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Predator signaling of multiple prey on different trophic levels structures trophic cascades

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Abstract

The capacity of an apex predator to produce non-consumptive effects (NCEs) in multiple prey trophic levels can create considerable complexity in non-consumptive cascading interactions, but these effects are poorly studied. We examined such effects in a model food web where the apex predator (blue crabs) releases chemical cues in urine that affect both the intermediate consumer (mud crabs seek shelter) and the basal prey (oysters are induced to grow stronger shells). Shelter availability and predator presence were manipulated in a laboratory experiment to identify patterns in species interactions. Then, experimentally induced and uninduced oysters were planted across high- and low-quality habitats with varying levels of shelter availability and habitat heterogeneity to determine the consistency of these patterns in the field. Oyster shell thickening in response to blue crab chemical cues generally protected oysters from mud crab predation in both the lab and in field environments that differ in predation intensity, structural complexity, habitat heterogeneity and predator composition. However, NCEs on the intermediate predator (greater use of refugia) opposed the NCEs on oyster prey in the interior of oyster reefs while still providing survival advantages to basal prey on reef edges and bare substrates. Thus, the combined effects of changing movement patterns of intermediate predators and morphological defenses of basal prey create complex, but predictable, patterns of NCEs across landscapes and ecotones that vary in structural complexity. Generalist predators that feed on multiple trophic levels are ubiquitous, and their potential effects on NCEs propagating simultaneously to different trophic levels must be quantified to understand the role of NCEs in food webs.

Keywords: blue crabs; chemical ecology; inducible defenses; non-consumptive effects; oysters; risk cues

Introduction

Predators often have large effects on prey populations and community structure by directly consuming lower trophic levels (consumptive effects, CEs) or by inducing prey to alter traits such as their morphology, behavior, or physiology (non-consumptive effects, NCEs). Both CEs and NCEs can cascade through food webs as higher-level predators alter the density or characteristics of their (intermediate) prey, which in turn affects prey at even lower trophic levels. Classic ecological studies revealed the broad effects of CEs (Paine 1969, Carpenter and Kitchell 1993, Estes, Tinker et al. 1998) whereas more recent work suggests NCEs produce significant population and community level effects (reviews by Preisser, Bolnick et al. 2005, Weissburg, Smee et al. 2014). Yet, although NCEs can influence community structure (Peacor et al. 2012), disease prevalence (Finke 2012), and evolutionary processes (Dingemanse, Barber et al. 2020), context-dependency in how NCEs emerge has made it difficult to predict their occurrence or magnitude. The list of factors suggested to modulate how NCEs operate includes prey state (Matassa, Donelan et al. 2016), sensory mode and environmental effects on sensing (Weissburg, Smee et al. 2014), and predator hunting style (Schmitz, Krivan et al. 2004). Importantly, nearly all experimental approaches to examining NCEs in trophic webs proceed from the largely implicit assumption that apex predators affect only prey at the next lowest trophic level, even though predators commonly exploit prey at different trophic levels. Although studies on intraguild predation (e.g., Sitvarin and Rypstra 2014) and multiple predator effects (e.g., Davenport and Chalcraft 2013, Trussel et al. 2017) explore NCEs through the lens of competitive interference or as cascades that produce NCEs on basal trophic levels indirectly, rarely have ecologists examined NCEs that occur when a predator directly affecting multiple trophic levels (but see Zhang et al. 2021; Appendix S1: Figure S1). For instance, chemical cues (kairomones) from starfish predators alter shell morphology of intermediate consumers such as predatory gastropods but also induce shell thickening in bivalves (the basal resource) that constitute the prey of these gastropods (Freeman 2007, Gosnell, Spurgin et al. 2017). Reactions of prey at different trophic levels to the same predator species may be widespread because of the high abundance of generalist predators in ecosystems and the variety of taxa that contain such predator species.

Trophic cascades in which NCEs emerge as a result of a single predator signaling across multiple trophic levels (multi-level predator signaling) will depend on the interplay between how the apex predator affects each prey species, and may create strong context-dependency in the benefits of prey defenses. Mobile intermediate consumers frequently react to apex predator cues (chemical, auditory, visual, or tactile cues of predator presence) by reducing activity levels and food consumption (Lima and Dill 1990) or by moving away from predators into alternate habitats (Sih 1984). Although reduced feeding by intermediate consumers can benefit basal prey species (e.g., Griffen, Toscano et al. 2012), moving into alternate habitats may have negative consequences for co-inhabiting basal prey if such behavior increases encounter rates between the two species. In contrast, sessile basal prey often rely on morphological or chemical changes to deter predators such as the production of spines (Padilla and Savedo 2013) or toxins (Karban and Myers 1989). Such induced defenses are a widespread phenomenon seen across numerous plant and animal taxa (Tollrian and Harvell 1999). These responses to an apex predator should increase basal prey survival, but the extent to which basal prey NCEs also aid against intermediate consumers and how this response interacts with intermediate consumer NCEs from apex predators largely is unknown. Because many basal prey organisms are immobile and defend against consumers using plastic morphological or chemical defenses (Hay 1996), it seems likely that responding to predation risk for higher order consumers might also offer protection from intermediate consumers. For example, thrips consume less plant tissue when plants have increased their production of chemical defenses following herbivory by spider mites (Agrawal, Kobayashi et al. 1999). However, aside from insects (e.g. Agrawal et al. 1999) and plants (e.g. Hay 2009), field studies that directly examine the survival benefits of induced defenses are rare despite the wide number of animal, fungi, and protist basal species (but see Smee and Weissburg 2006, Belgrad et al. 2021), while even fewer studies link NCEs to natural field patterns (Peacor et al. 2022). Thus, theoretical models that may explore the effects from such NCEs frequently lack the data necessary to parameterize them (Larsen 2012).

Environmental factors that affect predator foraging success and perceived risk in response to predator cues likely also play a strong role in shaping the magnitude of survival benefits provided NCEs produced by apex predators that affect multiple prey trophic levels. In particular, habitat quality and structural complexity may have profound effects on predatory interactions by offering predation refuges or altering encounter rates between predators and prey. For instance, the shelter provided by structurally complex oyster reefs enables mud crabs to hide from toadfish better than in structurally simple reefs, and strengthens the overall predator avoidance response to reduce predation rates on oysters and clams (Grabowski 2004, Grabowski and Kimbro 2005). However, if higher order predators drive intermediate consumers into refuges with basal prey, then morphological defenses in basal prey induced by predator cues may no longer be efficacious even though they might be more necessary as a result of increased intermediate consumer density. The result would be non-uniform patterns of NCEs across space.

