

## SIMULATED PREDATOR EXTINCTIONS: PREDATOR IDENTITY AFFECTS SURVIVAL AND RECRUITMENT OF OYSTERS

NESSA E. O'CONNOR,<sup>1,4</sup> JONATHAN H. GRABOWSKI,<sup>2</sup> LAURA M. LADWIG,<sup>1</sup> AND JOHN F. BRUNO<sup>3</sup>

<sup>1</sup>*Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, North Carolina 28557 USA*

<sup>2</sup>*Gulf of Maine Research Institute, 350 Commercial Street, Portland, Maine 04101 USA*

<sup>3</sup>*Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3300 USA*

**Abstract.** The rate of species loss is increasing at a global scale, and human-induced extinctions are biased toward predator species. We examined the effects of predator extinctions on a foundation species, the eastern oyster (*Crassostrea virginica*). We performed a factorial experiment manipulating the presence and abundance of three of the most common predatory crabs, the blue crab (*Callinectes sapidus*), stone crab (*Menippe mercenaria*), and mud crab (*Panopeus herbstii*) in estuaries in the eastern United States. We tested the effects of species richness and identity of predators on juvenile oyster survival, oyster recruitment, and organic matter content of sediment. We also manipulated the density of each of the predators and controlled for the loss of biomass of species by maintaining a constant mass of predators in one set of treatments and simultaneously using an additive design. This design allowed us to test the density dependence of our results and test for functional compensation by other species.

The identity of predator species, but not richness, affected oyster populations. The loss of blue crabs, alone or in combination with either of the other species, affected the survival rate of juvenile oysters. Blue crabs and stone crabs both affected oyster recruitment and sediment organic matter negatively. Mud crabs at higher than ambient densities, however, could fulfill some of the functions of blue and stone crabs, suggesting a level of ecological redundancy. Importantly, the strong effects of blue crabs in all processes measured no longer occurred when individuals were present at higher-than-ambient densities. Their role as dominant predator is, therefore, dependent on their density within the system and the density of other species within their guild (e.g., mud crabs). Our findings support the hypothesis that the effects of species loss at higher trophic levels are determined by predator identity and are subject to complex intraguild interactions that are largely density dependent. Understanding the role of biodiversity in ecosystem functioning or addressing practical concerns, such as loss of predators owing to overharvesting, remains complicated because accurate predictions require detailed knowledge of the system and should be drawn from sound experimental evidence, not based on observations or generalized models.

**Key words:** *Callinectes sapidus*; *crab*; *Crassostrea virginica*; *ecosystem functioning*; *extinction*; *foundation species*; *identity effect*; *Menippe mercenaria*; *oyster*; *Panopeus herbstii*; *predator–prey interaction*; *trophic interaction*.

### INTRODUCTION

As the global rate of extinction continues to climb upward (Regan et al. 2001), human-induced extinctions have been disproportionately concentrated toward higher trophic levels (Pimm et al. 1988, Lawton and May 1995, Petchey et al. 2004, Byrnes et al. 2007). Activities that remove predator species, such as fishing, can lead to ecological extinctions that can have dramatic cascading effects within a system (Pauly et al. 1998, Jackson et al. 2001, Bruno and O'Connor 2005).

Manuscript received 8 December 2006; revised 26 June 2007; accepted 27 June 2007. Corresponding Editor: P. T. Raimondi.

<sup>4</sup>Present address: Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland; and Department of Zoology, Ecology and Plant Science, University College Cork, Distillery Fields, North Mall, Cork, Ireland. E-mail: n.oconnor@ucc.ie

Virtually all communities contain several species of predator. To understand the effects of the continued loss of predator species we must, therefore, also understand the “emergent effects” of multiple predators (Polis and Strong 1996, Sih et al. 1998). There are various mechanisms by which predators interact with each other. Decreased predator diversity may reduce the magnitude of their consumptive (Cardinale et al. 2003, Duffy et al. 2003, Gamfeldt et al. 2005) or nonconsumptive (i.e., trait-related) (Sih et al. 1998, Schmitz et al. 2004, Wojdak and Luttbeg 2005, Byrnes et al. 2006) effects. Conversely, increased predator diversity could result in intraguild predation and nonconsumptive effects that dampen cascading effects on lower trophic levels (Strong 1992, Siddon and Witman 2004, Finke and Denno 2005).

Many processes depend on the influence of a particular taxon rather than species richness per se

(Grime 1998, Downing and Leibold 2002, O'Connor and Crowe 2005). The question of whether one (or a few) "key" species is driving an observed effect of biodiversity loss is fundamental for the accurate interpretation of results (Wardle and Grime 2003, Worm and Duffy 2003). It is, therefore, essential to design experiments that separate species richness from identity effects and avoid confounding them, as has been done in previous studies (Huston 1997, Wardle and Grime 2003, O'Connor and Crowe 2005).

Removal experiments explore the direct effects on ecosystem properties when a species is no longer present. These experiments have the potential to address effects of realistic extinctions on ecosystem functioning (Schläpfer and Schmid 1999, Wardle et al. 1999, Diaz et al. 2003, Hooper et al. 2005). When a species is removed from a system, the overall biomass of predators is also reduced. Any effect of the loss of species is, therefore, confounded by the loss of biomass (Likens 1985, Lawton and Brown 1994, Ruesink and Srivastava 2001). The only way to test conclusively for the loss of species is to include controls for this loss of biomass. A combination of "additive" and "replacement" type designs test for effects of species richness, identity, and mass (Connolly 1988, Sih et al. 1998, Benedetti-Cecchi 2004, Ives et al. 2005). Simultaneously removing certain species and increasing the mass (density) of remaining species also allows us to examine further the potential for apparent ecological "redundancy" or in-built "biological insurance" within the system and to test the density dependence of results.

The southeastern coast of the United States is characterized by soft-sediment dominated estuaries inhabited by several species of predatory crabs such as the blue crab (*Callinectes sapidus*), stone crab (*Menippe mercenaria*), and mud crab (*Panopeus herbstii*). These crabs are widely harvested commercially (Kennedy and Cronin 2006). The blue crab is considered a key species in this system (Hines et al. 1990, Eggleston et al. 1992, Ebersole and Kennedy 1995, Micheli 1997, Seitz et al. 2001). During the 1990s, their populations declined by 40–80% (Lipcius and Stockhausen 2002). It is, therefore, increasingly relevant and interesting to examine the potential role of other predatory crab species in the absence of blue crabs (e.g., Silliman and Bertness 2002). If blue crabs were to become ecologically extinct, it is unclear whether other species such as stone and mud crabs would assume their functional roles in the system. Some predator species that do not appear to have strong effects when predator diversity is high may become important when a key species, such as blue crab, is removed. In systems such as oyster reefs, remaining crab species could compensate for the loss of blue crab either by increasing their consumption rates of prey or by increasing in mass or density; however, this has yet to be tested.

