanddab, California halibut (*Paralichthys californicus*), starry flounder (*Platichthys stellatus*), fantail sole (*Xysteurys liolepis*), and California tonguefish (*Symphurus atricauda*) into one group given their similar lifestyles and because of low replication among the individual species during the survey period. Additionally, we mapped out flatfish probabilities to assess similarity in the spatial distribution of flatfish probabilities between the 2005 fish survey and 1988-2012 water quality datasets using the spatial modeling procedure described above (see **Modeling the extent of suitable flatfish habitat**).

3.6.4 Developing a Dissolved Oxygen Anomaly. To scale up to the estuary-wide hypoxic condition we developed a dissolved oxygen anomaly (DOA) to identify hypoxic periods in the estuary that could be correlated with the fish assemblage. The

DOA was calculated using the entire ESNERR water quality record (1988-2011) at stations (n = 6) that were sampled within the fish sampling range along the main channel of the estuary by calculating Z-scores: Global Mean – Raw DO (mg $*L^{-1}$) $*Global\ SD^{-1}$. The average monthly value among all the sampling stations was used for a single monthly value that represented the DO condition for the estuary for that month. We used the DOA, which is simply a standardized variance value, because we wanted to assess the estuary-wide DO condition for monthly sampling and not individual sites because of high spatial and temporal variability (Hughes et al. 2011). We defined hypoxic as any negative DOA value and normoxic as any positive DOA value.

3.6.5 Spatiotemporal associations of the DO Anomaly and the estuarine fish assemblage. We next investigated how variation in DO conditions in the estuary explained patterns in fish species richness and abundance of English sole and speckled sanddabs (number of individuals $*ha^{-1}$). Only data from standardized trawl surveys (1991-2003) that had surveyed deep channel habitats from both the lower and upper estuary within a month of each other were used in the analysis. We used the three month running average of dissolved oxygen anomaly (DOA) to characterize each sampling date as either hypoxic (negative DOA) or normoxic (positive DOA). We tested for the effects of hypoxia (hypoxic or normoxic) and region (lower v. upper) on species diversity (fish species richness) and the abundance of the two target flatfish species (English sole and speckled sanddabs). The estuary was divided into

lower and upper estuary based on known hydrological, oceanographic (Largier et al. 1997, Nidzieko and Monosmith 2013) and biological (Hughes et al. 2011) breaks, including differences in the severity of hypoxia and eutrophication (Figure 3.S2). We used a series of t-tests SPSS version 20 (IBM, Armonk, NY) to determine differences in hypoxia and region. We first pooled all data among hypoxic (n = 16) and normoxic (n = 34) regimes and compared them using an independent samples t-test. Next, we compared the paired samples (upper and lower estuary) during hypoxic and normoxic conditions, respectively, to determine specific habitat use during the two regimes using a paired samples t-test. Last, we compared fish variables in the lower and upper estuary, respectively, during hypoxic and normoxic conditions using an independent samples t-test. For independent samples t-tests, we tested for the assumption of equal variance using a Levene's Test for equality of variances, if the test was significant (P < 0.10) then we used a Welch's t-test of unequal variances.

3.6.6 Offshore English sole population and fishery relationships with the DO Anomaly. To complete the land-to-sea connection and to validate that variable nursery conditions can have consequences to offshore fish populations, we correlated the DOA with fisheries-independent annual to triennial bottom-trawl surveys from the National Marine Fisheries Service (NMFS). Zimmermann (2006) contains detailed description of the NMFS bottom trawl survey methods. NMFS trawl surveys report catch per unit effort (CPUE) using standardized trawls at various depths. Trawl surveys are typically performed during summer months (June to September). We used

the standardized mean CPUE (kg *trawl^{-1}) from all depths (36-460 m) for each year sampled (1989-2012, n = 16) using only trawls where English sole was caught. For cross-validation purposes we used the same analytical approach and applied it to a functionally similar flatfish species, Rex sole (*Glyptocephalus zachirus*) that does not use the Elkhorn Slough estuary as nursery grounds. To further validate the nursery hypoxia effects to English sole in Monterey Bay (36.5ºN and 37ºN, and east of -122.5ºW) we compared NMFS trawl data for English sole and Rex sole in Half Moon Bay (37.0ºN and 37.5ºN, and east of -123.5ºW), a nearby region outside of the nursery range of Elkhorn Slough (Brown 2006).