Here we tested how signaling of an apex predator on prey at multiple trophic levels affects basal prey survival across space and habitat structure, and produces context dependency in the efficacy of prey defenses. We use a tri-trophic system where the apex predator produces a behavioral NCE in the intermediate consumer and a morphological NCE in the basal prey. Specifically, we examined how basal prey survival depends on the predation risk imposed by intermediate prey and apex predator presence using both lab and field studies in a variety of habitats where physical structure determines the ability of intermediate prey to mitigate risk. Most research that examines trophic cascades within NCEs treat apex predators as if they only directly shape the next lower trophic level. These experiments demonstrate how an important, ubiquitous general apex predator directly shapes multiple trophic levels via the same cue, producing complex, but predictable context dependent changes in populations. Overall, this work elucidates the role of generalist predators in food webs and develops predictions on the strength of consumptive and non-consumptive effects in heterogenous environments.

Methods

The model system

The model system is a simple food web composed of blue crabs (*Callinectes sapidus*), mud crabs (*Panopeus herbstii*), and oysters (*Crassostrea virginica*). Blue crabs are the apex predator and consume the intermediate mud crab predator and compete with mud crabs for the basal resource, oysters (Hill and Weissburg 2013b). The intermediate predator, mud crabs, are small, cryptic xanthid crab predators that occupy the interstices of oyster beds at high densities and prey on a number of bivalve species, particularly newly settled oysters (Lee and Kneib 1994). Xanthid and other non-portunid crabs can make up approximately 43% of the diet of blue crabs which are voracious predators of mud crabs (Fitz and Wiegert 1991), particularly those outside refuge habitats deep within the reef structure (Belgrad and Griffen 2016). However, blue crabs can be effective oyster consumers, and bivalves can constitute up to 30% of a blue crab's diet (Fitz and Wiegert 1991). Blue crabs suppress foraging in several intermediate predators, including mud crabs (Grabowski 2004, Hill and Weissburg 2013a,b), and stimulate morphological changes in oysters (Robinson, Lunt et al. 2014). Urine from blue crabs contains dietary metabolites that are responsible for suppression of mud crab foraging (Weissburg, Poulin et al. 2016, Poulin, Lavoie et al. 2018), and is also the likely source of metabolites that increase the thickness and strength of oysters in their presence (Scherer, Lunt et al. 2016). Previous research also indicates that the potential response of oysters to mud crab cues is insignificant relative to blue crab cues (Robinson, Lunt et al. 2014). Therefore, the presence of blue crabs in the field is highly likely to cause oyster shells to be substantially stronger than they would be in their absence even with mud crabs in the vicinity.

Oysters, the basal prey, are ecological engineers whose shells form structurally complex reefs that serve as habitat for a variety of other sessile and mobile species (Lenihan, Peterson, et al. 2001). Oysters in the wild naturally experience both spatially and temporally diverse patterns of cue exposure due to strong variation in predator presence (Lenihan, Peterson, et al. 2001), variation in hydrology that transports cues (Weissburg, Smee et al. 2014), and the occurrence of multiple major and ongoing oyster spawn events throughout the late spring – early fall (Ingle 1951). This causes oysters to exhibit a wide range of shell strengths that is further magnified as not all oysters that are currently being exposed to an apex predator, and are in the midst of building defenses, will be consumed. In fact, differences in shell strength can vary 5x between individuals of the same brood within a site (Reustle and Smee 2020). Consequently, it is common for groups of oysters to coexist that have encountered different predator regimes and have developed different shell strengths while simultaneously being exposed to an apex predator that is currently shifting the foraging of an intermediate consumer (Reustle and Smee 2020). It is therefore relevant to understand both the degree of benefit provided by induction to blue crab chemical cues and how this benefit is affected by changes to mud crab behavior produced by these same cues.

Oyster culturing for induction

Oysters (*Crassostrea virginica*) were cultured as spat-on-shell at the Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, AL starting in late May 2019 using standard techniques (Congrove, Wesson et al. 2009). Oyster larvae were settled onto sun-bleached oyster shells to create spat-on-shell. After 3 days, when oyster spat were ~1.0 mm they were exposed to either urine from predatory blue crabs or empty cage controls in four flow-through holding tanks (length = 2.4 m, width = 0.9 m, water depth = 0.4 m) supplied with unfiltered seawater pumped directly from the Gulf of Mexico. The number of spat per shell varied from ~5 – 40 and we elected to not alter initial density to mimic natural settlement during the induction period. Oysters were suspended above the tank bottom in oyster aquaculture baskets (64 x 23 x 14 cm with 140 spat covered shells basket^{-1}) to prevent sediment buildup from suffocating oysters. Seven oyster baskets were present in each tank (28 total).

Spat were exposed to blue crab predator cues by holding four live caged adult blue crabs (*Callinectes sapidus*) in two of the tanks (8 crabs total), whereas the remaining two tanks contained empty cages (control) to mimic conditions where oysters regularly experience predator cues or are limited in their exposure from cues. Water volumes and crab densities were informed from established procedures (Belgrad et al. 2021) that produced shell strengths well within the range exhibited in the field (< 0.1 – 5.0 < N/mm; Lombardi, Chon et al. 2013, Reustle and Smee

2020; see Results, Appendix S1: Figure S2). Each crabs in each tank was held in a cage (32 x 23 x 14 cm) to prevent crabs from consuming the experimental oysters or each other. Every crab was fed one adult oyster daily (~5.0 cm length) to maximize predator cue intensity as experimental oysters would be exposed to urine from predators and metabolites from damaged conspecifics. This ensured that oysters were exposed to the most natural set of cues indicative of a predation event, and which produces a strong response in oysters (Scherer et al. 2016, Scherer, Garcia et al. 2017). Crabs were replaced during the experiment as needed due to mortality. Experimental oyster baskets were rotated around the crab cages daily to reduce differences in oyster growth due to proximity to predator cues, and no differences among cages were found. The induction period was 2 months, at which point the spat ranged 7.1 – 32.6 mm in diameter.

Shell morphology measurements

We sampled subsets of oysters to confirm that our predator cue treatments were causing control and induced oysters to exhibit different shell morphologies. Two shells were taken from every basket and three live spat were chosen from each shell for measuring spat shell characteristics after two months (number of individuals = 84 for each cue exposure treatment; 56 shells and 168 spat total). Spat shell properties were assessed by measuring shell size and shell crushing force (Robinson, Lunt et al. 2014, Scherer, Lunt et al. 2016). Oysters at these early life stages are roughly round, and we measured the shell length from the umbo to the outer shell edge to the nearest 0.01 mm using digital calipers. We took care to only measure individuals that were not bounded by cohorts to reduce any confounding effects on growth due to space limitation.

The force required to break each spat shell was quantified by a penetrometer attached to a charge amplifier (Kistler force sensor 9203 and Kistler charge amplifier 5995). The sensor probe was placed in the center of the shell, perpendicular to the shell surface. Gentle, consistent pressure was applied until the shell cracked, and the maximum force applied by the sensor to break the shell (N) was recorded. These methods are a standard proxy of shell hardness (Robinson, Lunt et al. 2014). We divided shell crushing force by shell length to produce a size-standardized metric of shell strength (i.e., standardized crushing force, N mm^{-1}) because larger individuals naturally have a stronger shell as a byproduct of their larger size (shell thickness).