Oysters, such as the eastern oyster (*Crassostrea virginica*), have been commercially exploited such that

native beds are being depleted both regionally and globally (Rothschild et al. 1994, Kennedy et al. 1996, Peterson et al. 2003). Degradation of oyster reef habitat has resulted in the loss of not only commercial oyster harvests but also the many ecosystem services they provide, such as increasing water quality by filter feeding, decreasing erosion by stabilizing sediments, and providing hard substrate and structured habitat for benthic organisms (Bahr and Lanier 1981, Dame et al. 1984, Newell 1988, Luckenbach et al. 1997, Eggleston et al. 1998, Peterson et al. 2003). The dramatic decline of such foundation species threatens the diverse communities they facilitate (Bruno and Bertness 2001, Lenihan et al. 2001, Bruno et al. 2003, Grabowski et al. 2005). Understanding the interactions among predators (crabs) and prey (oysters) is necessary to understand the dynamic processes that regulate this vulnerable system (Fig. 1).

We conducted a field experiment to test for the effects of loss of predator species on a shared prey species. Using native oyster reefs as a model system, we tested the effects of predators on an important foundation species and its associated habitat. We manipulated the number and identity of three predator species (blue crab, stone crab, and mud crab) using cage enclosures and measured their independent and interactive effects on three processes: (1) juvenile oyster survival, (2) oyster recruitment, and (3) percentage of organic matter of sediment. This design permitted us to test the following four models: (1) predator identity, but not species richness, affects oyster survival, oyster recruitment, and sediment organic matter; (2) the blue crab is a key predator in this system; (3) increased densities of other predators cannot compensate for the loss of a key predator; and (4) the effects of predator identity are density dependent in this system.

## MATERIALS AND METHODS

### *Study system*

The experiment was conducted at Hoop Pole Creek, Bogue Sound, North Carolina, USA. Hoop Pole Creek is a wildlife refuge in a sheltered estuarine area between the mainland and barrier islands. The area includes salt marshes, seagrass beds, native and restored bivalve beds including clams (*Mercanaria mercanaria*) and oysters (*Crassostrea virginica*), and extensive mudflats. Our experiment was conducted on intertidal mudflats adjacent to native oyster beds. Blue crabs, mud crabs, and stone crabs are among the most abundant benthic predators in this system and are easily manipulated at an appropriate scale (Grabowski 2004, Hughes and Grabowski 2006).

### *Experimental design*

The experiment ran from May to October 2005, incorporating the expected peak oyster spawning and recruitment periods (Southworth and Mann 2004). In total, 15 treatments (including a procedural control),

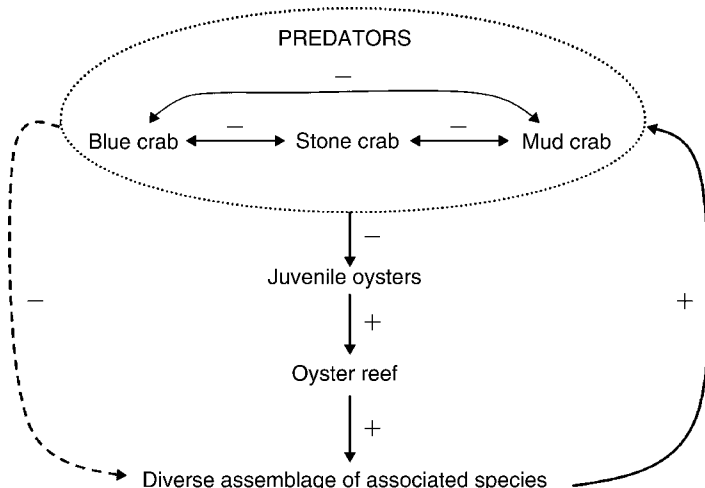


FIG. 1. Interaction web of the model system, illustrating the positive and negative direct (solid lines) and indirect (dashed lines) effects among species and functional groups. The figure includes potential intraguild effects among predators (blue crab *Callinectes sapidus*, stone crab *Menippe mercenaria*, and mud crab *Panopeus herbstii*) and the role of reefs of the eastern oyster *Crassostrea virginica* in facilitating a diverse assemblage of species, including the predatory crabs, thus forming a negative feedback loop.

each replicated in five experimental plots, were established, and subsets of these treatments were used to test specific hypotheses (Table 1). The first set of treatments included the simulated removal of 0, 1, 2, or 3 species in a fully factorial design. The factorial design facilitates examination of all independent and possible interactive effects of the loss of each species. To test if the number of species or the identity of species present affected the processes under examination, a nested design partitioned the variance of identity nested in richness (Schmid et al. 2002, Giller et al. 2004). A two-factor nested analysis was performed on all treatments where one or two species had been removed. The first factor, “number of species lost,” is fixed, and the second, factor “identity of the species,” is nested in the first. Planned comparisons tested directly if the blue crab is a key predator species by analyzing all treatments with and without blue crab and comparing them to the other treatments that had different numbers of species present. The “presence of blue crabs” is a fixed factor, and the second factor, “other treatments,” is nested in the first.

To simulate realistic extinction scenarios, species were present in experimental plots at densities based on natural abundance patterns. Predator densities within our plots were based on previously collected data and estimated to be within the range of densities typical in nature (e.g., Zimmerman et al. 1989, Lenihan et al. 2001, Grabowski et al. 2005; J. H. Grabowski, unpublished data). All three species have commonly been observed together in areas of similar size to our experimental cages (J. H. Grabowski, personal observation). Cages (described in *Materials and methods: Field and laboratory methods*) were necessary to maintain the treatments in each experimental plot. The treatment containing all crabs contained one blue crab, one stone crab, and four mud crabs. The average individual mass of each species was 12 ( $\pm 3.4$ ) g for blue crabs (30–70 mm carapace width, CW), 28 ( $\pm 2.4$ ) g for stone crabs (30–60 mm CW), and 1.5 ( $\pm 0.3$ ) g for mud crabs (10–20 mm CW); values are means  $\pm$  SE. Individual crabs used in the

experiment were deliberately not all the same size, but were all within the specified size range that is representative of native crab assemblages at this location.

In a second set of treatments, the overall mass of predators present was constant, and the number and identity of species of predators was manipulated as before. Specifically, each treatment that had one or two

TABLE 1. Experimental treatments simulating extinction of 0, 1, 2, and 3 species of predatory crabs.

Treatment code	No. species excluded	Identity of species excluded
a) Species number vs. identity		
A	0	0
B	1	blue crab
C	1	stone crab
D	1	mud crab
E	2	blue and stone crab
F	2	blue and mud crab
G	2	stone and mud crab
H	3	blue, stone, and mud crab
b) Compensation for mass		
I	1	blue crab†
J	1	stone crab†
K	1	mud crab†
L	2	blue and stone crab†
M	2	blue and mud crab†
N	2	stone and mud crab†
c) Cage effect		
O	0	0 (no cage/manipulation)

Notes: Subsets were compared to test specific hypotheses. (a) Treatments used to assess if the number or identity of species affected oyster survival and recruitment and organic matter content. A subset of these treatments (B, C, D, E, F, G) was analyzed in a nested ANOVA to differentiate between the effects of species number and identity. A separate analysis compared treatments with (A, C, D, G) and without blue crabs (B, E, F, H). (b) Comparison of treatments with compensation for loss of mass (I, J, K, L, M, N) to treatments without (B, C, D, E, F, G) tested the effects of loss of mass of predators resulting from their exclusion. (c) Comparison of the treatment with all crabs present and a cage (A) with the treatment that has no cage or experimental manipulation (O) tested for cage effects.