We then characterized each year prior to the NMFS trawl survey by taking the average annual DOA in Elkhorn Slough. Using cross correlation analysis in R version 3.0.2, we determined the time lag (in years) that had the greatest correlation between the annual DOA (predictor variable) calculated from monthly means across sites and English sole CPUE (dependent variable). Once the lag was determined, for each trawl survey we characterized the corresponding year as being hypoxic ($\geq$ 6 months hypoxia) or normoxic (< 6 months hypoxia), we defined a hypoxic year based on the mean number of hypoxic months in the estuary (μ = 5; Figure 3.S6). We then compared the offshore English sole and Rex sole CPUE between hypoxic (n = 10) and normoxic (n = 9) years in Elkhorn Slough using an independent samples t-test using SPSS version 20 (IBM, Armonk, NY). Additionally, we compared English sole sub-adult and young adult recruitment to the offshore population of English sole between hypoxic (n = 9) and normoxic (n = 6) years in Elkhorn Slough by selecting

age-1 (< 2 y) and age-2 (< 3 y) individuals (< 250 mm female, < 220 mm male), most likely to have used Elkhorn Slough as a nursery the year prior to recruitment. We performed independent samples t-tests for both Monterey Bay and Half Moon Bay, respectively. For independent samples t-tests, we tested for the assumption of equal variance using a Levene's Test for equality of variances, if the test was significant ($P < 0.10$) then we used a Welch's t-test of unequal variances.

Last we determined the lag relationship between nursery hypoxia and the commercial offshore fishery. We used the DOA to determine the number of hypoxic months for the English sole nursery in Elkhorn Slough and correlated it with the reported English sole landings from California Department of Fish and Wildlife (www.dfg.ca.gov) for the Monterey Bay region one year later using residual linear regression ($n = 23$ years). We assumed that all new sub-adult and adult recruits would be targeted by bottom trawls along with older size classes since the minimum required net mesh size for the commercial fishery was 115 mm (Pacific Fishery Management Council; PFMC 2014), and most English sole reach a minimum size of 120 mm by the time they reach age-1 according to Von Bertalanffy parameters from Elkhorn Slough (Smith and Nitsos 1969), which is around the time when English sole begin to emigrate from the estuary. We considered our analysis to detect nursery hypoxia effects on the English sole offshore fishery robust, since 53.1% of fish are older juveniles or young adults < 3 y of age (< 25 cm) according to the NMFS trawl surveys, which accounts for the majority of fish in the population. To remove the confounding effects in annual fishing effort on the total English sole landings we

calculated the residuals from the correlation of fish landings and year, and used the residuals as the dependent variable in the final analysis (see Graham 2003 for a description of residual regression).

3.6.7 Identifying drivers of dissolved oxygen. We explored the key correlates of hypoxia by using continuous water quality monitoring stations within the estuary that sample for DO, temperature, and salinity. This also served as a cross-validation technique for the DOA, but used more temporally explicit continuously collected data (every 15 minutes) rather than spatially explicit data. We used ESNERR's South Marsh (upper estuary, Figure 3.S2*A*) and Vierra Mouth (lower estuary, Figure 3.S2*A*) water quality monitoring stations, which have been sampling since 1995 and 2001 (CDMO http://cdmo.baruch.sc.edu), respectively, with YSI (Yellow Springs Instruments, Yellow Springs, OH) data sondes. The Vierra Mouth site is closer to the mouth of the estuary and therefore more likely influenced by oceanographic processes, whereas the South Marsh site is located half-way up the estuary where residence times are higher and is more representative of mid to upper estuarine sites. We characterized hypoxia at each site by calculating the 10^{th} percentile of DO (an indicator of the level of hypoxia; Hughes et al. 2011) for an entire calendar year, and then used structural equation modeling (SEM) to explore the key direct and indirect correlates of hypoxia to test for direct and indirect effects (see Graham 2003 and Byrnes et al. 2011 for a description of SEM). By constructing path models we tested the hypothesis that environmental processes (El Niño and upwelling) regulate the

effects of anthropogenic nutrient loading on hypoxia at both stations in the upper and lower parts of the estuary. Models were reduced to eliminate insignificant factors ($P >$ 0.10). For the model we used annual means for ENSO, MBU, water temperature and salinity, total annual precipitation, mean annual nitrate and the and the annual 10^{th} percentile of DO as an indicator of hypoxia. Nitrate data was from water samples collected and averaged monthly from three monitoring stations near the estuary mouth where the estuary receives the greatest land-based nutrient load (Hughes et al. 2013). We used the annual values of each factor as replicates in the SEM for the upper (n = 15) and lower (n = 10) estuary. SEMs were calculated using SPSS Amos version 22.0 (IBM, Armonk, NY).

To determine the influence of ENSO on hypoxic conditions of northeast Pacific estuaries we selected six sites within the NERR system that have sampled DO continuously at multiple stations since 1997. These sites consisted of three California sites: Tijuana River Estuary/San Diego Bay, Elkhorn Slough, San Francisco Bay; South Slough, OR; Padilla Bay, WA; and Kachemak Bay, AK. We first characterized each year as El Niño (mean annual ENSO index > 0.50; n = 3; 1997 – 1998, 2002 – 2003, 2004 - 2005) or non-El Niño (mean annual ENSO index < 0.50; n = 3; 2001 – 2002, 2005 – 2006, 2008 - 2009). We determined the mean annual hypoxic condition (10^{th} percentile DO) for independent monitoring stations within each estuary (1 - 4 stations *estuary^{-1}; n = 19). We compared the mean annual hypoxic condition during El Niño v. non El Niño conditions at each station using a paired samples t-test.

