

Three-dimensional interstitial space mediates predator foraging success in different spatial arrangements

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Abstract. Identifying and quantifying the relevant properties of habitat structure that mediate predator–prey interactions remains a persistent challenge. Most previous studies investigate effects of structural density on trophic interactions and typically quantify refuge quality using one or two-dimensional metrics. Few consider spatial arrangement of components (i.e., orientation and shape) and often neglect to measure the total three-dimensional (3D) space available as refuge. This study tests whether the three-dimensionality of interstitial space, an attribute produced by the spatial arrangement of oyster (*Crassostrea virginica*) shells, impacts the foraging success of nektonic predators (primary blue crab, *Callinectes sapidus*) on mud crab prey (*Eurypanopeus depressus*) in field and mesocosm experiments. Interstices of 3D-printed shell mimics were manipulated by changing either their orientation (angle) or internal shape (crevice or channel). In both field and mesocosm experiments, under conditions of constant structural density, predator foraging success was influenced by 3D aspects of interstitial space. Proportional survivorship of tethered mud crabs differed significantly as 3D interstitial space varied by orientation, displaying decreasing prey survivorship as angle of orientation increased ($0^\circ = 0.76$, $22.5^\circ = 0.13$, $45^\circ = 0.0$). Tethered prey survivorship was high when 3D interstitial space of mimics was modified by internal shape (crevice survivorship = 0.89, channel survivorship = 0.96) and these values did not differ significantly. In mesocosms, foraging success of blue crabs varied with 3D interstitial space as mean proportional survivorship (\pm SE) of mud crabs was significantly lower in 45° (0.27 ± 0.06) vs. 0° (0.86 ± 0.04) orientations and for crevice (0.52 ± 0.11) vs. channel shapes (0.95 ± 0.02). These results suggest that 3D aspects of interstitial space, which have direct relevance to refuge quality, can strongly influence foraging success in our oyster reef habitat. Our findings highlight the importance of spatial arrangement in mediating consumptive pathways in hard-structured habitats and demonstrate how quantifying the three-dimensionality of living space captures aspects of habitat structure that have been missing from previous empirical studies of trophic interactions and structural complexity.

Key words: 3D printing; habitat complexity; habitat structure; interstitial space; predator–prey interactions; spatial arrangement.

INTRODUCTION

Across ecological systems, it is well understood that consumptive effects of predators on prey are inherently dependent upon physical attributes of the habitat in which they occur, where the availability of certain refugia leads to the differential survival of prey taxa (Crowder and Cooper 1982, Diehl 1992). Numerous studies in freshwater, marine, and terrestrial environs have shown that habitat structure, or the physical arrangement of structural components in space (McCoy and Bell 1991), modifies portions of the predation event, including prey encounter rates (Anderson 1984, Gibb and Parr 2010), predator and prey detection (Devereux et al. 2005, McCormick and Lönnstedt 2013), ability of predators to access prey (Anderson 1984, Toscano and Griffen 2013); and ultimately prey consumption (e.g., Longland and

Price 1991, Grabowski 2004, Warfe and Barmuta 2004, Andruskiw et al. 2008). Thus, identifying which attributes of habitat structure mediate consumptive effects will further understanding of how physical habitat can influence top-down control.

In aquatic environments, benthic invertebrates (e.g., corals, oysters, mussels), macrophytic vegetation, rock, and woody debris serve as structural components that provide potential refuge from predation (Kovalenko et al. 2012). Most studies investigating trophic interactions and habitat structure in aquatic systems have focused on the relationship between predator foraging success, or the ability of predators to consume their prey, and the density of structural components in some manner. Past experimental studies have either purposefully manipulated component density (e.g., Gotecitas and Colgan 1989, Nanjo et al. 2014, Carroll et al. 2015) or inadvertently altered the number of structural components as they manipulated other attributes (e.g., Vince et al. 1976, Grabowski 2004, Humphries et al. 2011). However, in some systems, the orientation of structural

Manuscript received 8 August 2016; revised 14 November 2016; accepted 24 January 2017. Corresponding Editor: Jonathan H. Grabowski.