† Includes increased mass of other crabs.

species removed also had an analogous treatment in which the mass of the remaining predators was increased to an equivalent mass to compensate for this loss. This design determined whether other species at unchanged or increased densities were able to fill a functional role of a removed species and also illustrated whether any effects of the loss of predators are density dependent. In essence, replacement and additive designs were used concurrently (Connolly 1988, Sih et al. 1998, Benedetti-Cecchi 2004). To control for caging artifacts (Underwood and Denley 1984), we included experimental plots without cages, and the data from these plots were compared with the treatments that simulated the presence of all three predators at natural abundances.

#### *Field and laboratory methods*

Experimental plots were established in an area of ~750 m<sup>2</sup> and were assigned to treatments randomly. Cages were square, 50 × 50 × 50 cm, and made of plastic mesh (5 mm aperture) attached to a frame of polyvinyl chloride (PVC) 20 mm diameter pipes. A hydraulic pump was used to dig trenches in the mud so that cages could be fixed into the sediment to prevent the emigration of the experimental predators. The cages were buried at least 10 cm into the sediment, and reinforcing steel rods (rebar) were placed through the PVC pipes at two opposite corners of each frame. Plots that controlled for cage effects were marked with rebar and PVC but did not have a cage. To create suitable habitat for the predators and substrate for juvenile oyster recruitment, ~20 L of oyster shells were added to each plot. The oyster shells were collected previously from sites close to the study site and contained no living benthic fauna. All predators were collected with crab traps at the experimental site and surrounding area. After a week, predators were added to the cages as required for each treatment, and lids were fastened with cable ties. The cages were inspected regularly during the experiment, and treatments were maintained.

*Juvenile oyster survival rates.*—Juvenile oysters (<20 mm wide) were bought from a local oyster hatchery. Individual oysters were tethered to small marking flags with nylon thread and glue and kept alive in through-flow water tables. Ten individuals were added to each experimental plot ( $n = 5$  plots). The number of juvenile oysters surviving in each plot was recorded after 24 hours and analyzed as percentage survival.

*Oyster recruitment and organic matter content of sediment.*—After five months, we collected some of the original dead oyster shells from each experimental plot. Twenty shells were sampled randomly from each treatment. The number of oysters on each shell was counted, and an average was calculated for each plot ( $n = 5$ ). To standardize comparisons of recruitment among treatments, horizontally positioned shells of approximately the same size class (average width  $94 \pm 2.9$  mm) were selected, and all oysters that recruited onto these shells were counted. Sediment samples were also

collected, one from each plot ( $n = 5$ ). A 75 mm diameter core was used to sample the top 10 cm of sediment. Sediment samples were frozen and at a later date were oven-dried, weighed, placed in a furnace at 500°C for 4 h, and reweighed. We estimated the percentage of organic matter of sediment based on loss of ignition, following Dean (1974).

#### *Statistical analyses*

Analysis of variance (ANOVA) was used to test all hypotheses. WinGMAV5 was used for computations (Underwood and Chapman 1998). Prior to performing ANOVAs, we conducted Cochran's test for homogeneity of variance and transformed heterogeneous data (Underwood 1997). Oyster percentage survival rate data were arcsine transformed, and oyster recruitment and organic matter data were square-root transformed. A Student-Newman-Keuls procedure was used to make post hoc comparisons among levels of significant terms (Day and Quinn 1989). An alpha significance level of 0.05 was used on all analyses.

#### RESULTS

##### *Distinguishing the influence of identity vs. species richness*

The identity of the predator (or particular combination of predators), and not the number of predators present, affected juvenile oyster survival ( $F_{1,4} = 5.39$ ,  $P < 0.003$ ; Appendix A, Fig. 2A), the number of oysters recruited ( $F_{1,4} = 14.86$ ,  $P < 0.000$ ; Appendix A, Fig. 2B), and the percentage of organic matter in sediment ( $F_{1,4} = 6.57$ ,  $P < 0.001$ ; Appendix A, Fig. 2C). None of the measured processes responded in a predictable manner in relation to the number of species of predators present. Thus the responses were idiosyncratic and were related clearly to the identity of the species of predator present.

##### *Testing the importance of blue crabs*

Planned comparisons of treatments with and without blue crabs showed that the presence of blue crabs reduced juvenile oyster survival significantly ( $F_{1,6} = 42.53$ ,  $P < 0.001$ ; Appendix B, Fig. 2A). The presence of blue crabs alone or with the other predators had a negative effect on oyster survival rates. In the longer experiments, however, a different pattern emerged. Oyster recruitment was not affected by the presence or absence of blue crabs but by the particular treatment (combination of predators removed) ( $F_{1,6} = 11.45$ ,  $P < 0.000$ ; Appendix B). Post hoc tests show that oyster recruitment was much lower in treatments with no predators removed and in treatments that removed stone crabs and mud crabs together, compared to all other treatments (Fig. 2B). The presence of blue crabs alone was similar to when all three predators were present, and all other experimental removals had a similar effect as when no predators were present. The simulated removal of either just stone crabs or just mud crabs led to an increase in the number of oysters recruiting, suggesting initially that they are both