3.7 SUPPORTING INFORMATION

3.7.1 METHODS

Descriptions of data sources. *Characterizing the Nursery Fish Assemblage*. We used a long-term (1970-2010) fish survey dataset from the Monterey Bay National Marine Sanctuary's (MBNMS) Sanctuary Integrated Monitoring Network (SIMoN, http://sanctuarysimon.org/projects/project_info.php?projectID=100116&site=true) to determine the effects of variable environmental conditions on the structure and distribution of the fish assemblage that inhabits Elkhorn Slough. The dataset incorporates data from a number of studies, combining deep channel surveys using otter trawls (N = 626) and shallow margin surveys using beach seines (N = 318) surveys to sample the fish assemblage at various sites within Elkhorn Slough (Figure S2*A*). The dataset captures a high degree of temporal and spatial variability, which makes it ideal to test the effect of varying water quality on the fish assemblage. We limited analysis to those deep channel (n = 8) and shallow margin (n = 10) sites that had been consistently sampled at least 28 and 16 times, respectively, through the entire time series, and had also been sampled for water quality around the time of sampling.

Both otter trawl (deep channel habitat) and beach seine (shallow margin habitat) efforts were located along the entire main channel of the estuary (Figure 3.S2*A*). Trawl net size (4.8 m head rope, 3.8 cm stretch mesh, 1.3 cm codend liner) and sampling area (typically run at 1.5-3 knots for 10 minutes) was consistent throughout the entire study period. Net size of the beach seines varied from 8 m to

100 m, making it difficult to standardize for abundance, so only presence/absence analyses were used for seine surveys. Beach seines were assigned to the nearest water quality station. Each fish sampling event (otter trawls and beach seines) was located < 1 km and < 30 days to the nearest water quality sampling event. If there were multiple sampling events within the same 30 day period at the same sampling station, we either combined them (presence/absence data) or used an average (abundance data) to ensure independence among replicates. It was assumed that the monthly water quality sample was a good indicator for the overall water quality condition for the fish sample.

Water quality parameters. We used several data sets that span from 1970-2012 (Smith 1973, 1970-1972; Nybakken et al. 1977, 1974-1976; Elkhorn Slough National Estuarine Research Reserve (ESNERR) water quality monitoring program, 1988-2012). These data were collected monthly at various stations around the estuary (Figure 3.S2*A*). The parameters we used for analyses in this study were daytime dissolved oxygen (DO) (mg $*L^{-1}$), nitrate (mg $*L^{-1}$), temperature (°C), and salinity (ppt), as these were factors known to affect fish presence in estuarine environments (Emmett et al. 1991). We used the raw monthly values for DO, temperature, salinity, and nitrate for correlations with the fish assemblage.

Climate and Oceanographic Indices. We used El Niño Southern Oscillation (ENSO; http://www.esrl.noaa.gov/psd/enso/mei/table.html), Pacific Decadal Oscillation

(PDO; http://jisao.washington.edu/pdo/PDO.latest), North Pacific Gyre Oscillation (NPGO; http://www.o3d.org/npgo/npgo.php) and local Monterey Bay Upwelling (MBU; http://www.pfeg.noaa.gov/products/PFEL/modeled/indices/upwelling/NA/upwell_menu_NA.html) indices, to investigate the relative effects of large scale climate variation on the Elkhorn Slough water quality and fish assemblage over the past 40 years. These indices are reported as mean monthly values, so we matched the month of each fish and water quality sample to the corresponding ENSO, PDO, NPGO, and MBU indices, and used those in the statistical analyses.