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components (Bartholomew et al. 2000, Horinouchi et al. 2009, Barrios-O'Neill et al. 2015) or their external shape (Coull and Wells 1983, Dionne and Folt 1991, Beukers and Jones 1997, Warfe and Barmuta 2004), has further been shown to influence predator–prey outcomes, and even under conditions of constant density (e.g., Dionne and Folt 1991, Barrios-O'Neill et al. 2015). Thus, density alone may not fully capture the attributes of habitat structure that influence predator foraging success and these studies suggest that the role of spatial arrangement (i.e., orientation and shape) in mediating consumptive effects might be currently underappreciated.

Despite the potential importance of orientation and shape in mediating predator–prey interactions, it remains unclear how to describe these arrangements in a manner that captures the dimensions of refugia that are relevant to both predator and prey. Interstitial space, or the space within or between objects, may provide an appropriate target for measurement because “living space” represents the usable portion of structure available to organisms as potential refugia while simultaneously being a product of spatial arrangement. Several studies have previously documented that space created by structural components (e.g., algal fronds, corals, macrophyte blades, rocks) is a strong predictor of faunal abundance in both freshwater and marine habitats (Friedlander and Parrish 1998, Warfe et al. 2008, Ory et al. 2012), alluding to the importance of interstitial space as refugia from predation. Additionally, space-focused metrics have been shown to predict prey habitat preference (Hacker and Steneck 1990, Finstad et al. 2007, Martin et al. 2012), which often reflect anti-predator behavior to reduce predation risk (Lima and Valone 1991).

To date, few manipulative studies have used space-focused measures to assess whether spatial arrangement influences predator–prey interactions and those studies that do have had mixed success in predicting outcomes. These inconsistent findings may result from using one-dimensional or two-dimensional measurements to describe the true three-dimensionality of interstitial space available for prey (e.g., Bartholomew et al. 2000, Almany 2004, Humphries et al. 2011, Wong 2013, Barrios-O'Neill et al. 2015). For example, the most commonly used space-focused metric (Sp/Pr index) compares a single linear measure of space opening (width or height) to some aspect of predator size to assess refuge quality (Bartholomew et al. 2000). Humphries et al. (2011) attributed the failure of the Sp/Pr index to predict prey survivorship in their study because it did not account for the total available “volume” within a structure. Toscano and Griffen (2013) reported that location of prey within an angled “crevice” strongly affected predator handling time and prey capture across a suite of predator sizes, presumably a result of the 3D aspects of the interstitial space utilized by the prey. Thus, considering interstitial space in a 3D perspective might offer more complete insight into predator–prey interactions by fully describing areas where prey hide and predators must access.

Variation in structural components is evident on oyster reefs, which are composed of live and dead shells that differ widely in both their three-dimensional orientation to other oysters as well as their external and internal shape. The spatial arrangement of shells creates interstices that are utilized by numerous benthic fauna, such as the abundant flatback mud crab (*Eurypanopeus depressus*, hereafter “mud crab”). These crustaceans use the available spaces between and within oyster shells as a refuge (Tolley and Volety 2005) from known predators such as the Atlantic blue crab (*Callinectes sapidus*; e.g., Grabowski et al. 2008, Hill and Weissburg 2013). Predator–prey interactions between space-dwelling fauna and their predators have been widely studied and the impacts of structural complexity on foraging success repeatedly noted on oyster reefs (e.g., Grabowski 2004, Grabowski et al. 2008, O'Connor et al. 2008, Hill and Weissburg 2013); however, the importance of 3D aspects of interstitial space, and thus spatial arrangement, have not been directly tested.

