Density-mediated indirect effects from active predators and narrow habitat domain prey

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Abstract. The hunting-mode–habitat-domain-range framework suggests that the mechanism driving trophic cascades (i.e., trait-mediated indirect interactions [TMIIs] vs. density-mediated indirect interactions [DMIIs]) should depend upon the functional traits of predators and prey. For example, trophic cascades containing active, broad habitat domain range (BHDR) predators interacting with narrow habitat domain range (NHDR) prey are predicted to arise primarily via TMIIs, because these prey should reduce their conspicuous activity in the presence of these predators. Unfortunately, this hypothesis is difficult to test given the strong bias against studies assessing trophic cascades containing NHDR prey. Furthermore, this hypothesis ignores evidence that (1) active predators can have high consumption rates on prey, (2) continuously responding to active predators foraging across broad areas is energetically costly for prey, and (3) cues from active, BHDR predators may not influence prey density. We examined the TMIIs and total indirect interaction (TII) produced during interactions between an active, BHDR ladybeetle predator (*Naemia seriata*) and its NHDR prey (scale insects). We exposed scale insects to nonlethal and lethal ladybeetle predators in laboratory mesocosms for 15 weeks. We measured the growth of the scale insect's host plant (cordgrass) and the population density of scale insects. Contrary to theory, nonlethal ladybeetles did not induce TMIIs. However, lethal ladybeetles increased cordgrass total and root dry biomass by 36% and 44%, respectively, suggesting the presence of strong DMIIs. Additionally, both lethal and nonlethal ladybeetles reduced scale insect population density. Our findings suggest that DMIIs, rather than TMIIs, can result from interactions between active BHDR predators and NHDR prey.

Key words: density-mediated indirect interactions; habitat domain range; hunting mode; nonconsumptive effects; predator traits; trait-mediated indirect interactions.

INTRODUCTION

Predators can shape community structure and function by indirectly influencing basal resources via trophic cascades (Paine 1980, Carpenter et al. 1985). Such cascades can be generated by predators consuming prey (density-mediated indirect interactions [DMIIs]; Abrams et al. 1996) or by predators inducing trait changes in prey (trait-mediated indirect interactions [TMIIs]; Schmitz et al. 1997, Werner and Peacor 2003, Preisser et al. 2005). Understanding the mechanism(s) by which predators induce trophic cascades (i.e., DMIIs vs. TMIIs) is important because the nature of this indirect interaction can critically influence ecosystem-level processes such as energy flow (Trussell et al. 2006*a*) and nutrient cycling (Schmitz et al. 2010, Hawlena et al. 2012). Although recent efforts have focused on trying to predict the nature of trophic cascades (i.e., whether driven by TMIIs, DMIIs, or both) based on the functional traits of predators (Schmitz et al. 2004, Schmitz 2005),

some contexts are poorly studied. For example, the hunting-mode–habitat-domain framework (Schmitz et al. 2004, Schmitz 2005, Preisser et al. 2007) suggests that the nature of trophic cascades can be estimated by identifying predator hunting mode (e.g., active vs. sit-and-pursue vs. sit-and-wait) and the habitat domain range of predators and prey (i.e., the extent that individuals move throughout the habitat). Yet, this framework remains largely untested for predator-prey interactions involving active, broad habitat domain range (hereafter, BHDR) predators interacting with narrow habitat domain range (hereafter, NHDR) prey.

Trophic cascades resulting from interactions between active, BHDR predators and NHDR prey are uncommon in the literature despite being common and ecologically important in nature. For instance, a meta-analysis on the hunting-mode–habitat-domain framework only classified five of 153 prey species as having an NHDR (Preisser et al. 2007), forcing these authors to omit NHDR prey from the analyses. However, NHDR prey (i.e., a species that selects only part of the entire available habitat; Schmitz et al. 2004) can be found in most ecosystems. In aquatic systems, ecosystem engineering bivalves have NHDRs (see Appendix D in Preisser et al. 2007), and

they frequently interact with active BHDR predators (e.g., green crabs; Smith and Jennings 2000; see Appendix E in Preisser et al. 2007). In terrestrial systems, scale insects and other agricultural insect pests known to destroy 13% of crops (Pimentel 1997), can be classified as having NHDRs. These pests are commonly attacked by active BHDR ladybeetle predators (see Preisser et al. 2007: Table 1, Romero and Koricheva 2011).

According to the hunting-mode–habitat-domain framework, such interactions (i.e., active BHDR predators hunting NHDR prey) should generate trophic cascades primarily via TMIIs, as these prey should reduce their conspicuous activity and shift their time budgets (i.e., reduce foraging) under predation risk (Schmitz et al. 2004, Schmitz 2005). Indeed, several studies suggest that prey decrease their activity in response to active predators (see Preisser et al. 2007: Fig. 1). However, the limited number of studies containing NHDR prey (Preisser et al. [2007] only identified five), makes the nature of these cascades unclear. Furthermore, two observations suggest that TMIIs may be weak under these scenarios. First, the cost of continuously responding to cues from active predators present throughout the habitat may be too high (Bouskila 2001, Schmitz et al. 2004, Schmitz 2005). Second, cues from active predators that reduced prey activity had no effect on prey population dynamics (e.g., prey density; see Preisser et al. 2007: Fig. 1). This surprising observation suggests that prey activity and performance may be disconnected in these contexts. Together these observations suggest that the nature of trophic cascades containing these types of interactions (when active BHDR predators meet NHDR prey) remains unclear, and more studies are needed to resolve these contrasting predictions.