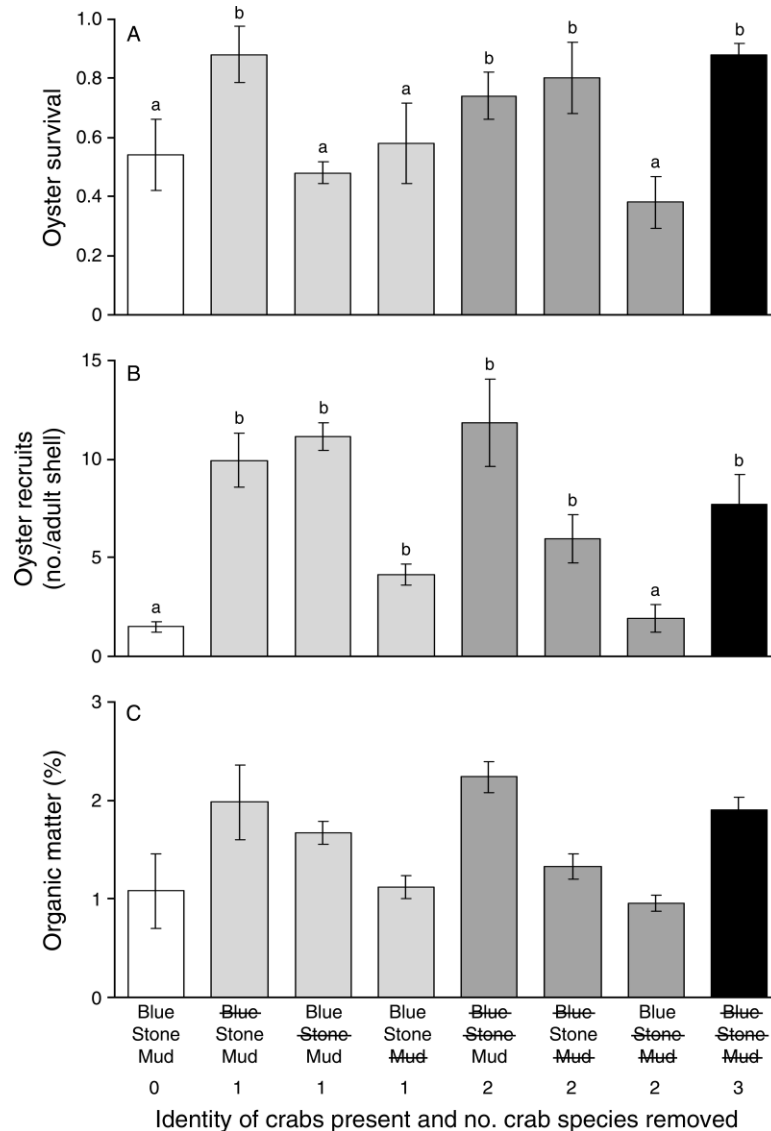


FIG. 2. Effect of loss of species richness and identity of oyster predator on (A) proportion of juvenile oysters surviving over 24 hours, (B) oyster recruitment rate over five months, and (C) percentage of sediment organic matter after five months;  $n = 5$  replicates of each treatment. In the case of recruitment, an average measurement was recorded for each plot. For the sediment samples, one core was taken from each of five replicate plots. Cores were 75 mm diameter and 100 mm deep ( $\sim 442 \text{ cm}^3$  sediment sampled from each plot). Values are untransformed means ( $\pm \text{SE}$ ). Letters (a, b) indicate groups of means that are statistically indistinguishable from each other (where letters differ,  $P \leq 0.05$ ). In the x-axis label, predatory crab species that have been removed have a line through the name.

important predators for this prey in this system. When both these species were removed together, however, the remaining blue crabs had a similar effect on oyster recruitment as when all three species are present.

The presence of blue crabs and the combination of predators removed both affected organic matter in sediment (blue crabs  $F_{1,6} = 6.9$ ,  $P < 0.039$ ; combination of predators  $F_{1,6} = 3.07$ ,  $P < 0.017$ ; Appendix B, Fig. 2C). Although the causative treatments could not be identified with post hoc tests, these results show a similar trend to oyster recruitment patterns and suggest that

there is a possible positive correlation between oyster recruitment and sediment organic matter.

*Compensation by other species for the loss of mass and testing for density dependence*

Other predators at increased densities could not compensate for the loss of blue crabs on juvenile oyster survival; none of the pairs of treatments with and without compensation differed from each other (Appendix C, Fig. 3A). We no longer detected an identity effect on oyster survival when comparing the data at

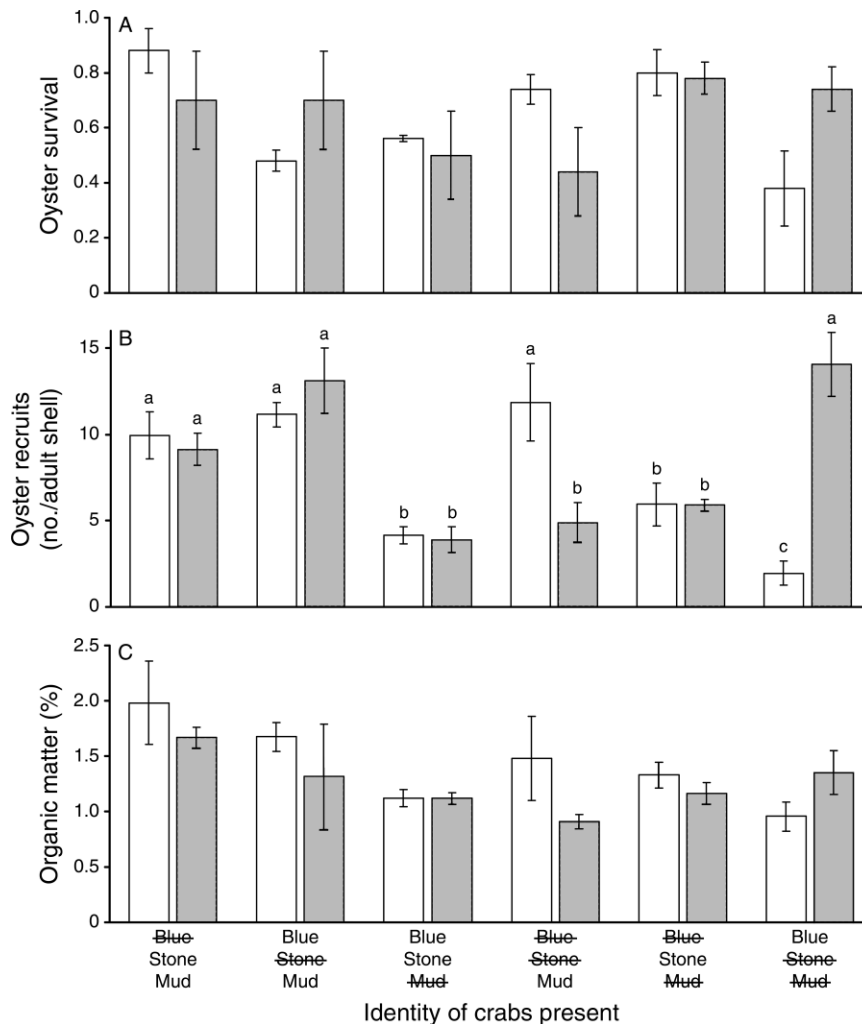


FIG. 3. Effect of loss of species richness and identity of oyster predator species with and without compensation for loss of mass of species on (A) proportion of juvenile oysters surviving over 24 hours, (B) oyster recruitment rate over five months, and (C) percentage of sediment organic matter after five months;  $n = 5$  replicates of each treatment. In the case of recruitment, an average measurement was recorded for each plot. For the sediment samples, one core was taken from each of five replicate plots. Cores were 75 mm diameter and 100 mm deep ( $\sim 442 \text{ cm}^3$  sediment sampled from each plot). Values are untransformed means ( $\pm$ SE). Open bars represent treatments that do not include compensation for loss for biomass. Gray bars represent treatments that include compensation of biomass for the removed predators. Letters (a, b, c) indicate groups of means that are statistically indistinguishable from each other (where letters differ,  $P \leq 0.05$ ). In the  $x$ -axis label, predatory crab species that have been removed have a line through the name.

lower and increased densities because these results were more variable than those without replacement (the additive design).