We utilized an oyster reef system to test the hypothesis that the 3D interstitial space provided by different shell spatial arrangements modified the ability of reef-associated predators to consume their space-utilizing mud crab prey without altering structural density. First, we examined whether the 3D interstitial space of oyster shell alters the foraging success of various predators on mud crab prey in the field. Second, we investigated trophic interactions between blue crab predators and mud crab prey in mesocosms under conditions of varying 3D interstitial space. To accomplish this, individual interstices were manipulated with respect to either orientation or internal shape of shell structural components, representing two distinct types of spatial arrangements displayed by oyster shells on a reef. In one set of field and mesocosm experiments, interstitial space was manipulated by changing only the orientation of identically sized shells. In a second set, interstitial space was manipulated by modifying internal shape of shells kept at the same orientation. By doing so, we provide a first experimental investigation on the relationship between 3D aspects of interstitial space and refuge quality as measured by predator–prey outcomes.

MATERIALS AND METHODS

Structural components

As the morphology of natural oyster shell is highly variable, we utilized 3D printing across all experiments to produce identical oyster shell mimics for use in shell orientation and internal shape treatments. The consistent replication of structural components provided by 3D printing allowed for the control of external spatial properties and density of shell structure in trials while manipulating only interstitial space. Shell mimics were created from the 3D image of a 60 mm high, left oyster valve that was digitized using a high-resolution, short-range, laser scanner. While

keeping the ratios of shell dimensions constant, the valve was resized to create different shell sizes when necessary. A Replicator+ 3D printer (MakerBot, Brooklyn, New York, USA) with polylactic (PLA) or acrylonitrile butadiene styrene (ABS) plastic was used to produce individual shells.

To manipulate the 3D interstitial space of shell mimics, components were attached to flat surfaces and either the orientation or the internal shape of shell mimics was varied, producing different levels of interstitial space (Table 1). Together, shell mimics and the flat surfaces to which they were attached simulated oyster shells adjacent to either an articulated right valve or another oyster. To change interstitial space via orientation, shell mimics of the same shape (Fig. 1a) were attached at angles of 0°, 22.5°, and 45° (Fig. 1b), representing a range of angles observed from natural oyster clumps (E. Salewski, *unpublished data*). Angle of shell orientation was measured between the attachment surface and the body axis (from umbo to tip of the ventral margin). Although 0° orientations are horizontal relative to the attachment surface, the tip of shell mimics are slightly elevated allowing prey access to interstices. To change interstitial space via internal shell shape, mimics were modified internally by inserting ~13.8 cm³ of Craft Smart oil-based modeling clay (Michaels, Irving, Texas, USA; Fig. 1c) and then attached to a base plate at an angle of 0° creating either a “crevice” or “channel” shaped interstice (Fig. 1d). Shell mimics with added clay approximated both differences in oyster shell shape (e.g., channel) as well as the additional occupancy of space by attached epifauna, such as mussels (e.g., crevice), which commonly utilize the spaces within and between oyster shells (Toscano and Griffen 2013).

Methods of assessing 3D interstitial space are not well developed and a single measure that simultaneously describes an interstice’s size and shape does not currently exist. Thus, interstitial space size, or the volume created between the shell mimic and the base to which it was attached (see Fig. 1), and interstitial space shape, or the form of the interstice created by the shell mimic and its base, were measured using two independent methods to describe the 3D aspects of each interstitial space.

TABLE 1. Mean (\pm SD) three-dimensional interstitial space size (cm³) and internal shape (i.e., length, width, height) of oyster shell mimics used in mesocosm and field tethering experiments.

Spatial arrangement	Space size (cm ³)	Space shape, length, width, height (cm)
Shell orientation		
0°	9.6 \pm 0.2	4.76, 3.04, 1.31
22.5°	17.4 \pm 0.6	4.42, 3.06, 2.91
45°	20.4 \pm 0.8	3.02, 3.05, 4.68
Internal shell shape		
Crevice	9.4 \pm 1.2	2.60, 4.23, 1.91
Channel	9.6 \pm 0.7	6.25, 1.51, 1.55

Interstitial space size was determined by inserting modeling clay into an interstitial space, removing it, and determining its volume (cm³) using displacement. For interstitial space shape, the length (i.e., depth), width, and height of an interstice were assessed to provide a relative measure of its form. Five replicate shells were measured to determine each orientation and shape and the mean values are reported (see Table 1).