In our study, we assessed the population and community-level effects of interactions between active, BHDR predators and NHDR prey (i.e., a trophic cascade hypothesized to be generated primarily by TMIIs according to the hunting mode-habitat domain framework). We exposed scale insects (i.e., NHDR prey) to lethal and nonlethal ladybeetles (i.e., active BHDR predators) in mesocosms for 15 weeks and monitored the population density of adult and crawler (i.e., juvenile) scale insects. To test if the resulting trophic cascade was mediated by TMIIs or DMIIs, we examined the effects of these interactions on basal resources by assessing the growth of the scale insect's host plant, *Spartina foliosa* (hereafter cordgrass).

MATERIALS AND METHODS

Ladybeetle and scale insect habitat domain range classification

A primary goal of this study was to assess the impacts of an active BHDR predator on the population dynamics of an NHDR prey. To classify the habitat domain ranges of our predator (the ladybeetle, *Naemia seriata*) and prey (scale insects, *Haliaspis spartinae*), we compared "the portion of the entire habitat used by the predator relative to that of the prey" (Schmitz 2005). Using this definition, we classified this ladybeetle as a BHDR predator because we have observed it occurring across several habitats in the field including all cordgrass tissues (e.g., leaves and stems), marsh sediment, and another salt marsh plant species, *Sarcocornia pacifica* (S. A. Rinehart, *personal observation*). In contrast, the specialist scale insects are restricted to the adaxial surface of cordgrass leaves as adults (Boyer and Zedler 1996; S. A. Rinehart and J. D. Long, *personal observation*). Furthermore, we observed that ladybeetles had a BHDR relative to scale insects in the mesocosm experiment we describe in *Collection sites and mesocosm set-up*. There, we assessed the location of ladybeetles daily, between 08:00 and 10:00 hours, on six separate days. We noted that on any given day, only 23% ± 0.02% (mean ± SE) of ladybeetles were found on cordgrass leaves. Meanwhile, scale insects were only observed on the adaxial surface of cordgrass leaves, indicating that ladybeetles were using more of the available habitat than their prey.

The scale insect, *H. spartinae*, is a feeding and habitat specialist on cordgrass leaves (Tippins and Beshear 1971, Boyer and Zedler 1996, Japoshvili and Russell 2012; S. A. Rinehart and J. D. Long, *personal observation*). Additionally, scale insects become immobile once they metamorphose, permanently limiting their habitat use (Boyer and Zedler 1996). However, the crawler (i.e., juvenile) life stage of scale insects is mobile, and may have a greater habitat domain range than adults due to its dispersal abilities (Boyer and Zedler 1996). Thus, to determine the habitat domain range of crawlers relative to ladybeetles with respect to range sizes of individual animals, we conducted a manipulative field experiment to examine the extent of crawler dispersal. In May 2012, we identified eight "source" cordgrass stems containing scale insects (116 ± 32 per stem) separated by at least 61 m at the San Dieguito Lagoon salt marsh restoration site (32°58′40.4″ N, 117°14′32.8″ W). We selected source stems that (1) had adult, female scale insects from which we hoped crawlers would emerge and disperse and (2) were in areas of low cordgrass stem density. The low cordgrass stem density at these sites allowed us to establish scale-insect-free areas around our source stems, thereby increasing the probability that crawlers dispersing onto experimental transplants originated from source stems. Additionally, we regularly removed scale insects from any non-experimental stems in a 30 m radius of source stems to further limit outside crawlers from entering our experiment.

On 25 May 2012, we transplanted sediment plugs, each containing a single cordgrass stem from which we removed all scale insects (plant height = 49 ± 1 cm; mean ± SE), from a common source location in San Dieguito Lagoon. We planted the cordgrass plugs in a line (i.e., at a constant elevation) at five distances from each source stem (0.05, 0.1, 1, 10, and 30 m). The leaves from transplanted stems at the 0.05 and 0.1 m distances

could touch the source stem. There were no other cordgrass stems present between our transplanted stems. We surveyed crawler abundance on all experimental stems (target and transplanted cordgrass) and removed any crawlers from transplanted stems every other week between 2 June 2012 and 10 August 2012. For each of our eight replicates, we conducted a goodness of fit test (one-way chi-square) to test the hypothesis that crawler recruitment onto our transplanted stems was the same, regardless of distance from target stem. Additionally, we conducted a goodness of fit test using the pooled data from all replicates. We found that 96.7% of crawlers dispersed 0.1 m or less, and no crawlers were ever observed on stems 30 m from source plants (see *Results*; Fig 1; Appendix S1: Table S1). Our findings strongly suggest that crawlers also have a narrow habitat domain range because their dispersal is highly localized.