There was an interactive effect of compensation for loss of biomass on oyster recruitment ( $F_{1,4} = 17.51$ ,  $P < 0.000$ ; Appendix C). Two pairs of treatments (with and without compensation for mass) differed from each other: experimental removal of blue and stone crabs together and removal of stone and mud crabs together (Student-Newman-Keuls tests,  $P < 0.05$ ; Fig. 3B, see also Fig. 2B). Having identified in the previous set of analyses that simultaneous removal of blue and stone crabs increased oyster recruitment significantly (Fig. 2B), removing these same crab species while also

compensating for their removal by elevating mud crab densities reduced oyster recruitment (i.e., this effect was attenuated).

The previous set of analyses also showed that the experimental removal of stone and mud crabs together had no effect on oyster recruitment (similar to when all three predators were present) (Fig. 2B). There was a significant difference in oyster recruitment, however, when stone and mud crabs were removed and blue crabs were at lower densities compared to when stone and mud crabs were removed and blue crabs were at increased densities. In fact, the highest oyster recruitment observed in the whole experiment was in this treatment. This difference demonstrated that although

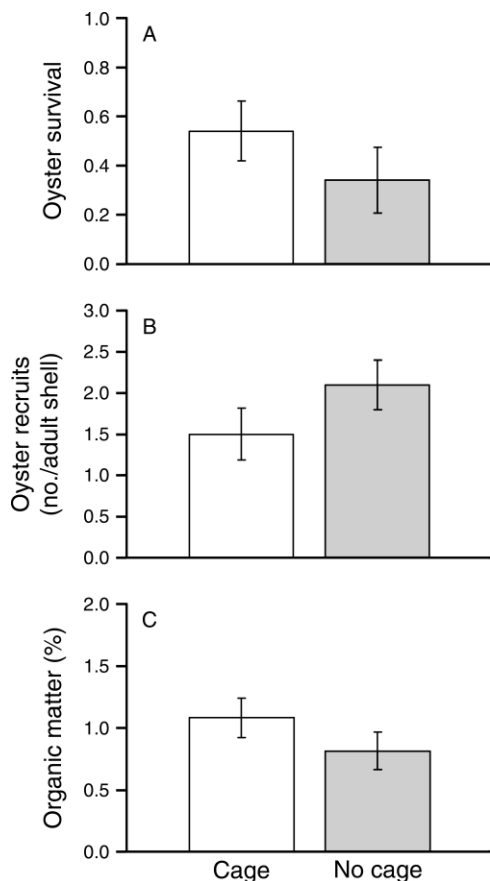


FIG. 4. Effect of cage on (A) proportion of juvenile oysters surviving over 24 hours, (B) oyster recruitment rate over five months, and (C) percentage of sediment organic matter after five months;  $n = 5$  replicates of each treatment. In the case of recruitment, an average measurement was recorded for each plot. For the sediment samples, one core was taken from each of five replicate plots. Cores were 75 mm diameter and 100 mm deep ( $\sim 442 \text{ cm}^3$  sediment sampled from each plot). Values are untransformed means ( $\pm$ SE). White bars represent the treatments with all three crab species present enclosed with a cage, and gray bars represent experimental plots with no cage (control).

blue crabs are important predators, at higher-than-estimated ambient densities their effect on the prey was completely mitigated, most likely due to negative intraspecific interactions. Collectively, these results suggest that the effects of predator identity on oyster recruitment are highly density dependent.

There was also an interactive effect of compensation for mass on sediment organic matter ( $F_{1,4} = 6.42$ ,  $P < 0.000$ ; Appendix C, Fig. 3C). In general, there was a similar trend to that seen in the oyster recruitment data, but the post hoc tests were not decisive. Comparisons between pairs of treatments also showed that there was a significant effect of compensation for mass in the treatments that involved the removal of blue and stone crabs as well as stone and mud crabs. This suggests that

the effect of predator identity on sediment organic matter is also likely density dependent.

#### Cage effects

There was no significant difference in juvenile oyster survival rates ( $F_{1,8} = 1.26$ ,  $P > 0.29$ ; Fig. 4A), the number of oysters recruiting ( $F_{1,8} = 3.06$ ,  $P > 0.12$ ; Fig. 4B), or sediment organic matter ( $F_{1,8} = 1.49$ ,  $P > 0.26$ ; Fig. 4C) in control plots (no cage, no predator manipulation) and treatments with cages and all three predators present at ambient densities.

#### DISCUSSION

We simulated the effect of extinction of three predators and found that the identity and not the richness of predator species affected the oyster prey populations and ecosystem properties (e.g., sediment organic matter). The effects of different species of predator on prey (and related processes) were both idiosyncratic and density dependent, and multiple-predator emergent effects were identified. This idiosyncratic effect of species loss is consistent with other studies in marine systems modeled in mesocosms (Emmerson and Raffaelli 2000, Bruno and O'Connor 2005) and in field experiments based on removal of primary producers (Allison 2004) and herbivores (O'Connor and Crowe 2005). This study presents one of the first field-based simulated multiple-predator removal experiments and strengthens the argument for idiosyncratic effects of species loss, that is emerging as a prevalent theme, in marine systems.

In our short-term experiment testing the effect of loss of predators on juvenile oyster survival, blue crabs appeared to be a key predator. Only the experimental removal of blue crabs (in any combination) affected juvenile oyster survival rates. Oyster recruitment and sediment organic matter accumulation (processes that occurred over five months), however, showed that all combinations of removals (single-species removal or more than one) had a positive effect except when stone and mud crabs were removed together (Fig. 2B). In other words, when the blue crab was the only predator present, it had the same effect as when all three predators were present. All predators affected oyster recruitment, and blue crabs again emerged as the dominant predator. The results also suggest that stone and mud crabs are at least partially redundant in this context. Clearly the number of oysters recruited was determined by the particular combination of predators present, and this could not be predicted based on species richness of predators. The singular effect of the removal of each predator on prey differed from the overall effect of a group of predators, depending of the identity of the predator. This has important implications for future models of food web dynamics where different predator species are often grouped as one functional unit.

Our findings are contrary to some other studies examining the effects of predators on shared prey.

Sokol-Hessner and Schmitz (2002) used a terrestrial system and examined all combinations of zero to three species of predator. The authors concluded that multiple-predator effects on prey mortality were generally the average of the corresponding single-species effects and suggested that predator species could be aggregated into a single functional unit. The emergent effects of species losses arising from complex trophic interactions, often through indirect effects, cannot, however, always be predicted by adding their individual impacts (Sih et al. 1998, Downing and Leibold 2002, Duffy et al. 2003, Worm and Duffy 2003).

Perhaps even more important, the density of each species of predator also emerged as a critical factor determining the outcome. Our findings show that in the absence of stone and mud crabs, blue crabs present at estimated ambient density affected oyster recruitment. This effect was no longer present when the density of blue crabs was increased to compensate for the loss of mass of the other two species. Mitigation of the strong effect of this dominant predator was most likely due to negative intraspecific interactions among blue crabs when densities are high. The exact mechanism responsible requires further study and should include tests for consumptive and nonconsumptive interactions between crabs (Werner and Peacor 2003). Increasing predator density is usually assumed to increase the suppression of prey populations; however, our results suggest that restoring the density of overharvested consumers, such as blue crabs, may potentially reduce their ecosystem impacts.