3.7.2 FIGURES

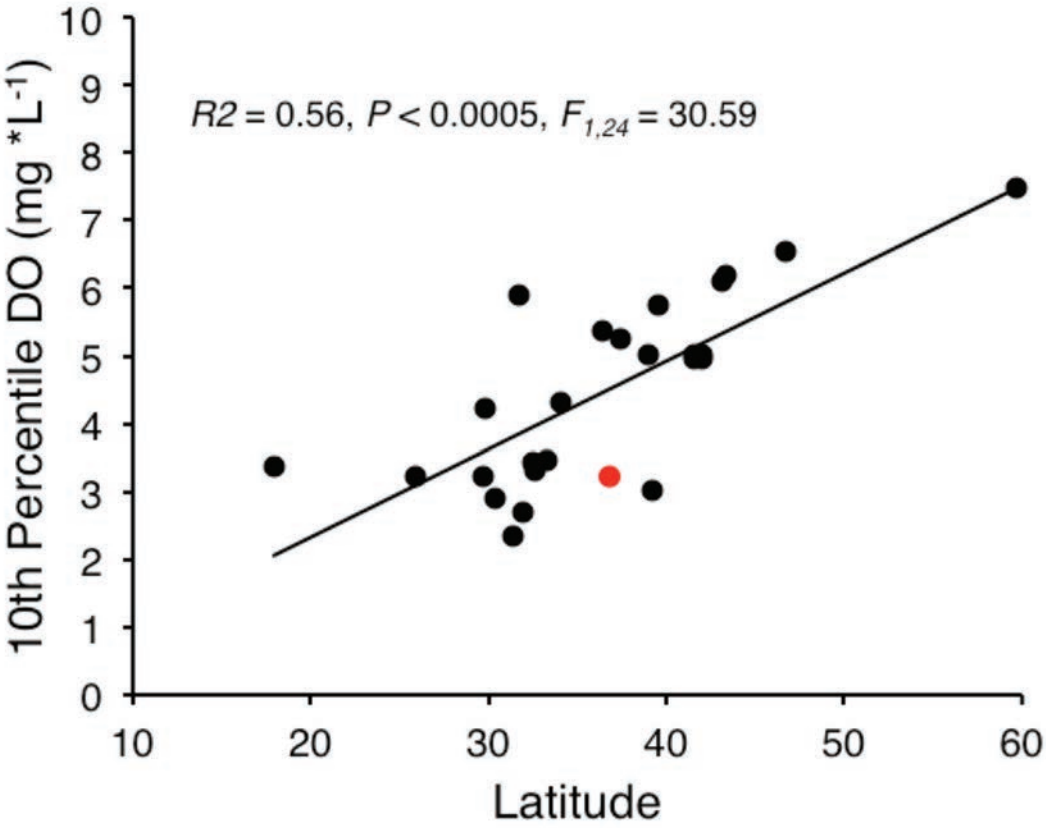

Figure 3.S1. Estuarine hypoxia in the United States. Relationship between latitude and hypoxia in United States estuaries (n = 27), measured as the 10th percentile DO (mg *L^{-1}) from continuously collected (15 – 30 min. intervals) data from 2009 – 2010 (CDMO). Each point represents an average over the two-year period from 3 – 4 monitoring stations within each estuary. The red point indicates Elkhorn Slough.

A) DO Spatial Model

B) Flatfish Probability Model

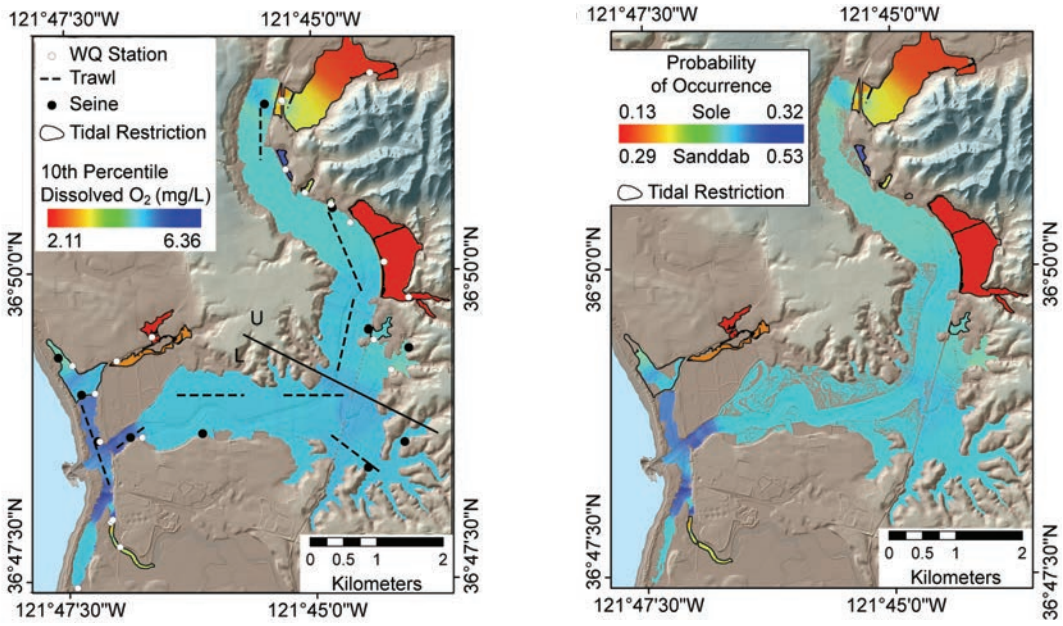

Figure 3.S2. (A) Survey locations for both water quality monitoring and fish sampling along with a spatial model of 10th percentile DO from 1988-2012. Tidal height is mean higher high water (MHHW) to indicate the greatest available habitat on an average day within Elkhorn Slough. Open circles (º) are the ESNERR water quality monitoring stations as well as the 2005 slough-wide sampling stations from Ritter et al. (2008). Dark circles (•) indicate locations for historical shallow margin (beach seine) surveys, dashed lines (----) indicate approximate locations of historical deep channel (otter trawl) surveys, and the solid line (—) indicates the division of upper (U) and lower (L) estuarine stations. Areas behind tidally restricted water control structures were indicated with a bold line drawn around the area. (B) Predicted probabilities of presence of English sole and Speckled sanddab based on logistic regression analysis. Spatial probabilities were calculated based on the interpolated 10th percentile of DO (mg *L^{-1}) collected monthly from the 1988-2011 (*n* = 252) ESNERR water quality database. Probability scales for each species were adjusted to conform to the interpolated DO values. Dark drawn lines indicate areas behind tidally restricted water control structures.