In addition to defining the habitat domain range, we needed to determine the hunting mode of ladybeetle predators. We classified ladybeetles in our system as active predators (i.e., predators that are continuously searching for prey), rather than sit-and-pursue predators (i.e., predators that remain in a fixed feeding location and rushes at prey in their vicinity) or sit-and wait predators (i.e., ambush predators that remain in a fixed location for a prolonged period whether hunting or not; Schmitz et al. 2004, Schmitz 2005). In our system, ladybeetles are active predators because they constantly move on, and between, cordgrass stems while foraging, even when scale insect densities are high on all stems in the area (S. A. Rinehart, *personal observation*). Our classification is also supported by the literature, as ladybeetles have previously been defined as active predators (Preisser et al. 2007, Romero et al. 2011).

Collection sites and mesocosm set-up

Using this model system, we conducted a mesocosm experiment at the San Diego State University Coastal and Marine Institute Laboratory (CMIL). On 28 May
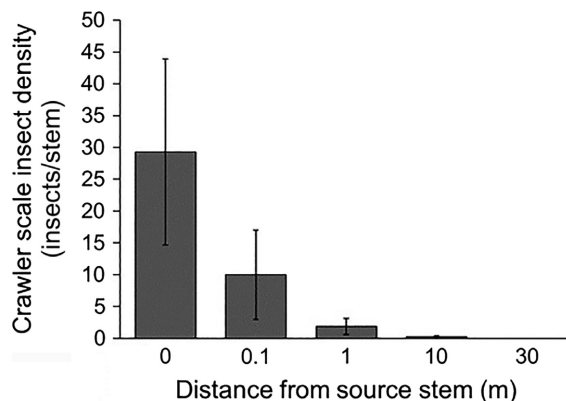

Fig. 1. Density (mean ± SE) of crawler scale insects dispersing to cordgrass stems at 0, 0.1, 1, 10, and 30 m from source stems. The samples size is eight for all distances.

2014, we collected sediment plugs (15 cm diameter × 15 cm deep) each containing a single cordgrass stem infested with scale insects from Sweetwater Marsh (South San Diego Bay; 32°38′15.8″ N, 117°06′37.5″ W). We planted cordgrass stems and field-collected sediment in 2.6-L plant pots with holes for drainage (Elite Nursery Containers; 300 Series). By using toothbrushes to remove scale insects, we standardized initial mean total scale insect density to 273 ± 19 insects/stem (mean ± SE). We collected ladybeetles from two sites, Sweetwater Marsh and San Dieguito Lagoon (32°58′40.4″ N, 117°14′32.8″ W).

We placed five potted plants from random treatments in each of our outdoor, 17-gallon tanks (1 gallon = 3.79 L) connected to CMIL's flow-through seawater system. Potted plants were randomly reallocated to tanks weekly. To further mimic natural salt marsh conditions, we connected tanks to a tide height control system that used a microcontroller to temporally match submersion and immersion in tanks to tidal conditions in San Diego Bay (Miller and Long 2015), allowing us to create mesocosm tidal conditions equivalent to those experienced by cordgrass in the field (i.e., at an equivalent tidal height of 1.5 m above sea level).

We randomly allocated potted cordgrass plants to one of four treatments: (1) lethal ladybeetles, (2) nonlethal ladybeetles, (3) scale insects only, and (4) no insects (i.e., no scale insects or ladybeetles). Every treatment except the no insect treatment contained scale insects. We introduced a single adult ladybeetle into each replicate for lethal ladybeetles and nonlethal ladybeetle treatments. At our sites, we commonly observe at least one ladybeetle on each cordgrass stem (S. A. Rinehart and J. D. Long, *unpublished data*) suggesting that our ladybeetle densities were realistic. We glued shut the mandibles of ladybeetles in the nonlethal ladybeetles treatment with 3M Scotch Super Glue Liquid (3M, St. Paul, Minnesota, USA). These were monitored for 10 min afterwards to ensure that their mandibles were sufficiently glued. Although unable to consume prey, non-lethal ladybeetles provide risk cues to prey in the absence of direct predation (Schmitz 1998, Kersch-Becker and Thaler 2015).

We replaced ladybeetles in lethal ladybeetle and nonlethal ladybeetle treatments each week because most ladybeetles died one week after gluing. In no insect treatments, we removed all scale insects from plants with a soft toothbrush and did not introduce a ladybeetle (Long and Porturas 2014). Each treatment was replicated nine times, except the scale insect treatment, which had one plant senesce during the second week of the experiment. To prevent ladybeetle dispersal among replicates, we covered all individual replicates with nylon insect mesh (54 cm tall × 50 cm, mesh size = 1 mm). Additionally, we monitored ladybeetle behavior between 08:00 and 10:00 hours on six days (20 June 2014, 22 June 2014, 25 June 2014, 26 June 2014, 2 July 2014, and 3 July 2014). On these days, we observed all lethal ladybeetle and nonlethal ladybeetle replicates and noted if the

ladybeetle was visible or not. If the ladybeetle was visible, we documented its habitat (e.g., cordgrass leaf, cordgrass stem, mud, or cage). The entire experiment was maintained for 15 weeks and ended 3 September 2014.