We analyzed the effect of predator cue exposure (present vs absent) on spat shell length and crushing force using separate generalized linear mixed models (GLMMs; R package: glmmTMB; R Development Core Team, 2020) with a Gamma distribution and variable dispersion (dispersion = 0.7439 – 0.9318). One model was created for each of these two response variables. Cue exposure treatment was treated as fixed effects whereas the shell individual spat settled on, nested in basket, nested in tank were treated as random effects (Bolker, Brooks et al. 2009). All interactions initially were included in every model here and below when possible, and nonsignificant interactions were removed stepwise, from the most complex interaction terms to the simplest, following the protocol of Crawley (Crawley 2013) to help resolve the significance of main effects and achieve the lowest Akaike information criterion (AIC) value. Assumptions of GLMMs were validated using the R package DHARMa (Hartig 2022).

Mesocosm survival experiment

After the 2-month spat grow out period, we conducted a mesocosm experiment to determine how induced predator defenses (i.e., changes in shell strength) altered oyster survival under different ecological contexts of habitat shelter (within reef shelter vs outside reef) and predator regime (apex predator present vs absent). Spat-covered shells from the above experiment were scraped so that each shell contained either four induced oysters grown with predator cues or four control oysters grown without predator cues to standardize individual predation risk. Eight shells from each of these two treatments were placed into six circular flow-through seawater tanks (diameter = 1.15 m, water height = 40 cm; 16 shells tank^{-1}; 64 spat tank^{-1}).

An artificial reef was located in the center of each tank, and four shells containing spat of each type were placed in each of the "reefs" that provided a refuge for oyster consumers (mud crabs, *Panopeus herbstii*) and an additional four shells containing oysters of each type were placed roughly 15 cm from tank wall. Artificial reef shelters were composed of a plastic basket (30 cm length x 20 cm width x 11 cm height) turned upside down and covered in sun-bleached oyster shells that were epoxied to envelop the basket outer edges. This roughly mimics the effect of a robust healthy reef with 3-dimensional structure where mud crabs can occupy interstitial spaces difficult for blue crabs to access. Each tank also contained seven mature mud crabs (mean ± SD carapace width = 2.52 ± 0.43 cm) to serve as intermediate consumers, with these densities being consistent with field measurements (Hill and Weissburg 2013a,b). Every mud crab cohort contained at least two individuals of each sex to match natural fine-scale sex ratios.

Apex predators (blue crabs) were added to half of the tanks whereas the remaining three tanks lacked blue crabs. Tanks with the predator treatment contained a single adult blue crab (mean ± SD carapace width = 14.8 ± 1.4 cm), which at this size commonly feeds on mud crabs but rarely upon oyster spat (Hines 2007, personal observations of all authors). As this experiment was focused on identifying how predator cues cause cascading effects through food webs, all blue crabs had their claws taped closed throughout the duration of the experiment so that blue crabs could provide chemical, visual, and mechanical predation risk cues without actually consuming the mud crabs. Blue crabs were fed a diet consisting of a single mud crab every day for a week prior to the start of the experiment to help ensure that blue crabs would produce urine containing metabolites mud crabs perceive as risk cues. Diet was standardized since cue perceptibility is affected by the amount of prey biomass consumed by a predator (Weissburg and Beauvais 2015). Every blue crab was replaced with a new, recently fed blue crab each day to ensure that the apex predator would continue to release chemical cues. Preliminary experiments found that blue crabs did not consume spat under these conditions.

The experiment began by allowing the blue crabs and oysters to acclimate in the tank for 30 min. Mud crabs were released in the tank center near the artificial reef after the acclimation period whereupon they immediately began traveling in all directions. Oyster survival was recorded after 48 hours. This experiment commenced July 30th 2019 and was repeated two additional times within that same week (9 replicate tanks distributed across 3 blocks). No individuals were used more than once (n = 1,152 spat, n = 126 mud crabs, n = 18 blue crabs total).

We examined the effects of oyster induction treatment (induced or control), oyster habitat location (sheltered in refuge or exposed) and apex predator status (present or absent) on oyster survival (proportion alive for each treatment within a tank, n = 16 spat) using a GLMM with a binomial distribution as the data was proportional. The final model set oyster induction treatment (predator, no predator), habitat location (on reef, exposed), predator status (apex predator, no predator) and their interaction as fixed effects. Shelter grouping nested within holding tank, nested in trial served as random effects. Non-significant terms were removed step-wise as described above for shell morphology assessment (See Appendix S1: Table S1; dispersion = 0.9876). Since our primary goal was to examine the effect of changes in shell morphology, we used a Wilcoxon Signed Rank test with Bonferroni corrections to examine differences between induced and non-induced shells. We computed the difference in survivorship of the two shell treatments for all spat in refuge and exposed locations for each tank, and tested this difference for the two locations in trials with and without apex predators.

As an additional analysis of inducement efficacy, risk ratios between induced and uninduced oysters were calculated ((consumed induced/total induced)/(consumed control/total control)) for each habitat location and predator status treatment. We compared risk ratios across shelter and predator presence using a GLMM with a Tweedie distribution. Tank nested in trial were treated as random effects (R package: glmmTMB; see Appendix S1).

Field survival experiment on high quality reef

We conducted a field experiment on a healthy intertidal oyster reef to test whether our laboratory findings were consistent with effects in the field. We cultured another batch of oysters in late July 2020 with and without predator cues for one month. Spat were settled onto 4.5 x 4.5 cm marble tiles, but the procedure was otherwise as described for the previous laboratory experiment. Six spat-covered tiles were taken from both cue exposure treatments after one month of culturing to confirm that the predator cues induced morphological changes in the oysters (size range = 8.2 – 15.7 mm). Five spat from each tile had their shell characteristics measured and statistically analyzed using the same methods as described for the mesocosm experiment (n = 30 spat per treatment).

Oysters were scraped to 10 spat per tile to standardize individual predation risk, and transported in aerated coolers filled with seawater to a large (>1 km long x ~ 10 m wide x ~ 1m high) healthy oyster reef at Skidaway Island, GA (31°57'52.2"N; 81°00'49.4"W) on August 11th 2020. This reef is a contiguous set of ~10-100 m live oyster patches separated by regions of mud and shell hash containing smaller oyster clusters. The site is located along the Wilmington River in upper Wassaw Sound, adjacent to the Skidaway Institute of Oceanography and bordered by a robust *Spartina alterniflora* zone. Mud crab densities within the oyster reef are roughly 30 m^{-2} and resident mud crabs experience heavy predation by blue crabs particularly in areas of bare substrate and shell hash (Hill and Weissburg 2013a,b).