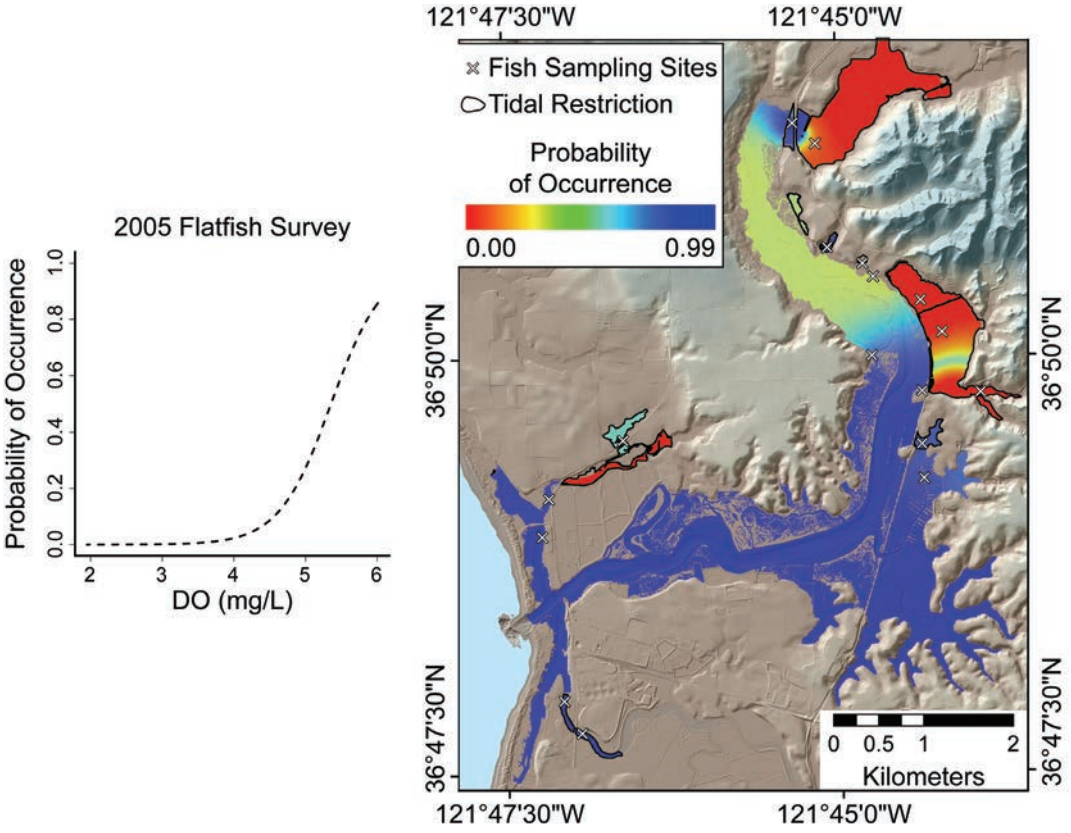

Figure 3.S3. Logistic regression analysis of the predicted probability of flatfish occurrence during 2005 shallow margin surveys in Elkhorn Slough as a function of 10^{th} percentile of DO from 1989-2011 (n = 33). Tidal height is mean higher high water (MHHW) to indicate the greatest available habitat on an average day within Elkhorn Slough. See Table 3.S2 for statistical results. Areas behind tidally restricted water control structures were indicated with a bold line drawn around the area.