For the duration of the experiment, we included two types of cage controls, an uncaged scale insect and uncaged no insect treatment ($n = 9$). Cage controls were compared to equivalent caged treatments to test for caging effects on scale insects and cordgrass. We used two-sample t tests to compare adult and crawler scale insect density in the caged scale insect and uncaged scale insect treatments. Similarly, we used two-sample t tests to compare all cordgrass performance metrics in caged no insect and uncaged no insect treatments. However, we lost one stem dry biomass replicate from the uncaged scale insect treatment, giving us a sample size of eight for this treatment.

Effects of nonlethal and lethal ladybeetles on scale insects

To assess the nonlethal and total predator effects of ladybeetles on the local population dynamics of scale insects, we monitored the density of juvenile (hereafter "crawler") and adult scale insects weekly. Crawlers and adults can be distinguished by their morphology (crawlers lack the white waxy tests of adults) and their mobility (adults are immobile). Scale insect density was log transformed prior to analysis. We then compared adult, crawler, and total scale insect density using separate repeated-measures ANOVA with treatment as a fixed factor and time as the repeated measure. We ran separate analyses on adult, crawler, and total scale insect density to test for any differential effects on the two scale insect life stages. We visually inspected our data using the resulting covariance matrix to ensure our data met the test's assumptions. We conducted all statistical analyses in JMP v. 13 (SAS, Cary, North Carolina, USA).

In addition, we calculated the effect sizes of the total predator effect (i.e., TPE) and the nonconsumptive effect (i.e., NCE) of ladybeetle predators on the cumulative total (i.e., crawlers and adults) scale insect-days (i.e., SD) at week 15, for all replicates in the experiment using the following equation (see Ruppel [1983], Fournier et al. [2006], and Ohnesorg et al. [2009] for similar metrics):

$$SD = \sum (X_{i+1} - X_i)[(Y_i + Y_{i+1}) \div 2].$$

Here, X_i and X_{i+1} represent consecutive sampling dates, while Y_i and Y_{i+1} represent corresponding total scale insect density estimates (after Ruppel 1983). We selected this approach because it produces a single metric that accounts for both scale insect density as well as the variation in scale density through time (Ruppel 1983, Ohnesorg et al. 2009). Cumulative total scale insect-day calculations (SD) for each replicate were then used to calculate the effect sizes of the TPE and NCE using the following equations (see Peacor and Werner [2004],

Trussell et al. [2006b], and Hughes et al. [2012] for similar metrics):

$$TPE = 1 - \left(\frac{SD_{lethal\ ladybeetle}}{SD_{scale\ insect}} \right)$$

$$NCE = 1 - \left(\frac{SD_{nonlethal\ ladybeetle}}{SD_{scale\ insect}} \right).$$

These metrics were used to determine the per capita (ladybeetle) reduction of scale insect density caused by predator type (lethal vs. nonlethal) on a proportional basis. For example, TPE = 0.35 would indicate that lethal ladybeetles decreased scale insect density by 35%. Likewise, NCE = −0.20 would indicate that nonlethal ladybeetles increased scale insect density by 20%.

The numerators of these proportions were obtained from each individual replicate for the given treatment, while the denominator for both proportions was the mean of the given scale insect variable for the Scale Insect treatment (see Wojdak et al. [2005] and Trussell et al. [2006b] for similar approaches). We calculated the corresponding effect sizes for each replicate in the lethal ladybeetle and nonlethal ladybeetles treatments.

Direct effects of scale insects on cordgrass

In addition to assessing the impacts of ladybeetle predators on scale insect populations, we also examined the correlation between cumulative total scale insect-days and final cordgrass dry biomass. At the end of the experiment, we removed whole plants from pots and dissected them into three tissue types: leaves, stems, and roots. Each tissue type was dried at 60°C and then weighed. We used linear regressions to examine the relationship between cumulative total scale insect-days and log-transformed cordgrass stem, root, leaf, and total dry final biomass. Because of relatively high within-treatment variability, we examined the overall relationship of scale insect density and plant biomass, independent of treatment.

Indirect effects of lethal and nonlethal ladybeetles on cordgrass

To examine the indirect interaction between ladybeetles and cordgrass, we compared leaf biomass, stem biomass, root biomass, and total dry cordgrass biomass between treatments using one-way ANOVAs with Tukey's HSD post-hoc tests where necessary. Prior to analysis, we inspected our data to ensure that they did not violate test assumptions, and transformed data when necessary.