Sixty pairs of induced and control spat covered tiles were zip tied to 60 pieces of 1.5 m long rebar. Rebar were set in 15 transects that ran perpendicular to the shoreline with over 5 m separation between each transect. Each transect contained 4 rebar poles planted in the following locations: the upper tidal zone of the reef, the lower tidal zone of the reef, the reef edge, and in the mud with at least 1 m distance from the reef. Poles typically were separated from each other by 1.5 m (total transect length = 7 – 8 m), and were planted so that the spat tiles rested just above the substrate. Individual survival of all oysters was checked 48 hrs after planting (n = 1,200 spat total; 150 spat $treatment^{-1}$).

Field survival experiment on low quality reef

We performed another field experiment on degraded, low quality oyster reefs on Dauphin Island, AL to determine if the ecological relationships produced from induced defenses remain consistent across habitat quality. Oyster spat taken from the first culturing period (also used in mesocosm experiment) were planted on three small (5 - 10 m long x 3 m wide) oyster reefs in early September 2019. Oyster reefs were within 50 m of each other and composed of dead shell hash on a bed of sand with no mud crabs found among the hash (30°16'42.6"N; 88°07'14.7"W). No live oyster clusters were found within at least 500 m of the site. Spat covered shells were scraped to 10 individuals per shell as before. Forty-four pairs of induced and control shells were zip tied to poles and set in 11 transects total (3 – 5 transects per reef depending on its size). Transects contained four poles, with 1 m separation between each pole, and maintained the same relative reef locations as the previous field experiment (upper reef, lower reef, reef edge, and bare substrate). Individual survival of all oysters was checked 48 hrs after planting (n = 880 spat total; 110 spat/treatment).

The effect of oyster induction treatment and oyster habitat location on spat survival (proportion alive on each shell or tile, n = 10 spat) was analyzed using a GLMM with a binomial distribution, separately for each reef type (R package: lme4). As before, the full model contained main effects of induction, habitat and their interactions treated as fixed effects and pole nested within transect nested within reef as random effects, with non-significant terms excluded in the final model (Appendix S1: Table S1; dispersion = 0.9655 - 1.0005). We used pairwise tests to compare survivorship of induced vs. non-induced spat at each location, with a Wilcoxon Signed Rank test with Bonferroni corrections. We calculated the difference in survivorship between induced on non-induced spat on the paired tiles of each pole at a given location.

Similarly, risk ratios were calculated between induced and control spat covered shells and analyzed using separate GLMMs with Gamma distributions for high- and low-quality reefs. Habitat location was treated as a fixed effect while transect nested within reef served as random effects (see Appendix S1).

Results

Oyster spat induction

Growing oyster spat in the presence of risk cue from blue crabs significantly increased spat shell strength by roughly 67%, with a standardized crushing force (force divided by shell size) of approximately 2.5 N/mm in spat exposed to blue crabs compared to 1.5 N/mm in spat reared without crabs (Appendix S1: Figure S2A; $z = 4.64$, $P < 0.001$, $N = 84$ per treatment).

Overall spat size was similar in blue crab and control treatments spat (Appendix S1: Figure S2C; z = -1.28, P > 0.05, N = 84 per treatment). Similar results were obtained for the oyster spat reared for the high-quality reef field experiments; normalized crushing force of spat induced with blue crab cues exceeded that of spat reared without blue crabs by almost 1 N/mm and there was no difference in size between treatments (Appendix S1: Figure S2B,D).

Survival of induced and non-induced spat in mesocosm with refugia and apex predators

Cue exposure and location of spat both influenced oyster spat survival, and apex predator presence and location interacted to influence the survival of spat to create additional complexity (see below). The main effects of induction (induced vs control) and the location of spat (sheltered in refuge vs exposed) both produced significant increases in spat survival (Figure 1, Appendix S1: Figure S3; Table S1). Induced oysters survived better in all treatments. On average the risk ratio of predation of induced vs control was 0.82, indicating induced spat had an 18% survival advantage, although the difference was not always dramatic.

The effect of location (sheltered in refuge or exposed) was robust. The survival of spat exposed outside refuge was 2.5x higher than that of sheltered spat, on average. Although the main effects of the predator treatment were not significant, there was a tendency for spat to survive better in the presence of the apex predator, presumably because chemical (and possibly other) cues suppressed mud crab foraging for oysters. The significant interaction between apex predator presence and spat location (see below) also obscures the predator effect.

Apex predator presence and spat location interacted significantly to affect survivorship (Figure 1; Table 1). In the presence of an apex predator, exposed spat on average had 238% higher survival probability than spat in the refuge and there was no significant effect of induction treatment. Without the apex predator, exposed oysters on average only had 43% higher survival probability than spat in the refuge, but survivorship differed as a function of induction treatment. As noted, the induction treatment was particularly efficacious in improving spat survivorship (relative to controls) for exposed spat without apex predators.

Survival of induced spat in high- and low-quality reefs

Oyster spat survival on high quality oyster reefs ranged from 40-80%, and following removal of non-significant terms (Appendix S1: Table S2), was a function of location and induction treatment (Figures 2a and Appendix S1: Figure S4a), with a significant interaction (Table 2). Generally, spat survival increased with distance away from the reef center. In parallel with the mesocosm data, spat reared with blue crab chemical cues survived better in all locations except for the reef center, which is a mud crab refuge. On average, the risk ratio of predation of induced vs control was 0.66, indicating induced spat had a 34% higher survival probability, but the survival advantage was nonexistent in the reef center and increased to roughly 27-58% at the other locations (Appendix S1: Figure S4a).

Patterns of survival in low quality habitats (Figure 2b) were quantitatively different from that of healthy reefs, with additional qualitative differences in location effects and of inducement. Mean oyster spat survival in the low-quality reef habitat was roughly half that in the higher quality habitat. In these low-quality reefs, only induction treatment (Table 2) had significant effects on spat survival. In contrast with patterns on high quality reefs, neither location nor the induction*location interaction had significant effects on survivorship. Induced spat had a modest, but consistent survival advantage at every location that was, on average, 8% greater than control spat (risk ratio = 0.92; Appendix S1: Figure S4b). The lack of a location effect differs from the pattern seen on high quality reefs where locations away from the reef center conferred greater survival and enhanced the positive effect of induction.

Discussion

Nearly all studies of cascading NCEs implicitly assume that effects are propagated by species adjacent to each other in the trophic chain; that is, apex predators affect intermediate predators that affect basal resources. Perhaps this view is a legacy from the perspective provoked by the initial discovery of trophic cascades mediated by direct density dependent interactions, but it ignores the fact that generalist predators may directly influence traits of organisms at multiple trophic levels below them. Thus, NCEs may propagate through multiple pathways simultaneously and flow directly from apex predator to basal prey in addition to affecting the intermediate consumer. Our results demonstrate that apex predators can, in addition to increasing the survival of basal prey through altering intermediate consumers behavior and distribution, also generate survival benefits for basal prey by another mechanism: directly causing basal prey to change to be less frequently consumed. Yet, these effects can be context dependent as a function of habitat quality that alters interactions among predators and prey. Our results indicate distinct survivorship and NCE patterns in heterogenous habitats of varying quality or structural complexity that affect the behavior of the intermediate consumer.