Further, when blue and stone crabs were removed and mud crabs were present at estimated ambient density, oyster recruitment increased, suggesting that mud crabs were weak predators. When the density of mud crabs was increased to compensate for the loss of mass of blue and stone crabs, however, the number of oysters recruiting was significantly less. This indicates that at increased densities mud crabs fulfilled at least some of the role of the more dominant predators (blue and stone crabs), suggesting another potential level of redundancy in the system. Whether mud crabs would indeed increase in densities in the absence of blue and stone crabs remains to be tested. Our results are the first step in examination of such a scenario and show clearly that it warrants exploration, possibly by a large-scale, field-based removal of blue crabs. The species we investigated display territorial, antagonistic behavior (Beck 1997, Clark et al. 1999, Grabowski and Powers 2004), so that removal of one species could result in compensation by another. This may already be occurring given that both stone crabs and blue crabs have been fished heavily over the past several decades. However, long-term data sets recording mud crab densities throughout these periods do not exist to the best of our knowledge.

Most previous theoretical and empirical studies examining the effects of predators have concluded that knowledge of the identity of a particular species is

crucial to predict the impact of predators on prey populations (Paine 1992, Chalcraft and Reserits 2003, Thébault and Loreau 2003, Petchey et al. 2004). Our study strengthens this conclusion and also shows that the density of predators and their intraguild and intraspecific interactions must also be considered, especially since density and identity effects can be coupled. Knowledge of the identity of species within each trophic level and the number of individuals of each species is, therefore, necessary to predict the emergent effects of multiple predators on their shared prey.

Properties such as organic matter transformation are not often considered in the context of species loss and ecosystem functioning (Giller et al. 2004). Incorporation of such processes will help merge the traditional fields of ecosystem ecologists and food web ecologists and bring further understanding to community ecology and the effects of species loss in general. A change in organic matter is indicative of a shift in organic carbon within the system and can have consequences for the nutrient dynamics of a whole system. The similar trend in response to treatments of oyster recruitment and organic matter suggests that there is a positive, albeit correlative, relationship between oyster recruitment and organic matter in the associated sediment. This demonstration of an indirect effect of loss of predators on a basal resource may be a consequence of changes in the abundance of oysters, possibly linked to their production of biodeposits. In particular, these oysters produce pseudofeces (material filtered out of the water column and rejected before ingestion), thus facilitating the transfer of organic matter such as algal cells into the sediment (Newell and Langdon 1996). This indirect effect suggests that predator effects result in trophic cascades in this system because the loss of predators (determined by identity and density) affects oyster recruitment, which appears linked to the amount of organic matter in sediment.

Nontrophic interactions are important factors determining the structure and dynamics of systems based on habitat-modifying species (Jones et al. 1994, Bruno and Bertness 2001, Grabowski 2004). Oyster reefs are important recruitment and refuge sites for many species, including juvenile predators such as blue crabs (Eggleston et al. 1998, Posey et al. 1999). These predators could, therefore, affect indirectly the persistence of their own habitat. The positive link between oysters, the foundation species, and their associated assemblages are counteracted by the negative effects of predatory species preying on juvenile oysters. The strength of these opposing effects must be included in any predictive model.

As with all removal experiments, we must be careful in drawing conclusions regarding the application of the results to larger spatial and temporal scales (Bengtsson et al. 2002, Hooper et al. 2005). This study provides a significant contribution toward understanding the role of predators and predator diversity in realistic food



webs; however, future research is required to test explicitly the context dependency of our results (Hooper et al. 2005, O'Connor and Crowe 2005). Although removal experiments have limitations, they are the most appropriate method of simulating species loss (Symstad et al. 1998, Diaz et al. 2003, Petchey et al. 2004). Human-driven species loss tends not to be random; therefore, research yields more useful results when targeted species are included in extinction simulations (Grime 1998, Wardle 1999, Diaz et al. 2003, Srivastava and Vellend 2005), such as those used in our experiment.

Our findings have practical implications regarding the conservation and management of marine reserves (Fairweather 1991, Kennedy et al. 1996, Kennedy and Cronin 2006). Our study provides insight into the role of predators that are normally heavily fished, except in reserves where they often occur in higher abundances, and examines their impact through trophic processes on a foundation species. This kind of basic research, aimed at examining the effects of loss of heavily harvested species, provides information necessary for reserve managers to make better-informed decisions and improves the effectiveness of environmental restoration (Peterson and Lipcius 2003).

A recent synthesis of both biodiversity–ecosystem functioning and predator–prey interactions research (Ives et al. 2005) has paved the way for greater communication between these subdisciplines. Examining the effect of predators on multiple trophic levels and multiple processes is, therefore, imperative to understand the relationship between biodiversity and ecosystem functioning in order to construct a more predictive framework.

#### ACKNOWLEDGMENTS

We thank everyone who provided technical assistance, including J. Kertesz, S. Lee, Z. Long, J. McGhee, M. O'Connor, E. Selig, M. Stallworth, J. Wall, I. Donohue, the staff of UNC-IMS, and the North Carolina Coastal Federation. The comments from two anonymous reviewers also greatly enhanced this manuscript. This research was funded by the National Science Foundation (OCE0326983 and OCE0327191) to J. F. Bruno and supported by NC Sea Grant (R/MG-0512).