We calculated the relative strengths of the total indirect interaction (TII) effect and the trait-mediated indirect interaction (TMII) of ladybeetles on cordgrass stems, leaves, roots, and total dry biomass. We calculated

TIIs and TMIIs using the quantitative methods outlined by Peacor and Werner (2004) (also see Trussell et al. [2006b] for similar metrics):

$$TII = \left(\frac{C_{\text{lethal ladybeetle}}}{C_{\text{scale insect}}} \right) - 1$$

$$TMII = \left(\frac{C_{\text{nonlethal ladybeetle}}}{C_{\text{scale insect}}} \right) - 1$$

where C is final cordgrass biomass for the different tissue types. A TII = 0.35 indicates that lethal ladybeetles increased cordgrass biomass by 35%. Likewise, TMII = −0.20 indicates that nonlethal ladybeetles decrease cordgrass biomass by 20%. The numerators of the proportions above were obtained from each individual replicate for the given treatment, while the denominator for both proportions was the mean of the given cordgrass variable for the scale insect treatment (see Wojdak et al. [2005] and Trussell et al. [2006b] for similar approaches). We calculated effect sizes for each replicate in the lethal ladybeetle and nonlethal ladybeetles treatments.

<center>RESULTS</center>

Scale insect habitat domain range classification

A major crawler dispersal event occurred during our experiment examining crawler dispersal between 15 June and 27 July 2012. During this time, scale insect densities on source stems increased by nearly an order of magnitude from starting scale densities (115 ± 38 to $1{,}090 \pm 195$ insects/stem; mean ± SE). Over this nine-week study, 331 crawlers appeared on our transplanted stems. Of these, 96.7% of crawlers were observed on transplanted stems whose leaves could touch source stems (i.e., transplanted stems at 0.05 and 0.1 m from target plants; Fig. 1). An additional 2.5% of crawlers were found at the next closest transplant distance (1 m). Two individual crawlers were found on transplanted stems at 10 m and crawlers were never observed on transplanted stems at 30 m. For each of our eight source stems, the number of crawler recruits differed between all transplant distances (goodness of fit tests, $P \leq 0.003$; Appendix S1: Table S1), with more crawlers recruiting to transplanted stems close to source stems. This same pattern was observed when data were pooled from all eight replicates ($\chi^2 = 596$, df = 4, $P < 0.001$; Appendix S1: Table S1).

Effects of nonlethal and lethal ladybeetles on scale insects

Ladybeetles in our mesocosm experiment appeared to be habitat generalists. We found ladybeetles on all available habitats in our experiment, including the cage, cordgrass stems, cordgrass leaves containing scale insects, and the mud (the mean percentage of time ladybeetles were found on each of these habitats was ($36.6\% \pm 0.05\%$, $29.2\% \pm 0.06\%$, $23\% \pm 0.03\%$, $11.1\% \pm 0.08\%$, respectively; mean ± SE).

Cages did not influence scale insect density (caged vs. uncaged, 434 ± 59 and 321 ± 71 per stem [mean ± SE]; $t_{15} = -1.489$, $P = 0.157$), crawler scale insect density (caged vs. uncaged, 151 ± 36 and 194 ± 67 per stem; $t_{15} = -0.101$, $P = 0.921$), or total scale insect density (caged vs. uncaged, 585 ± 87 and 514 ± 128 per stem; $t_{9.1} = -0.455$, $P = 0.670$). Thus, we excluded the uncaged scale insect treatments from further analyses.

Overall, adult scale insect density depended upon time ($F_{14,\ 391} = 5.27$; $P < 0.0001$) and treatment ($F_{2,\ 391} = 554.28$, $P < 0.0001$; Fig. 2a; Appendix S1: Table S2). Additionally, time and treatment had an interactive effect on adult scale density ($F_{44,\ 391} = 34.28$, $P < 0.0001$). Adult scale insects displayed negative population growth in the presence of lethal ladybeetles, with adult scale insect density decreasing by 99% in this treatment during our 15-week study (Fig. 2a). In contrast, adult scale insects displayed positive population growth in treatments lacking lethal ladybeetles with the magnitude of increase depending upon treatment (increasing by 40% and 150% in nonlethal ladybeetle and scale insect treatments, respectively).

Crawler density depended upon time ($F_{14,\ 391} = 159.72$, $P < 0.0001$), treatment ($F_{2,\ 391} = 371.48$, $P < 0.0001$), and their interaction ($F_{44,\ 391} = 25.48$, $P < 0.0001$; Fig. 2b; Appendix S1: Table S3). We observed two clear crawler dispersal events that peaked around weeks 4 and 11. Lethal ladybeetles reduced crawler density by 96%, while nonlethal ladybeetles decreased crawler density by 55% during the study. In contrast, crawler density increased by 49% in our treatment lacking ladybeetles (i.e., scale insect treatment).