Induced defenses operate in field conditions to increase survival generally.

Chemical cues induce a host of potential changes to morphology of prey that may confer survival benefits. This is true for a large variety of different taxa including crustaceans (Dodson and Havel 1988), mollusks (Scherer, Lunt et al. 2016, Gosnell, Spurgin et al. 2017), insects (Benard 2004), and amphibians (Relyea 2004, Hagman, Hayes et al. 2009). Geographic differences in prey properties have been linked to predator presence, suggesting these morphological changes provide enough benefits to structure entire populations (Hay 2009). However, direct field tests of the benefit of predator-induced morphological changes are rare and primarily limited to vegetation (e.g. Hay 2009) or insects (e.g. Agrawal et al. 1999), and are nearly nonexistent in mollusks or across different levels of natural habitat complexity (but see Smee and Weissburg 2006, Belgrad, Combs et al. 2021).

Our findings reveal that morphological changes in oysters produced after exposure to an apex predator, blue crabs, significantly increased oyster survivorship in both mesocosms and a variety of field habitats (Figures 1 and 2). Presumably, differences in handling time or ability to break the shell results in either a preference of intermediate predators to non-induced forms, or the greater shell strength of induced spat decreases predation success. In both primary field sites, induced oysters survived significantly better than oysters reared without induction, suggesting that the morphological defense is robust and effective in habitats that differ in structural complexity and potential access to refuges.

The consistent positive effect of inducement occurred not only in areas of differing structure, but also with different predator assemblages. Mud crabs were the most abundant oyster predators in the high-quality reef and were the only oyster predators encountered within the reef matrix when deploying the experiment. In contrast, the low-quality habitats contained an entirely different assemblage of dominant intermediate consumers (oyster drills instead of mud crabs). Here, no mud crabs were found when deploying the experiment and both oyster drills and adult blue crabs were observed consuming our experimental oysters in the region (Belgrad, Combs et al. 2021).

Survival benefits of induced defenses are context dependent and affected by intermediate consumers risk perception and habitat characteristics

The benefit conferred by induction in these different contexts asserts that induced defenses can be effective across a wide range of community types that differ in both biological and physical contexts affecting NCEs. Induction treatment had a significant effect in mesocosms and both field habitats, conferring a general increase in survival across most conditions, but also showing considerable context dependence. Survival benefits in the mesocosm occurred in all conditions but with a range of relative benefits depending upon the presence of an apex predator and whether oysters were located in refuge habitats (Figure 1). On healthy natural reefs, there was an interaction of cue with location; the induction treatment increased oyster survival except in reef interior where the survival of induced vs. non-induced spat was equal. Generally, top predator mobility and ability to access different habitats can affect the expression of trophic cascades across ecotones (Grabowski 2004, Byers, Holmes et al. 2017).

The strong spatial variation in oyster spat survival in high quality reefs contrasts with the consistently modest and spatially uniform survival benefit of inducement in the low-quality reef habitat representative of degraded, disturbed habitats with low structural complexity (Table 2; Figures 2 and Appendix S1: Figure S4). The results on these different reef types clearly show induction provided a survival advantage across a large range of predation intensities; the predation intensity on oysters in the healthy reef was less than half that on the degraded reef. On healthy reefs, oyster spat had a survival probability roughly 230% greater than those of spat in the degraded reefs. Interestingly, studies of plant chemical defenses suggest survival benefits of induced defenses disappear when predation rate is high (Baldwin 1998), but our results provide a counter example where defenses continued to provide survival benefits and indicate the general conditions where induced defenses are beneficial are not entirely clear.

The context dependence of the survival benefit of induction appears related to habitat structural complexity that affects the behavior of the intermediate consumer and the apex predator, as evidenced by how closely oyster survivorship patterns at the healthy, high heterogeneity field site matched patterns observed during mesocosm trials. High complexity reef matrix protects mud crabs from blue crab predation in both mesocosm and field experiments, where predation rates on mud crabs in shell hash and bare substrate are 2-5 higher than in reef habitats (Hill and Weissburg 2013a). Apex predators may deprive basal prey (oysters) of induction benefits by modifying the distribution of intermediate consumers (e.g., mud crabs) to seek less risky habitats of the reef interior, which is not a favored foraging area for blue crabs. In the presence of apex predators, the survival of induced spat within the artificial refuges of mesocosms was low and similar to control spat, indicating these defenses were overwhelmed by increased local predation pressure and lack of alternative foraging options. Additionally, induced spat were not protected in the reef interior of the high-quality reef, although they were in all other locations. Thus, there was strong spatial variability in the efficaciousness of induced defenses across this heterogenous habitat (Appendix S1: Figure S4a).

In contrast, the degraded reef appears to lack sufficient structural complexity to provide a refuge for oyster spat predators (or oyster predators elect to not use potential refuge sites), thus there is no interaction of location and other variables as in the mesocosm experiment and the experiment on healthy reefs. Structural complexity is well known as a major driving force behind the foraging success of a variety of predators including fish, crabs, and snails where the absence of such structure leads to high predation rates (Pennings 1990, Warfe and Barmuta 2004, Toscano and Griffen 2013). Notably, induction conferred strong survival benefits in structurally homogeneous, open regions of healthy reefs and regions that were degraded. In this manner, induced defenses may be an effective aid for populations to colonize new territory or replenish degraded habitats. Although there is a wealth of literature on plant chemical defenses preventing other species from colonizing regions and allelopathy is well known to improve invasion success (e.g., Bais, Vepachedu et al. 2003), little is known on how induced defenses from predators may improve prey establishment success, particularly for animals.

Differences in oyster survivorship patterns between the low quality and high-quality sites are unlikely to be a result of abiotic or pathogenic differences due to the extremely high tolerance oysters have for changing abiotic conditions and the short time scale of the experiments. Oyster survivorship differences across reef type are also unlikely to be due to differences among oysters across annual spawning batch or settlement substrate (tile vs shell). Oysters used in the low-quality reefs and mesocosm experiments were from the same batch, but exhibited dissimilar survivorship patterns. Oysters from a different batch but placed in a high-quality habitat displayed similar survivorship patterns as to the mesocosm experiment, suggesting that differences in reef structure and not batch accounted for observed differences in oyster survivorship. Degraded reefs frequently are associated with different animal communities and lower prey densities (Rodney and Paynter 2006) that are likely partially responsible for the low overall survival of oysters as predators would home in on any oysters available. Yet, the relatively consistent survivorship of induced and control oysters across all stakes and locations (Figures 2b and Appendix S1: Figure S4b) suggests the homogenous character of low-quality reefs with its surrounding habitat also plays a strong role in governing these patterns.