Field tethering experimental design

Two tethering experiments were conducted to test whether the 3D interstitial space of shell mimics affects the ability of reef-associated predators to consume tethered mud crabs in the field. In the first tethering experiment (hereafter, “field-orientation”), 3D interstitial space was manipulated by changing the orientation of shell mimics (Table 1). Identical 60 mm shell mimics were arranged into one of three orientations (0°, 22.5°, 45°; Fig. 1a, b), representing three different levels of interstitial space. In the second field tethering experiment (hereafter, “field-shape”), 3D interstitial space was manipulated by changing only the internal shape of shell mimics (Table 1). Shell mimics (80 mm) with contrasting internal shapes (crevice or channel) were kept at the same orientation (0°; Fig. 1c, d).

Both field experiments were conducted using mud crabs (9.5–16.0 mm carapace width [CW], see Appendix S1) tethered to ceramic tiles (32 \times 32 cm) with a single shell mimic of the appropriate treatment and level attached by the umbo to the corner of ceramic tiles with WaterWeld epoxy putty (J-B Weld, Atlanta, Georgia, USA). One shell was attached to each tile, thereby allowing predators unobstructed access to each mimic. In the lab, a tether of monofilament line (7.6 cm, 2.79 kg test) was attached to the top of each prey’s carapace using Super Glue (Loctite, Düsseldorf, Germany). Tethers were attached at least one day prior to experimentation and individual mud crabs were maintained in separate containers until use. A metal stake and hollow plastic bead were used to secure the free end of the monofilament line to the center of each square ceramic tile through a hole in both the bead and plate, preventing prey items from using any structure other than the artificial shell mimic. Mud crabs were collected from an oyster reef in Tampa Bay, Florida, USA (27°89.296’ N, 82°54.076’ W) by hand three days before being used in each experiment. The prey size-range used in these experiments fell within the local size distribution of mud crabs (Appendix S1; Fig. S1).

The field-orientation and field-shape experiments were conducted on the same intertidal oyster reef in upper Tampa Bay (28°00.166’ N, 82°37.514’ W), but on two different dates: 19 September 2014 and 7 December 2015. Additionally, a set of plates with shell mimics of 45° orientation was included in the December field-shape experiment to provide a relative comparison of predation intensity between experiments (see below). The study site