Total scale insect density (the sum of adults and crawlers) depended upon time ($F_{14,\ 391} = 7.95$, $P < 0.0001$), treatment ($F_{2,\ 391} = 559.7$, $P < 0.0001$), and their interaction ($F_{44,\ 391} = 34.60$, $P < 0.0001$; Fig. 2c; Appendix S1: Table S4). Lethal ladybeetles reduced total scale insect density on cordgrass by 98% and nonlethal ladybeetles reduced total scale insect density by 7%.

The NCE of ladybeetles on the cumulative total scale insect-days comprised 43% of the TPE of ladybeetles (Table 1). Again, we chose to use cumulative total scale insect-days rather than final scale insect density because it allowed us to account for the total potential scale insect effects on cordgrass over the experiment.

Direct effects of scale insects on cordgrass

Cages had no effect on cordgrass stem biomass (caged vs. uncaged, 1.11 ± 0.18 and 1.32 ± 0.24 g [mean ± SE]; $t_{13.84} = -0.401$, $P = 0.347$), leaf biomass (caged vs. uncaged, 0.69 ± 0.08 and 0.68 ± 0.13 g; $t_{12.28} = 0.248$, $P = 0.596$), and total tissue biomass (caged vs. uncaged, 4.83 ± 0.461 and 6.87 ± 1.06 g; $t_{11.03} = -1.117$, $P = 0.144$). There was a tendency for cages to reduce

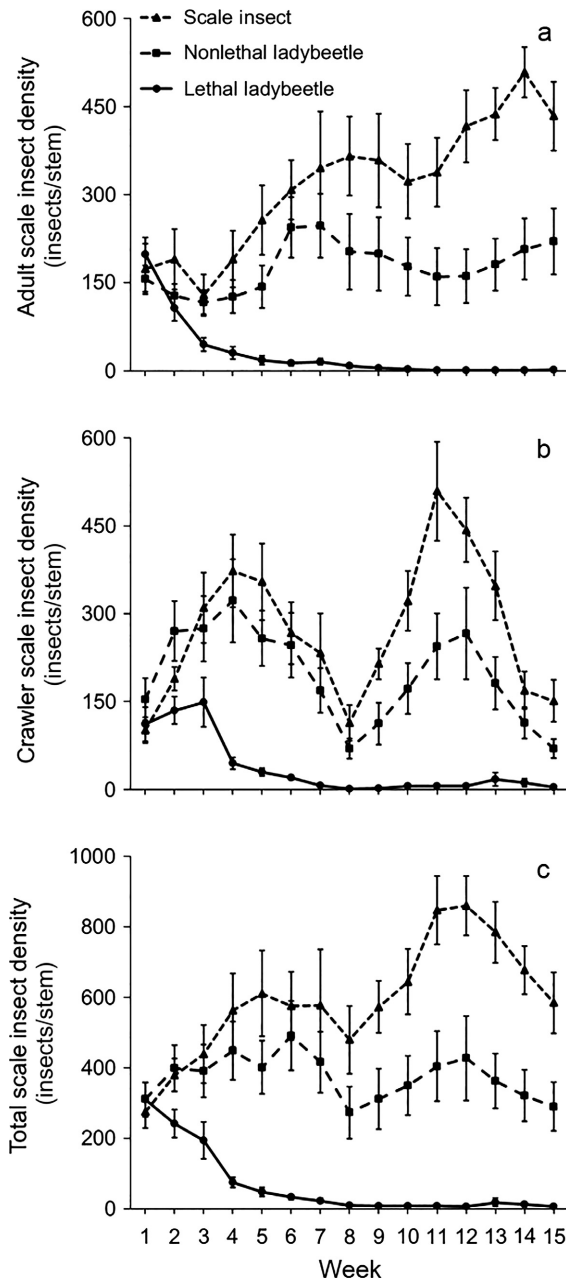FIG. 2. Density (mean ± SE) of (a) adult, (b) crawler, and (c) total scale insects per cordgrass stem in all treatments over 15 weeks. The sample size is nine for the lethal and nonlethal ladybeetle treatments and eight for the scale insect treatment.

TABLE 1. Calculated NCE and TPE effect sizes using cumulative total scale insect-days.

Parameter	NCE	TPE
Cumulative total scale insect-days	0.38 (0.11)	0.89 (0.02)

Notes: NCE, nonconsumptive effect; TPE, total predator effect. Values shown for NCE and TPE represent mean effect sizes. Values in parentheses are ±1 SE of calculated effect sizes. Sample size was nine.

$R^2 = 0.269$, $P < 0.001$; Appendix S1: Fig. S1b), stem (linear regression, $R^2 = 0.196$, $P < 0.001$; Appendix S1: Fig. S1c), and leaf biomass (linear regression, $R^2 = 0.093$, $P = 0.026$; Appendix S1: Fig. S1d).