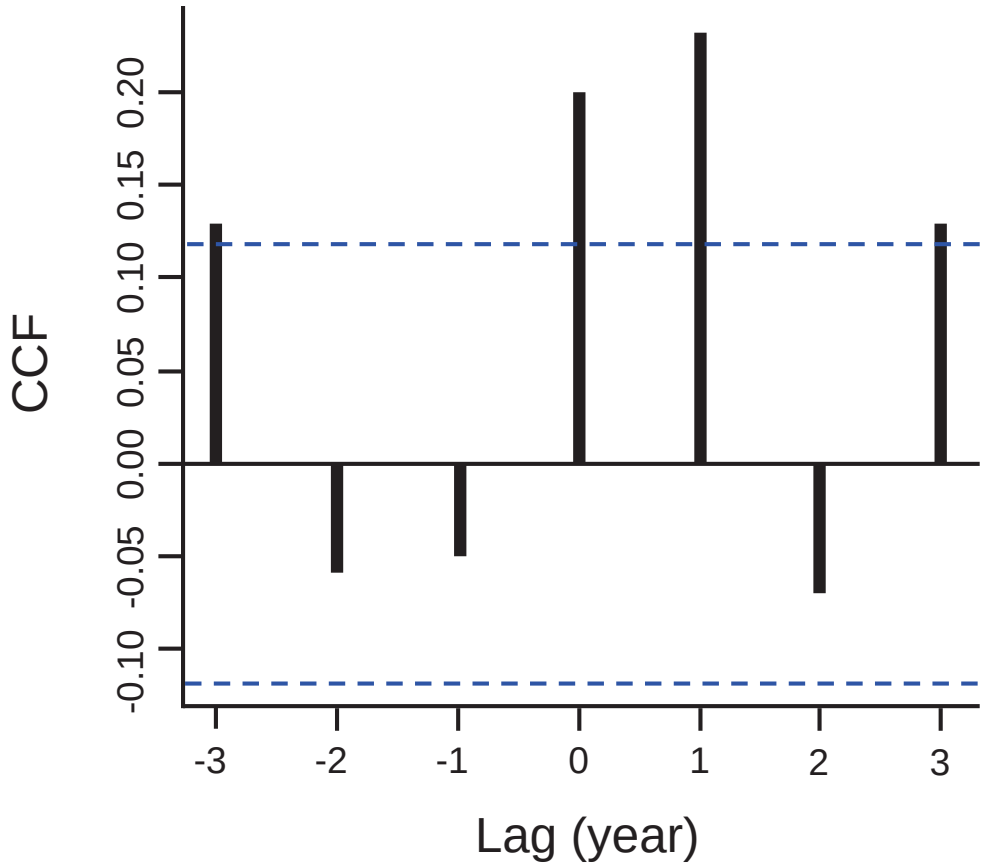

Figure 3.S4. Cross-correlation analysis determining the lag (years) of offshore English sole CPUE (kg *trawl^{-1}) with the greatest cross-correlation function (CCF) to the mean annual DOA in Elkhorn Slough. The dashed blue line indicates the threshold for significant correlations ($P < 0.05$).

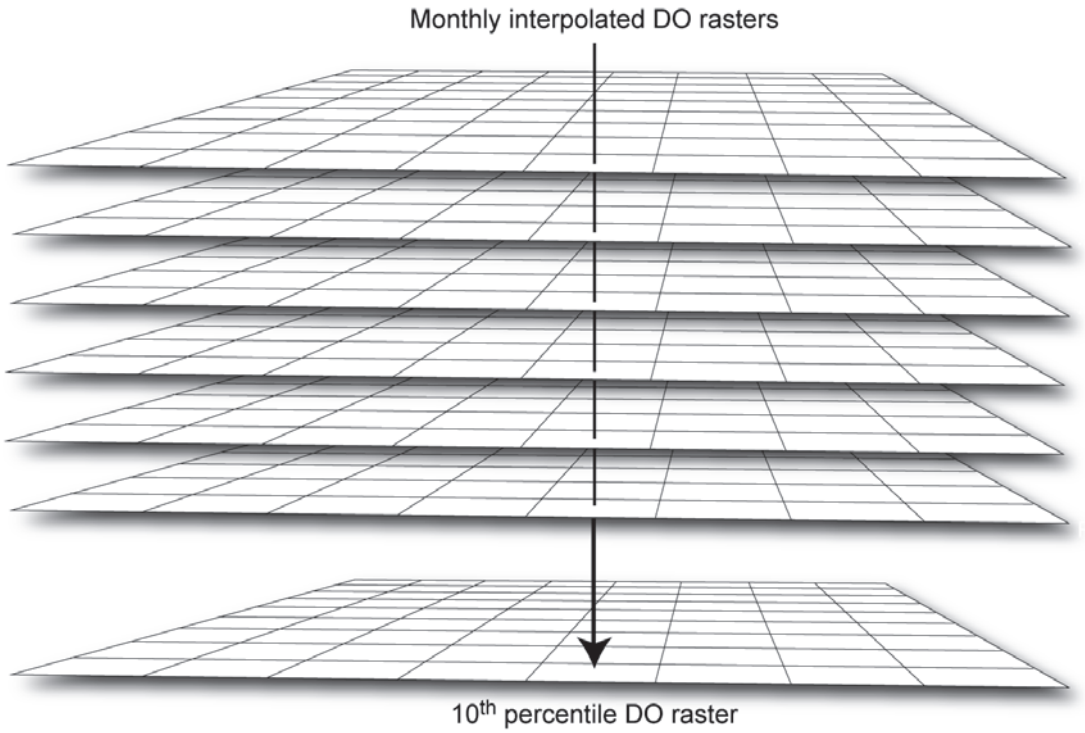

Figure 3.S5. Graphical representation of same-cell analysis among monthly interpolated dissolved oxygen rasters (i.e.; raster stack), used to calculate 10th percentile DO for the entire sampling period.