Predator multi-trophic level signaling produces complex effects

Positive effects of NCEs on basal prey often result from trophic cascades when apex predators reduce foraging behavior or alter the distribution of intermediate consumers (Preisser, Bolnick et al. 2005, Weissburg, Smee et al. 2014). Our results are consistent with these findings as blue crabs changed mud crab foraging and led to increased oyster survival in mesocosm experiments, and oyster survivorship in the field matched these foraging patterns. However, NCEs on intermediate predators did not extend into refuge habitats as noted in earlier work (Grabowski 2004, Grabowski and Kimbro 2005), as mud crabs continued to consume oysters in refuge habitats in both the lab and field. Here, a different NCE mechanism provided a benefit to basal prey; exposure to the apex predator (blue crab) caused a morphological change in the basal prey (oysters), which in turn improved oyster survival in both the lab and field.

One likely explanation for the higher predation we observed in reef structure than in previous studies (Grabowski 2004, Grabowski and Kimbro 2005) is differences in hunting mode of the apex predator, which has strong effects on behaviorally mediated NCEs (Schmitz et al. 2004). The apex predator commonly used in previous research on trophic interactions in this system is the toadfish, *Opsanus sp.,* which hunt by ambushing crabs and preferentially consume individuals deep within the reef. In contrast. the blue crabs used in this study are active foragers that preferentially consume individuals outside the reef and cause mud crabs to spend longer periods within shelter than toadfish exposure (Belgrad and Griffen 2016). Blue crabs therefore likely limit mud crabs foraging options to within reefs where individuals were free from predation. Further, Grabowski and Kimbro (2005) used hard clams *Mercenaria mercenaria* as their basal prey. Hard clams have behavioral response to crab predators that may have also limited their susceptibility to predation in refuge habitats when crabs are present (Smee and Weissburg 2006).

Recent multipredator work in this system also indicates that greater habitat complexity can increase oyster consumption when prey are avoiding predators in a region, and demonstrates that the strength of these trophic cascades depends on the apex predators involved (Schweiss and Rakocinksi 2020). Additionally, unlike earlier studies in this system, our experiments provided intermediate consumers a choice on where to consume basal prey and quantified spatial differences in consumption rates rather than centering all prey in the system within the same type of habitat, effectively exploring how edge effects and patchy heterogenous habitat structure these trophic interactions.

Blue crabs produce chemical cues that induce responses of both intermediate predators and basal prey. Mud crabs respond to blue crab chemical cues contained in urine to avoid risk (Weissburg, Poulin et al. 2016, Poulin, Lavoie et al. 2018). Oysters also respond to chemical cues in blue crab urine (possibly the same molecules) by creating more predation resistant shells (Robinson, Lunt et al. 2014, Scherer, Lunt et al. 2016). Thus, chemical cues from apex predators affect both of the other elements in this cascade, increasing the complexity of interactions in ways not captured under the assumption that cascades only operate via sequential pairwise interactions.

In general, the predator avoidance responses of both the intermediate consumer and the basal prey enhances NCEs to decrease predation pressure on the basal prey and stabilize what is a trophic cascade with intra-guild predation. This happens in some contexts, such as reef edges and open areas where mud crabs or other intermediate consumers reduce foraging effort and induced spat have a clear survival advantage (Figures 1 and 2). However, the behavioral effects observed on mud crabs tends to oppose the morphological effect on oysters in sheltered habitats. Here, the interplay between environmental context and responses of both the intermediate predator and basal prey determines the final effect on basal prey (oyster) survival. Much effort over the decades has been focused on characterizing trophic cascades and NCEs in increasingly reticulated food webs (e.g. Schmitz 1998, Sitvarin and Rypstra 2014, Trussell et al 2017, Zhang et al. 2021). However, such studies generally examine these interactions under homogenous habitat settings, which may prevent many of the trophic interactions that are likely typical of reticulated food webs.

Our results suggest signaling by generalist apex predators across multiple trophic levels can produce complex, but predictable trophic interactions across different quality habitats as summarized in Figure 3. In low quality – homogenous habitat, omnivorous or generalist apex predators (blue crabs) have a large direct effect on both intermediate consumer (mud crab) and basal prey (oyster) populations due to the lack of refuge within the habitat. Intermediate consumers also have a modest direct effect on basal prey because of the diminished presence of refugia, but this is tempered by apex predators also feeding heavily on existing intermediate consumers. Both apex predators and intermediate consumers produce moderate trait effects in basal prey populations by inducing prey to build defenses, thereby creating refuge from predation in a habitat that provides little shelter itself. This pattern is repeated across the habitat as patches are homogenous and transition to new patches does not change any predation risk. Thus, basal prey in low quality habitats have low survivorship consistently throughout the habitat as well as low variability in the trait effect. However, this trait effect substantially increases survivorship, helping prey gain a foothold in this area.

In high quality – heterogenous habitat, apex predators have modest direct effects on both intermediate consumer and basal prey populations. This consumption is modest and primarily focused on intermediate consumers due to the high shelter availability and overall abundance of both prey species. Apex predators produce large trait effects on intermediate consumer populations as high-quality habitat has high shelter availability for intermediate consumers to utilize. This also causes intermediate consumers to have high direct consumptive effects on basal prey within sheltered patches. Consequently, the trait effects on basal prey from intermediate consumers and apex predators is small in sheltered patches because intermediate consumers are in high concentration here and find it less costly to expend energy overcoming basal prey defenses than exposing themselves to apex predators consuming undefended prey in unsheltered patches. Trophic patterns of unsheltered patches in high quality habitat are the inverse of those in sheltered patches and similar to the trophic patterns as those in low quality habitat. Importantly, the magnitude of the effects in unsheltered patches among low- versus high-quality habitat do not match completely because individuals in high quality habitat also can utilize sheltered habitat whereas individuals in low quality regions have no such option. Of note, direct consumption of basal prey in unsheltered habitat in high quality regions is less than in low quality habitat because intermediate consumers predominantly utilize sheltered patches whereas apex predators have more intermediate consumers to consume and also drive intermediate consumers to shelter. Basal prey continue to have greater trait effects in unsheltered than sheltered regions, which can help prey expand the area of sheltered regions. Therefore, high quality regions are characterized with high prey survival and relatively high trait effects on basal prey populations, but strong spatial variability in both survival and trait effects that are associated with shelter availability or habitat complexity. This multi-trophic level signaling also has consequences for experimental analysis of NCEs. For trophic chains that operate similarly to the system examined here, not considering the additional, non-sequential NCE of apex predators on basal prey underestimates the spatial gradient in NCEs across ecotones.