Indirect effects of nonlethal and lethal ladybeetles on cordgrass

After the 15-week experiment, treatment influenced total cordgrass biomass (Fig. 3a, $F_{3,31} = 6.320$, $P = 0.002$). In the absence of ladybeetle predators, scale insects reduced total and root biomass by 46% and 44%, respectively (in scale insect vs. no insect treatments, Fig. 3a, b). Lethal ladybeetles had considerable impacts on cordgrass total and root biomass, increasing their biomass by 36% and 44%, respectively, relative to scale insect treatments ($F_{3,31} = 4.233$, $P = 0.013$; Fig. 3b); but they did not influence cordgrass stem or leaf biomass (Fig. 3c, d). Nonlethal ladybeetles did not significantly influence cordgrass biomass; however, there was a slight trend for nonlethal ladybeetles to increase cordgrass root and total biomass. Finally, the calculated effect sizes of the TII and the TMII of ladybeetles on cordgrass stems, leaves, roots, and total cordgrass biomass were not different from zero (Table 2).

DISCUSSION

The hunting-mode-habitat-domain framework predicts that interactions between active broad habitat domain range (BHDR) predators and narrow habitat domain range (NHDR) prey should result in trait-mediated indirect interactions (TMIIs; Schmitz et al. 2004, Schmitz 2005). Although nonlethal ladybeetles suppressed scale insect density, this nonconsumptive effect did not have a cascading influence on cordgrass productivity. Rather, only lethal ladybeetles induced a trophic cascade that enhanced cordgrass biomass (Fig. 3). Together, these observations suggest that, in contrast to theory, the trophic cascade in this system was generated primarily by DMIIs and not TMIIs.

Trophic cascades generated via TMIIs may be rare for food chains containing active, BHDR predators interacting with NHDR prey for several reasons. First, the magnitude and timing of nonconsumptive effects (NCEs) that could lead to TMIIs may be lower and slower than consumptive effects under some situations. In support of this hypothesis, nonlethal ladybeetles reduced scale insect
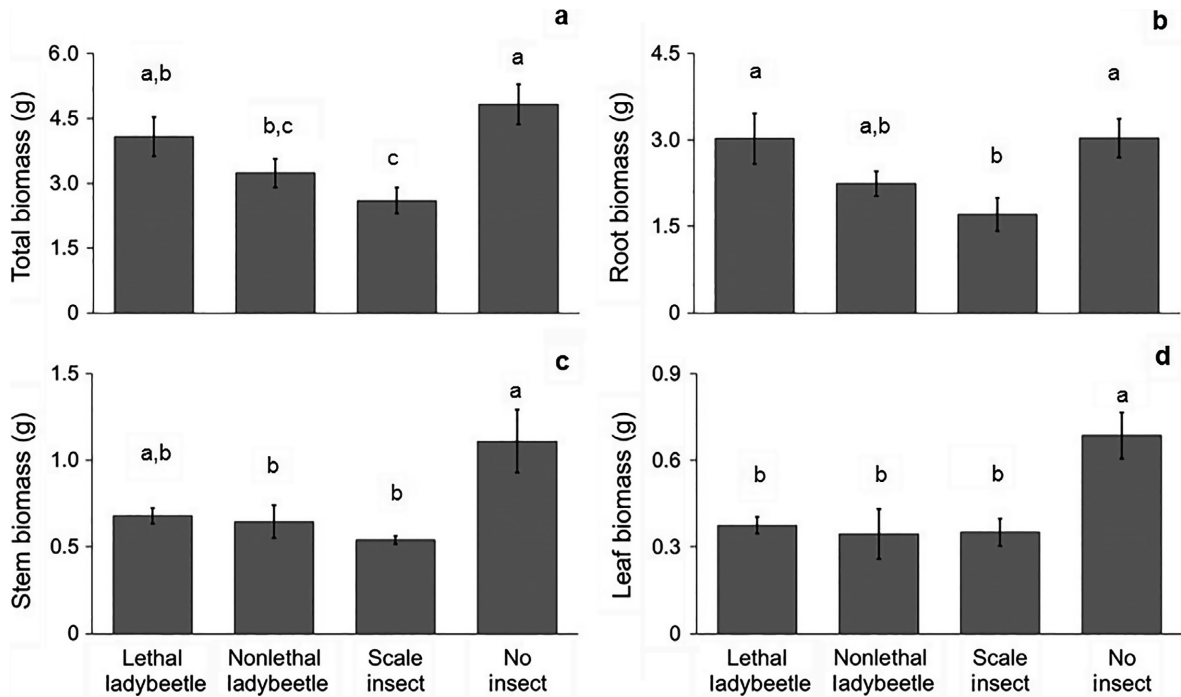
root biomass (caged vs. uncaged, $3.04 ± 1.00$ and $4.87 ± 1.02$ g; $t_{11.90} = -1.526$, $P = 0.077$). However, because (1) cages were applied to all other treatments and (2) we had no a priori reason to expect this effect to interact with treatments, we removed uncaged no insect treatments from further analyses.