#### LITERATURE CITED

- Allison, G. W. 2004. The influence of species diversity and stress intensity on community resistance and resilience. *Ecological Monographs* 74:117–134.
- Bahr, L. M., and W. P. Lanier. 1981. The ecology of intertidal oyster reefs of the South Atlantic coast: a community profile. U.S. Fish and Wildlife Service FWS/OBS/81.15. Washington, D.C., USA.
- Beck, M. W. 1997. A test of the generality of the effects of shelter bottlenecks in four stone crab populations. *Ecology*, 78:2487–2503.
- Benedetti-Cecchi, L. 2004. Increasing accuracy of causal inference in experimental analyses of biodiversity. *Functional Ecology* 18:761–768.
- Bengtsson, J., K. Engelhardt, P. Giller, S. Hobbie, D. Lawrence, J. Levine, M. Vilá, and V. Walters. 2002. Slippin' and slidin' between the scales: the scaling components of biodiversity–ecosystem functioning relations. Pages 209–220 in M. Loreau, S. Naeem, and P. Inchausti, editors. Biodiversity and ecosystem functioning: synthesis and perspectives. Oxford University Press, Oxford, UK.
- Bruno, J. F., and M. D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. Pages 201–218 in M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. *Marine community ecology*. Sinauer, Sunderland, Massachusetts, USA.
- Bruno, J. F., and M. I. O'Connor. 2005. Cascading effects of predator diversity and omnivory in a marine food web. *Ecology Letters* 8:1048–1056.
- Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18:119–125.
- Byrnes, J. E., P. L. Reynolds, and J. J. Stachowicz. 2007. Invasions and extinctions reshape coastal marine food webs. *Public Library of Science ONE* 2(3):e295.
- Byrnes, J., J. J. Stachowicz, K. M. Hultgren, A. R. Hughes, S. V. Olyarnik, and C. S. Thornber. 2006. Predator diversity strengthens trophic cascades in kelp forests by modifying herbivore behaviour. *Ecology Letters* 9:61–71.
- Cardinale, B. J., C. T. Harvey, K. Gross, and A. R. Ives. 2003. Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters* 6:857–865.
- Chalcraft, D. R., and W. J. Resetarits. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? *Ecology* 84:2407–2418.
- Clark, M. E., T. G. Wolcott, D. L. Wolcott, and A. H. Hines. 1999. Intraspecific interference among foraging blue crabs *Callinectes sapidus*: interactive effects of predator density and prey patch distribution. *Marine Ecology Progress Series* 178: 69–78.
- Connolly, J. 1988. What is wrong with replacement series? *Trends in Ecology and Evolution* 3:24–26.
- Dame, R. F., R. G. Zingmark, and E. Haskin. 1984. Oyster reefs as processors of estuarine materials. *Journal of Experimental Marine Biology and Ecology* 83:239–247.
- Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance. *Ecological Monographs* 59:433–463.
- Dean, W. E. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. *Journal of Sedimentary Petrology* 44:242–248.
- Diaz, S., A. J. Symstad, F. S. Chapin, D. A. Wardle, and L. F. Huenneke. 2003. Functional diversity revealed by removal experiments. *Trends in Ecology and Evolution* 18:140–146.
- Downing, A. L., and M. A. Leibold. 2002. Ecosystem consequences of species richness and composition in pond food webs. *Nature* 416:837–841.
- Duffy, J. E., J. P. Richardson, and E. A. Canuel. 2003. Grazer diversity effects on ecosystem functioning in seagrass beds. *Ecology Letters* 6:637–645.
- Ebersole, E. L., and V. S. Kennedy. 1995. Prey preferences of blue crabs *Callinectes sapidus* feeding on 3 bivalve species. *Marine Ecology Progress Series* 118:167–177.
- Eggleston, D. B., L. L. Etherington, and W. E. Elis. 1998. Organism response to habitat patchiness: species and habitat-dependent recruitment of decapod crustaceans. *Journal of Experimental Marine Biology and Ecology* 223:111–132.
- Eggleston, D. B., R. N. Lipcius, and A. H. Hines. 1992. Density-dependent predation by blue crabs upon infaunal clam species with contrasting distribution and abundance patterns. *Marine Ecology Progress Series* 85:55–68.
- Emmerson, M. C., and D. G. Raffaelli. 2000. Detecting the effects of diversity on measures of ecosystem function: experimental design, null models and empirical observations. *Oikos* 91:195–203.
- Fairweather, P. G. 1991. Implications of supply-side ecology for environmental assessment and management. *Trends in Ecology and Evolution* 6:60–63.

- Finke, D. L., and R. F. Denno. 2005. Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters* 8:1299–1306.
- Gamfeldt, L., H. Hillebrand, and P. R. Jonsson. 2005. Species richness changes across two trophic levels simultaneously affect prey and consumer biomass. *Ecology Letters* 8:696–703.
- Giller, P. S., H. Hillebrand, U.-G. Berninger, M. O. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie, B. Malmqvist, M. T. Monaghan, P. J. Morin, and G. O'Mullan. 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. *Oikos* 104:423–436.
- Grabowski, J. H. 2004. Habitat complexity disrupts predator–prey interactions but not the trophic cascade on oyster reefs. *Ecology* 85:994–1004.
- Grabowski, J. H., A. R. Hughes, D. L. Kimbro, and M. A. Dolan. 2005. How habitat setting influences restored oyster reef communities. *Ecology* 86:1926–1935.
- Grabowski, J. H., and S. P. Powers. 2004. Habitat complexity mitigates trophic transfer on oyster reefs. *Marine Ecology Progress Series* 277:291–295.
- Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology* 86: 902–910.
- Hines, A. H., A. M. Haddon, and L. A. Wiechert. 1990. Guild structure and foraging impact of blue crabs and epibenthic fish in a subestuary of Chesapeake Bay. *Marine Ecology Progress Series* 67:105–126.
- Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75:3–35.
- Hughes, A. R., and J. H. Grabowski. 2006. Habitat context influences predator interference interactions and the strength of resource partitioning. *Oecologia* 149:256–264.
- Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460.
- Ives, A. R., B. J. Cardinale, and W. E. Snyder. 2005. A synthesis of subdisciplines: predator–prey interactions, and biodiversity and ecosystem functioning. *Ecology Letters* 8: 102–116.
- Jackson, J. B. C., et al. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293:629–637.
- Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. *Oikos* 69:373–386.
- Kennedy, V. S., and L. E. Cronin. 2006. The blue crab, *Callinectes sapidus*. Maryland Sea Grant College, University of Maryland System, College Park, Maryland, USA.
- Kennedy, V. S., R. I. E. Newell, and A. F. Eble. 1996. The eastern oyster *Crassostrea virginica*. Maryland Sea Grant College, University of Maryland System, College Park, Maryland, USA.
- Lawton, J. H., and V. K. Brown. 1994. Redundancy in ecosystems. Pages 225–270 in E.-D. Schulze and H. A. Mooney, editors. *Biodiversity and ecosystem function*. Springer-Verlag, New York, New York, USA.
- Lawton, J. H., and R. M. May. 1995. *Extinction rates*. Oxford University Press, Oxford, UK.
- Lenihan, H. S., C. H. Peterson, J. E. Byers, J. H. Grabowski, G. W. Thayer, and D. R. Colby. 2001. Cascading of habitat degradation: oyster reefs invaded by refugee fishes escaping stress. *Ecological Applications* 11:764–782.
- Likens, G. E. 1985. An experimental approach to the study of ecosystems. *Journal of Ecology* 73:381–396.
- Lipcius, R. N., and W. T. Stockhausen. 2002. Concurrent decline of the spawning stock, recruitment, larval abundance, and size of the blue crab *Callinectes sapidus* in Chesapeake Bay. *Marine Ecology Progress Series* 226:45–61.
- Luckenbach, M. W., J. A. Nestlerode, and G. M. Coates. 1997. Oyster reef restoration: developing relationships between structure and function. *Journal of Shellfish Research* 16:270–271.
- Micheli, F. 1997. Effects of predator foraging behavior on patterns of prey mortality in marine soft bottoms. *Ecological Monographs* 67:203–224.
- Newell, R. I. E. 1988. Ecological changes in Chesapeake Bay: are they the result of overharvesting the American oyster? Pages 536–546 in M. P. Lynch and E. C. Krome, editors. *Understanding the estuary: advances in Chesapeake Bay research*. Chesapeake Bay Research Consortium, Baltimore, Maryland, USA.
- Newell, R. I. E., and C. J. Langdon. 1996. Mechanisms and physiology of larval and adult feeding. Pages 185–229 in V. S. Kennedy, R. I. E. Newell, and A. F. Eble, editors. *The eastern oyster: Crassostrea virginica*. Maryland Sea Grant College, University of Maryland System, College Park, Maryland, USA.
- O'Connor, N. E., and T. P. Crowe. 2005. Biodiversity loss and ecosystem functioning: distinguishing between number and identity of species. *Ecology* 86:1783–1796.
- Paine, R. T. 1992. Food web analysis through field measurement of per-capita interaction strength. *Nature* 355:73–75.
- Pauly, D., V. Christensen, J. Dalsgaard, R. Froese, and F. Torres. 1998. Fishing down marine food webs. *Science* 279: 860–863.
- Petchey, O. L., A. L. Downing, G. G. Mittelbach, L. Persson, C. F. Steiner, P. H. Warren, and G. Woodward. 2004. Species loss and the structure and functioning of multitrophic aquatic systems. *Oikos* 104:467–478.
- Peterson, C. H., J. J. Grabowski, and S. P. Powers. 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitat: quantitative valuation. *Marine Ecology Progress Series* 264:249–264.
- Peterson, C. H., and R. N. Lipcius. 2003. Conceptual progress towards predicting quantitative ecosystem benefits of ecological restorations. *Marine Ecology Progress Series* 264:297–307.
- Pimm, S. L., H. L. Jones, and J. Diamond. 1988. On the risk of extinction. *American Naturalist* 132:757–785.
- Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. *American Naturalist* 147:813–846.
- Posey, M. H., C. M. Powell, T. D. Alphin, and E. Townsend. 1999. Use of oyster reefs as habitat for epibenthic fish and decapods. Pages 229–237 in M. Luckenbach, R. Mann, and J. Wesson, editors. *Oyster reef habitat restoration: a synopsis and synthesis of approaches*. Virginia Institute of Marine Sciences Press, Gloucester Point, Virginia, USA.
- Regan, H. M., R. Lupia, A. N. Drinnan, and M. A. Burgman. 2001. The currency and tempo of extinction. *American Naturalist* 157:1–10.
- Rothschild, B. J., J. S. Ault, P. Gouletquer, and M. Heral. 1994. Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Marine Ecology Progress Series* 111:29–39.
- Ruesink, J. L., and D. S. Srivastava. 2001. Numerical and per capita responses to species loss: mechanisms maintaining ecosystem function in a community of stream insects. *Oikos* 93:221–234.
- Schläpfer, F., and B. Schmid. 1999. Ecosystem effects of biodiversity: a classification of hypotheses and exploration of empirical results. *Ecological Applications* 9:893–912.
- Schmid, B., A. Hector, M. A. Huston, P. Inchausti, I. Nijs, P. W. Leadley, and W. Tilman. 2002. The design and analysis of biodiversity experiments. Pages 61–78 in S. Naeem, M. Loreau, and P. Inchausti, editors. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press, Oxford, UK.
- Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters* 7:153–163.