The potential for complex NCEs produced by predator signals not flowing sequentially from apex predator to basal prey are not limited to marine systems, or even to animal-to-animal interactions. For example, complex interactions have been observed in the tri-trophic system with omnivorous thrips that consume spider mites and cotton plants. Here, the interactions were initiated by the intermediate consumer as plants exposed to spider mite feeding would change their chemical composition and cause thrips (the apex predator) to eat less cotton plants and consume more mites instead (Agrawal, Kobayashi et al. 1999). Recent work in crop systems indicates that such NCEs may be useful in insect pest control in several insect-plant systems (Zhang et al. 2021). Importantly, unlike the traditional perspective where NCEs typically cascade from apex predators indirectly to basal prey, (e.g., Hill and Weissburg 2013b, Trussel et al. 2017), NCEs in generalist predator systems also can propagate from apex predator to basal trophic levels even in the absence of intermediate trophic levels. Such NCEs likely have profound effects on the recruitment, distribution, and persistence of lower trophic level species. NCEs from the same predator affecting multiple trophic levels directly are likely to be common in natural systems given the number of insects, crustaceans, fish, reptiles, birds, and mammals that are generalist predators. However, how widespread generalist predator (omnivore)-induced responses are, and their effects on other species, remains a major knowledge gap (Zhang et al. 2021).

The complex effects seen in this study are contingent upon mobile intermediate predators, mud crabs, altering patterns of habitat use in response to the apex predator. However, intermediate consumers, although mobile, may not always respond to apex predator cues with altered habitat or foraging preferences. Sea stars induce morphological changes in intermediate gastropod consumers that feed upon mussels (Gosnell and Gaines 2012), which also respond to the apex predator cues (Reimer and Tedengren 1996, Reimer and Tedengren 1997). Here, the intermediate consumer's response to the apex predator has little consequence for its ability to consume mussels, and so the effect of apex predator cues in the basal resource should be uniform as opposed to spatially heterogenous, all else being equal. Alternatively, if both the intermediate consumer and basal prey are mobile, one may find that NCEs caused by intermediate consumers on basal prey can contradict NCEs caused by apex predators if basal prey respond by moving away from the most immediate threat. Signaling by generalist predators to prey across multiple trophic levels therefore may create additional context dependence in the propagation of NCEs that will need to be considered. Just as understanding how a single prey species responds to multiple predators promoted a fuller understanding of direct density dependent effects, a full accounting of NCEs in communities will require understanding the effects of a single predator on multiple prey species in a food web.

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Table 1: Generalized linear mixed model of predator cue exposure, shelter availability, and apex predator presence on spat survival.

Source of variation	Estimate	z	*p*
Cue exposure	0.70	4.25	*<0.0001*
Shelter availability	4.81	9.12	*<0.0001*
Predator presence	0.28	0.58	0.5637
Predator x shelter	4.36	6.58	*<0.0001*
Cue x predator	-0.38	-.1.14	0.2510
Cue x shelter	0-0.68	-1.87	0.0611
Cue x predator x shelter	0.95	0.89	0.3713

Table 2: Generalized linear mixed models of predator cue exposure and location on spat survival within high- and low-quality reefs.

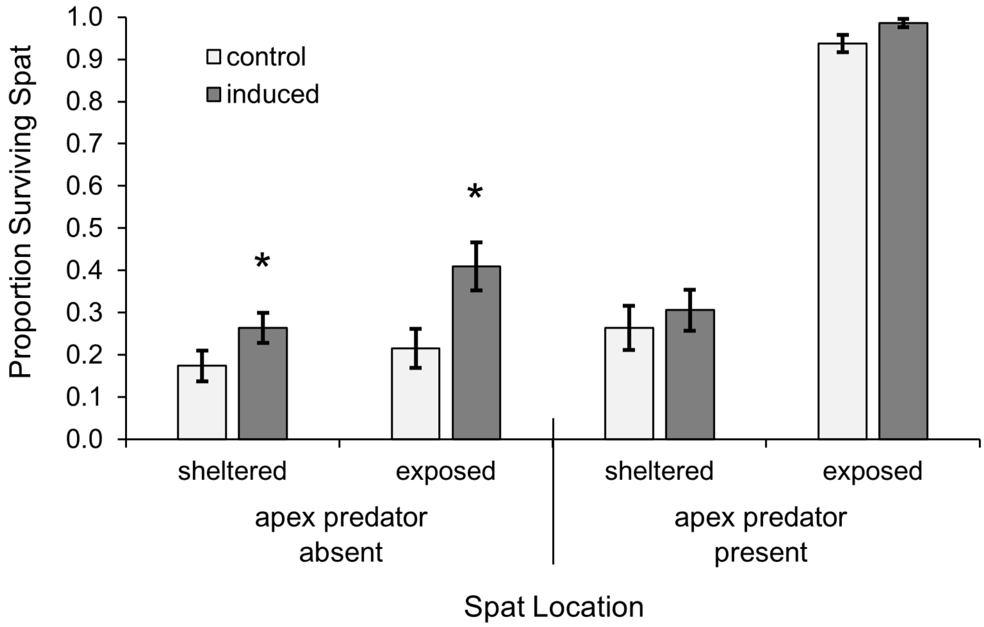
	High-quality reefs			Low-quality reefs		
Source of variation	Estimate	z	*p*	Estimate	z	*p*
Cue exposure	1.15	4.02	*<0.0001*	0.61	3.26	*0.0011*
Location	2.05	4.77	*<0.0001*	0.18	0.54	0.5874
Cue x location	1.06	2.80	*0.0050*	0.22	0.42	0.6776