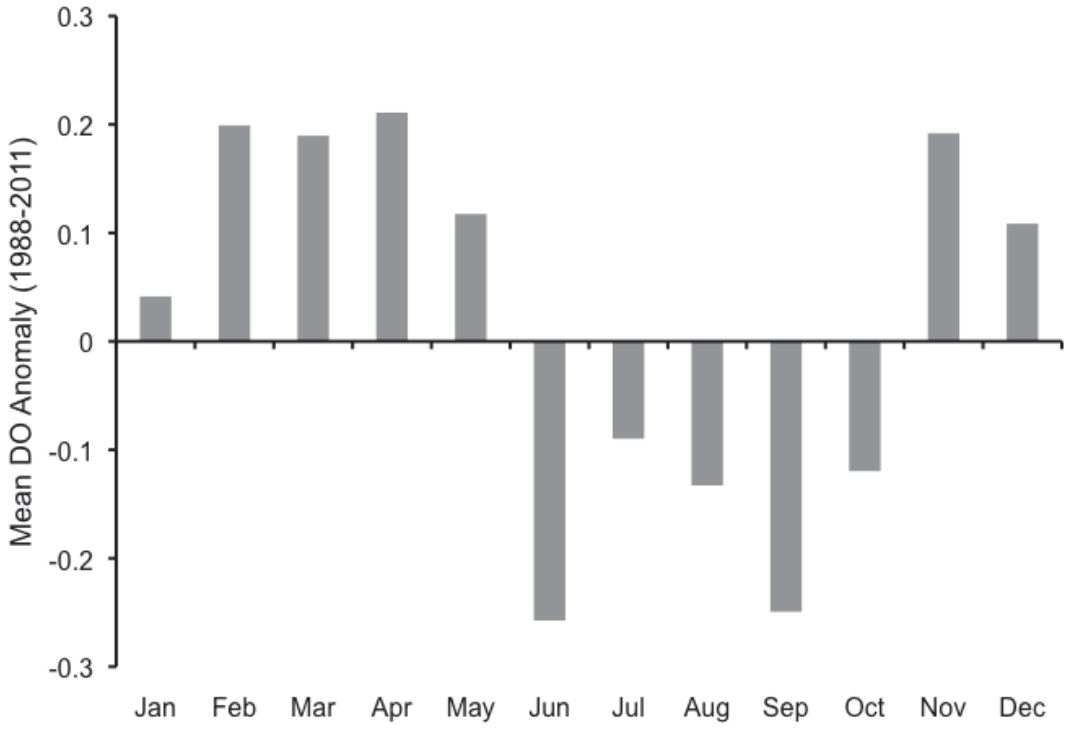

Figure 3.S6. The mean monthly DOA for all water quality monitoring stations in Elkhorn Slough from 1988-2011.

3.7.3 TABLES

Table 3.S1. Sequential logistic regression results testing the effects of DO, temperature, salinity, ENSO, PDO, local upwelling, and daily sampling effort on presence/absence data for (A) English sole (Figure 3.1A) and (B) speckled sanddab (Figure 3.1B) using surveys from both deep channel (n = 169) and shallow margin (n = 78) habitats. The best fitting model was confirmed using AIC weights and we reported the best fitted model using multiple logistic regression. Last, the model was reduced down to using only DO as the predictor to test for generality of DO effects. Significant values (P < 0.10) are in bold.

(A) English sole

Deep channel:

Best-fit Model					
Source	*Estimate*	*Std. Error*	*z value*	*P*	
DO	0.283	0.092	3.077	**0.002**	
Temperature	0.124	0.064	1.926	**0.054**	
Upwelling	0.018	0.004	4.424	**<0.0005**	
ENSO	0.326	0.209	1.559	0.119	
AIC = 202.7					

DO Model					
Source	*Estimate*	*Std. Error*	*z value*	*P*	
DO	0.256	0.087	2.942	**0.003**	

Shallow margin:

Best-fit Model					
Source	*Estimate*	*Std. Error*	*z value*	*P*	
Temperature	-0.350	0.187	-1.875	**0.061**	
Upwelling	0.020	0.008	2.692	**0.007**	
AIC = 49.55					

DO Model					
Source	*Estimate*	*Std. Error*	*z value*	*P*	
DO	0.409	0.215	1.906	**0.056**	

Table 3.S1. (continued)

(B) Speckled sanddab

Deep channel:

Best-fit Model				
Source	*Estimate*	*Std. Error*	*z*	*P*
DO	0.238	0.092	2.577	**0.010**
Temperature	-0.147	0.063	-2.315	**0.021**
ENSO	0.487	0.221	2.205	**0.027**
AIC = 212.8				

DO Model				
Source	*Estimate*	*Std. Error*	*z value*	*P*
DO	0.234	0.0917	2.551	**0.011**

Shallow margin:

Best-fit Model				
Source	*Estimate*	*Std. Error*	*z value*	*P*
DO	0.403	0.197	2.049	**0.040**
Temperature	-0.236	0.135	-1.755	**0.079**
PDO	1.329	0.512	2.595	**0.009**
Salinity	-0.134	0.057	-2.359	**0.018**
Sampling effort	0.512	0.271	1.890	**0.059**
AIC = 79.3				

DO Model				
Source	*Estimate*	*Std. Error*	*z value*	*P*
DO	0.347	0.158	2.2	**0.028**

Table 3.S2. Logistic regression analysis of the presence/absence of flatfish during two 2005 shallow margin surveys at 16 locations in Elkhorn Slough as a function of 10^{th} percentile of DO from 1989-2011 (n = 33) (Figure 3.S3).