- Seitz, R. D., R. N. Lipcius, A. H. Hines, and D. B. Eggleston. 2001. Density-dependent predation, habitat variation, and the persistence of marine bivalve prey. *Ecology* 82:2435–2451.
- Siddon, C. E., and J. D. Witman. 2004. Behavioral indirect interactions: multiple predator effects and prey switching in the rocky subtidal. *Ecology* 85:2938–2945.
- Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution* 13:350–355.
- Silliman, B. R., and M. D. Bertness. 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Sciences (USA)* 99:10500–10505.
- Sokol-Hessner, L., and O. J. Schmitz. 2002. Aggregate effects of multiple predator species on a shared prey. *Ecology* 83:2367–2372.
- Southworth, M., and R. Mann. 2004. Decadal scale changes in seasonal patterns of oyster recruitment in the Virginia sub estuaries of the Chesapeake Bay. *Journal of Shellfish Research* 23:391–402.
- Srivastava, D. S., and M. Vellend. 2005. Biodiversity–ecosystem function research: is it relevant to conservation? *Annual Review of Ecology, Evolution, and Systematics* 36:267–294.
- Strong, D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology* 73:747–754.
- Symstad, A. J., D. Tilman, J. Willson, and J. M. H. Knops. 1998. Species loss and ecosystem functioning: effects of species identity and community composition. *Oikos* 81:389–397.
- Thébault, E., and M. Loreau. 2003. Food-web constraints on biodiversity–ecosystem functioning relationships. *Proceedings of the National Academy of Sciences (USA)* 100:14949–14954.
- Underwood, A. J. 1997. *Experiments in ecology: their logistical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, UK.
- Underwood, A. J., and M. G. Chapman. 1998. WinGMAV5. Institute of Marine Ecology, University of Sydney, Sydney, Australia.
- Underwood, A. J., and E. J. Denley. 1984. Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. Pages 151–180 *in* D. R. Strong, D. Simberloff, L. G. Abele, and A. B. Thistle, editors. *Ecological communities: conceptual issues and the evidence*. Princeton University Press, Princeton, New Jersey, USA.
- Wardle, D. A. 1999. Is “sampling effect” a problem for experiments investigating biodiversity–ecosystem function relationships? *Oikos* 87:403–408.
- Wardle, D. A., K. I. Bonner, G. M. Barker, G. W. Yeates, K. S. Nicholson, R. D. Bardgett, R. N. Watson, and A. Ghani. 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monographs* 69:535–568.
- Wardle, D. A., and J. P. Grime. 2003. Biodiversity and stability of grassland ecosystem functioning. *Oikos* 100:622–623.
- Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100.
- Wojdak, J. M., and B. Luttbeg. 2005. Relative strengths of trait-mediated and density-mediated indirect effects of a predator vary with resource levels in a freshwater food chain. *Oikos* 111:592–598.
- Worm, B., and J. E. Duffy. 2003. Biodiversity, productivity and stability in real food webs. *Trends in Ecology and Evolution* 18:628–623.
- Zimmerman, R. J., T. J. Minello, T. Baumer, and M. C. Castiglione. 1989. Oyster reef as habitat for estuarine macrofauna. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFC-249, Silver Spring, Maryland, USA.

#### APPENDIX A

Results from ANOVA testing effects of the predator richness and identity treatments on juvenile oyster survival, oyster recruitment, and percentage organic matter (*Ecological Archives* E089-024-A1).

#### APPENDIX B

Results from ANOVA testing planned comparisons of different combinations of predators with and without blue crabs on juvenile oyster survival, oyster recruitment, and organic matter (*Ecological Archives* E089-024-A2).

#### APPENDIX C

Results from ANOVA testing effects of removing different combinations of species of predator with and without compensation for the loss of biomass on juvenile oyster survival, oyster recruitment, and organic matter (*Ecological Archives* E089-024-A3).