Cumulative total scale insect-days was inversely related to cordgrass total (linear regression, $R^2 = 0.291$, $P < 0.001$; Appendix S1: Fig. S1a), root (linear regression,

FIG. 3. Cordgrass (a) total tissue biomass, (b) root biomass, (c) stem biomass, and (d) leaf biomass in all treatments (mean ± SE). Biomass metrics represent dried cordgrass tissue mass (g). Within each panel, treatments with shared letters are not significantly different at significance level of α = 0.05 (Tukey HSD tests). Sample size is nine for all treatments accept for the scale insect treatment, which has a sample size of eight.

density by only 50% after 15 weeks, whereas it took lethal ladybeetles just 3 weeks to have the same effect (Fig. 2c). Because of the quick rates of consumption, cordgrass in the lethal ladybeetle treatment grew essentially scale-insect-free for 10 weeks. Thus, the relative importance of DMIIs and TMIIs may be tightly related to the rates at which predators impact their prey.

Second, the energetic costs required to respond continuously to an active predator moving through the habitat may be too high (Bouskila 2001, Schmitz et al. 2004). This is especially true for prey responding to BHDR predators, as their cues may persist throughout the environment, making it difficult for prey to accurately predict predation risk (Schmitz et al. 2004, Schmitz 2005, Preisser et al. 2007). In support of this observation, grasshoppers (*Melanoplus femurrubrum*) altered their diets and

TABLE 2. Calculated TMII and TII effect sizes using cordgrass biomass metrics.

Plant tissue	TMII	TII
Stem	0.19 (0.52)	0.26 (0.25)
Leaves	−0.02 (0.74)	0.07 (0.24)
Root	0.32 (0.38)	0.77 (0.76)
Total tissue	0.25 (0.25)	0.57 (0.52)

Notes: TMII, trait-mediated indirect interaction; TII, total indirect interaction. Values shown for TMII and TII represent mean effect sizes. Values in parentheses are ±1 SE of calculated effect sizes.

microhabitats in response to sit-and-wait (*Pisaurina mira*) and sit-and-pursue (*Rabidosa rabida*) predators, but they did not respond to cues from active spider predators (*Phidippus rimator*; Schmitz and Suttle 2001, Schmitz et al. 2004, Schmitz 2005). However, this reason seems unlikely for our model system because we observed strong NCEs on prey population density. Also, while some active predator cues can alter prey behaviors, these cues need not necessarily have cascading impacts on basal resources because they do not always alter prey population dynamics (e.g., prey density; Preisser et al. 2007). For instance, cues from red fox (*Vulpes vulpes*) did not impact the density or fecundity of house mice prey (*Mus domesticus*; Banks and Powell 2004). Similarly, cues from nearby wolf packs (*Canis lupus*) altered elk (*Cervus elaphus*) movement and vigilance, but did not impact elk demography (Middleton et al. 2013). However, this reason also seems unlikely for our model systems as we saw a negative impact of nonlethal predators on prey density.

Lethal ladybeetles, by indirectly enhancing cordgrass root biomass, may enhance cordgrass asexual reproduction, as cordgrass reproduces primarily through rhizomatous growth (Fang 2002). Furthermore, greater cordgrass biomass can increase salt marsh sediment stability and accretion (Pillay et al. 2011) and enhance spring regrowth (Hull et al. 1976, Lytle and Hull 1980, Hopkinson and Schubauer 1984). Therefore, by increasing cordgrass root productivity, lethal ladybeetles may have profound effects on both the structure and function of salt marsh

communities. Such effects may be particularly pronounced for cordgrass experiencing intermediate salinities where scale insects suppress cordgrass production (Long and Porturas 2014). Understanding the complex interactions between salt marsh predators, consumers, and primary producers may serve to inform restoration and management strategies. In southern California, where over 91% of natural salt marsh habitat has been lost or heavily degraded (Ferren 1990), restoration efforts frequently include transplanting cordgrass stems from preexisting sites to restoration sites. Such efforts have experienced variable success (Langis et al. 1991, Boyer and Zedler 1996, Zedler and Callaway 1999, Callaway and Zedler 2004). One factor impacting the success of restorations may be scale insect outbreaks (Boyer and Zedler 1996), as scale insects can reduce the productivity of cordgrass plants (Long and Porturas 2014). Therefore, identifying predators, such as ladybeetles, that can suppress scale insect populations and facilitate cordgrass growth may enhance marsh restoration success in southern California.

Current theory suggests that interactions involving active, BHDR predators and NHDR prey should result in TMIIs (Schmitz et al. 2004, Schmitz 2005). Although we did find NCEs of ladybeetles on scale insect population density, these NCEs did not manifest in TMIIs. Rather, we found that this interaction yielded strong DMIIs. The lack of a TMII may be due to the magnitude and timing of nonlethal ladybeetle effects compared to lethal ladybeetle effects. DMIIs may also be more common than predicted for these interactions because (1) behavioral anti-predator responses are energetically costly and (2) there is a disconnect between prey behavior/activity and population dynamics (Bouskila 2001, Schmitz et al. 2004, Schmitz 2005, Preisser et al. 2007). Overall, our findings suggest a need to further refine the predator hunting mode-habitat domain range framework to improve our predictions about when and where TMIIs are likely to be important.

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SUPPORTING INFORMATION