Source	*Estimate*	*Std. Error*	z value	*P*
DO	2.751	1.339	2.054	**0.040**

Table 3.S3. Deep channel survey results from t-tests testing for the effect of hypoxia and region on abundance of English sole and speckled sanddabs (abundance *ha^{-1}) and fish species richness (species *trawl^{-1}). (A) Independent samples t-test comparing hypoxic (n = 18) and normoxic (n = 32) periods on fish parameters (Figure 3.2*B*-*E*). (B) Paired samples t-test testing for differences among each sampling date for fish parameters during (i) hypoxic periods and (ii) normoxic periods (Figure 3.2F-I). (C) Independent samples t-test comparing fish parameters between hypoxic and normoxic periods for the (i) lower and (ii) upper estuary, respectively (Figure 3.2*F*-*I*). Significant values (P < 0.10) are in bold. Note: English sole had a reduced sample size because the analysis excluded sampling dates when no English sole were caught (n = 12 hypoxic, n = 28 normoxic).

A) Pooled (Hypoxia v. Normoxia)

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
English sole abundance*	-25.779	11.616	-2.219	29	**0.034**
Speckled sanddab abundance	-9.810	7.125	-1.378	48	0.175
Species Richness	-1.557	0.691	-2.253	48	**0.029**

*Welch's t-test of unequal variances

B) Paired (Lower - Upper estuary)

i. Normoxia

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
English sole abundance	-14.032	23.511	-0.597	13	0.561
Speckled sanddab abundance	12.834	12.092	1.061	15	0.305
Species Richness	-0.960	0.637	-1.506	15	0.153

ii. Hypoxia

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
English sole abundance	7.446	2.152	3.461	5	**0.018**
Speckled sanddab abundance	17.345	7.190	2.413	8	**0.042**
Species Richness	1.684	0.447	3.766	8	**0.005**

Table 3.S3. (continued)

C) Partitioned (Lower and Upper estuary)

i. Lower (Hypoxia v. Normoxia)

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
English sole abundance	-12.287	18.442	-0.666	18	0.514
Speckled sanddab abundance	-7.562	14.187	-0.533	23	0.599
Species Richness	-0.495	0.918	-0.595	23	0.595
*Welch's t-test of unequal variances					

ii. Upper (Hypoxia v. Normoxia)

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
English sole abundance*	-39.272	19.742	-1.989	13	**0.068**
Speckled sanddab abundance*	-12.074	5.814	-2.077	16	**0.054**
Species Richness	-3.139	0.894	-3.508	23	**0.002**
*Welch's t-test of unequal variances					

Table 3.S4. (A-C) Independent samples t-test comparing the one year lag effect of hypoxia (n = 9-10) and normoxia (n = 6) in Elkhorn Slough on fish caught in offshore Monterey Bay and Half Moon Bay waters for: A) the CPUE (kg *trawl^{-1}) of offshore English sole (Figure 3.4*A*), B) the CPUE (kg *trawl^{-1}) of Rex sole (Figure 3.4*B*), and C) adult recruitment (recruits *trawl^{-1}) of English sole (Figure 3.4*C*). Each year was categorized as hypoxic if the DOA was negative for ≥ 6 months (Figure 3.S6). (D) Linear regression analysis for residuals of annual English sole fishery landings (kg) in Monterey Bay as a function of hypoxic months in Elkhorn Slough lagged by one year (n = 23) Significant values (P < 0.10) are in bold.

A) English sole CPUE

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
Monterey Bay	-14.886	7.081	-2.102	14	**0.054**
Half Moon Bay*	-5.705	5.122	-1.114	6	0.308
*Welch's t-test of unequal variances					

B) Rex sole CPUE

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
Monterey Bay[1]	0.033	0.247	0.132	14	0.896
Half Moon Bay	-8.045	8.414	-0.956	14	0.355
[1]log-transformed data					

C) English sole adult recruit

Dependent variable	*Mean Difference*	*SE Difference*	*t*	*df*	*P*
Monterey Bay[1,*]	-0.302	0.157	-2.265	11	**0.045**
Half Moon Bay[1,*]	-0.071	40.128	-0.556	12	0.588
[1]log-transformed data, *Welch's t-test of unequal variances					

D) English sole fishery landings residuals v. number of hypoxic months

Dependent variable	R^2	ß	*df*	*F*	*P*
Hypoxic months	0.201	-9728	1	5.293	**0.032**

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