Figure Legends

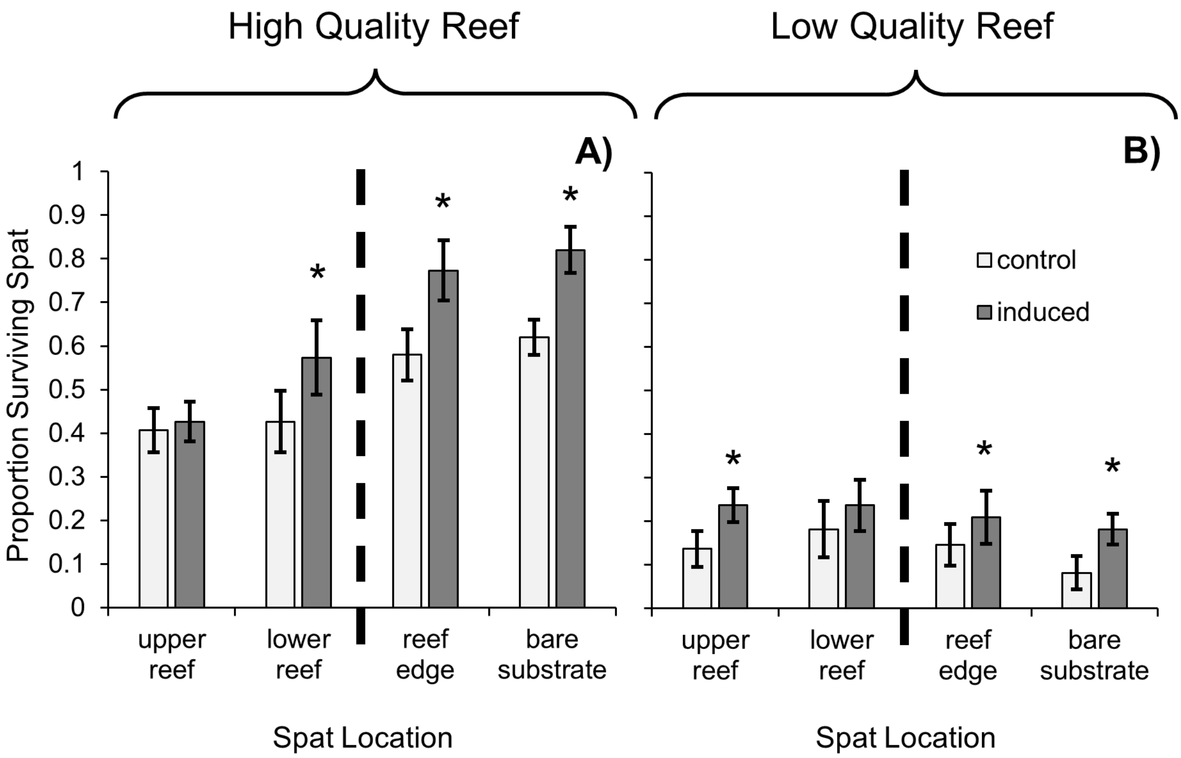
Figure 1: Mean ± SE proportion of surviving spat after 48 hrs in the mesocosm with predatory mud crabs (n = 9 replicates; 16 spat per treatment replicate). Spat were cultured either with predator cues (induced) or without predator cues (control) prior to their placement within (sheltered) and outside (exposed) artificial reef structures. Mesocosms contained either an adult blue crab to serve as an apex predator or no blue crab. Asterisks denote significant differences ($p < 0.05$) of pairwise comparisons across predator cue exposure treatments (induced vs control) using a Wilcoxan Sign Rank test.

Figure 2: Mean ± SE proportion of surviving spat after 48 hrs in the field on a **A)** high-quality and **B)** low-quality oyster reef (n = 15 and 11 replicates respectively; 10 spat per treatment replicate). Spat were cultured either with predator cues (induced) or without predator cues (control) prior to their placement on different regions of the reef habitat. Dashed lines delineate the reef border and denotes separation between oysters planted within the reef versus those planted outside. Asterisks denote significant differences from pairwise comparisons across predator cue exposure treatments using a Wilcoxan Sign Rank test.

Figure 3: Diagram of consumptive direct effects (black arrows) and non-consumptive trait effects (grey arrows) within model oyster reef communities across habitat quality depicting interactions when the apex predator causes a behavioral NCE in intermediate consumers and a morphological NCE in basal prey. Arrow thickness denotes relative size of the direct and trait effects.



ECY_4050_Figure 1.tif



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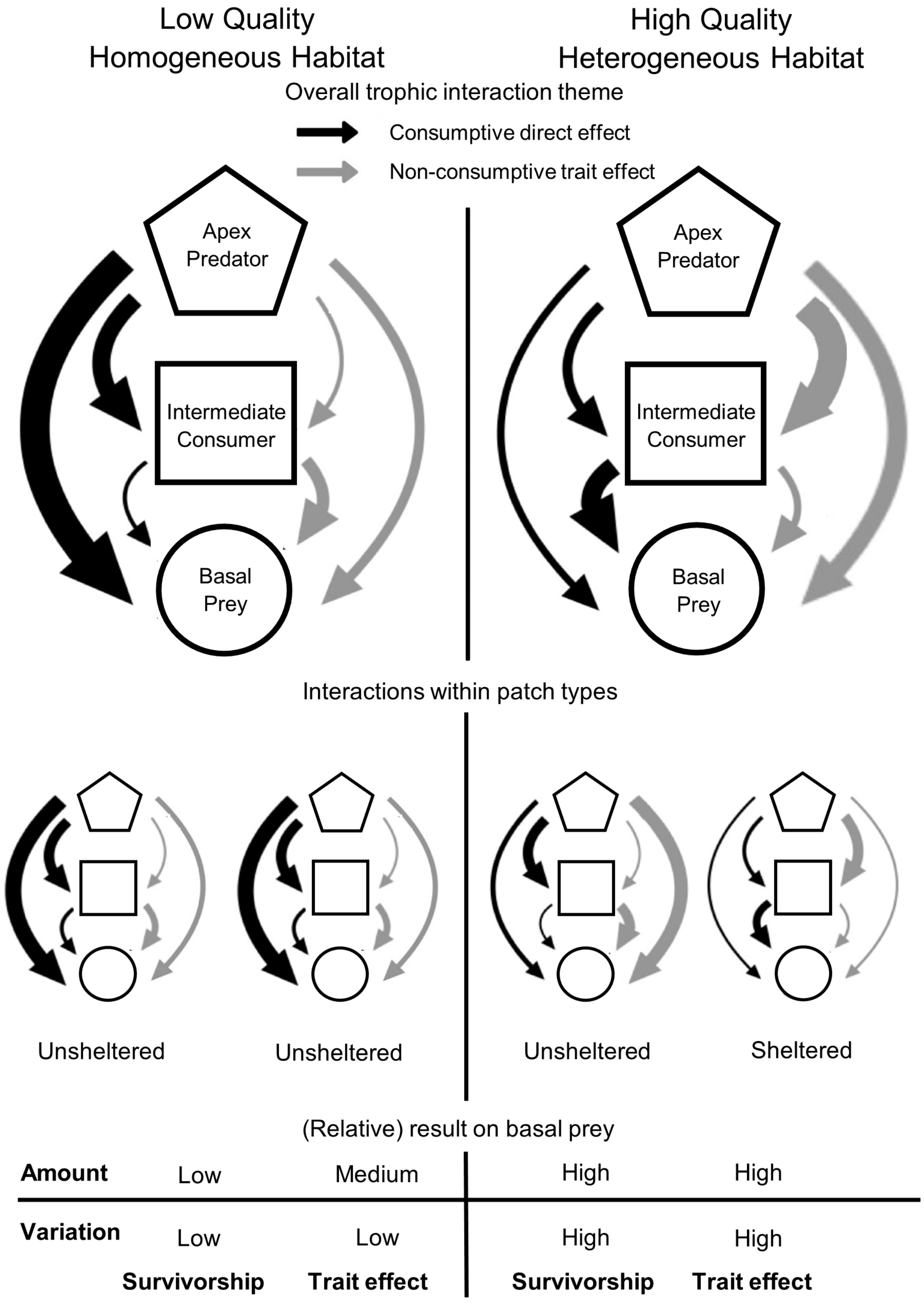

ECY_4050_Figure 3.tif