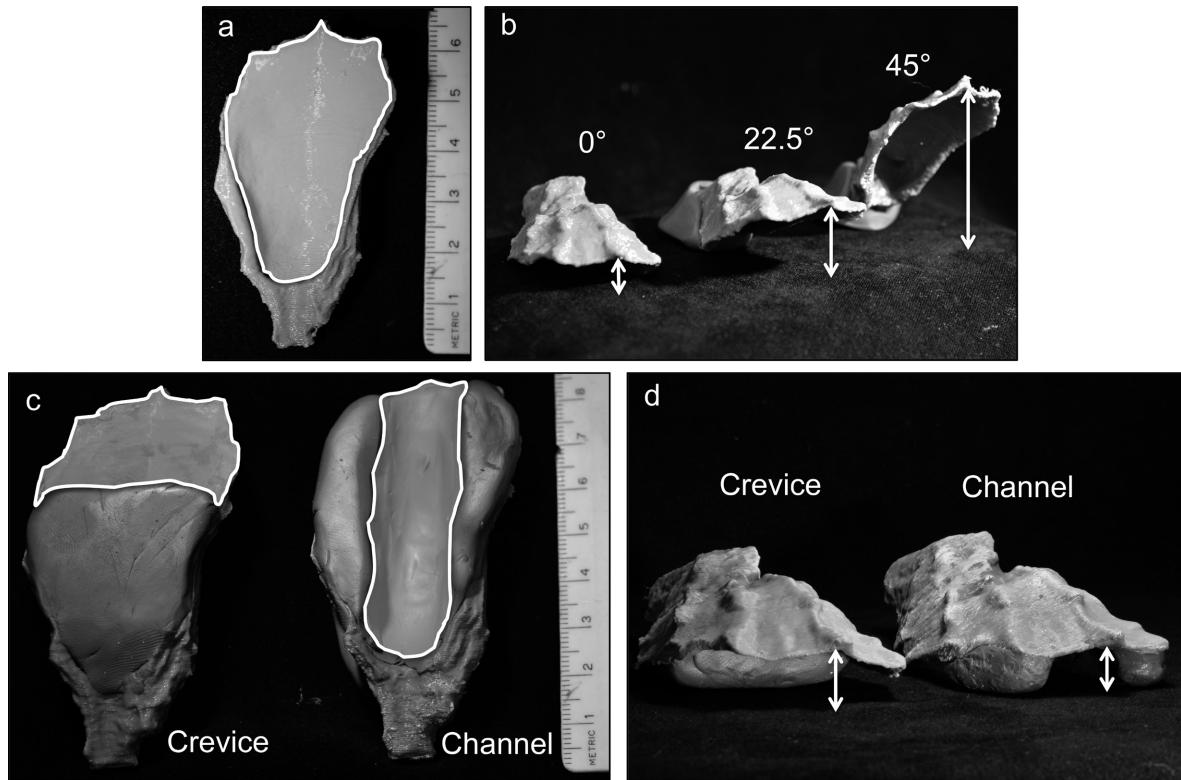


FIG. 1. Three-dimensional (3D)-printed oyster shell mimics with white outlines and arrows identifying each component's 3D interstitial space. (a) Underside of shell mimic (60 mm shell height [SH]) used for shell orientation experiments. (b) Oyster shell mimics (60 mm SH) oriented sequentially at angles of 0°, 22.5°, and 45°. (c) Underside of oyster shell mimics (80 mm SH) modified with modeling clay used for internal shell shape experiments. (d) The shell mimics containing the crevice and channel shaped interstices were fixed at 0° orientation.

is characterized by a mosaic of oyster reefs and mangrove islands, interspersed among a loose shell and mud bottom. Salinity typically varies between 10–25 psu and the faunal community is representative of other oyster reefs described in southwest Florida, USA (Tolley and Volety 2005), with *E. depressus* being one of the most abundant organisms at this site (S. Hesterberg, unpublished data). Mud crab predators, primarily sheepshead (*Archosargus probatocephalus*) and *C. sapidus*, have been observed foraging on the oyster reef (S. Hesterberg, personal observation). All orientation ($n = 35$ per angle) and internal shape ($n = 30$ for each of crevice and channel) tiles were haphazardly placed at approximately mean low water (MLW) and separated by at least 0.5 m.

As the composition and abundance of predators were expected to differ seasonally on oyster reefs (e.g., Stunz et al. 2010), an additional set of tethered mud crabs with shell mimics of 45° orientation ($n = 18$) was deployed during the December field-shape experiment. By doing so, these tiles provide a relative comparison of predation intensity between September and December experiments. We also rely on our mesocosm experiments to circumvent potential artifacts of tethering designs (see Peterson and Black 1994) and provide additional results to test our hypothesis.

The ability of various reef associated predators to capture mud crab prey (i.e., predator foraging success) was measured as prey survivorship, or the presence/absence of mud crabs on ceramic tiles after 24 h. Tile replicates were excluded from analysis if tiles were missing or overturned, mud crabs molted, or loose oyster shell moved onto the tile providing unintentional refuge. Additionally, the potential of mud crabs to escape from tethers was examined by placing tethered mud crabs in 37.9-L laboratory aquaria without a predator and recording the number of detached and surviving mud crabs after 24 h. No mud crabs were found dead or detached ($n = 12$).

Mesocosm experimental design

Experiments were conducted between July and October 2015 to test whether the 3D interstitial space of shell mimics modified the ability of blue crabs to consume their mud crab prey. Similar to the design of field tethering experiments, 3D interstitial space was modified by changing either the orientation or internal shape of shell mimics that prey could utilize as refugia in separate mesocosm experiments (Table 1). However, prey were not tethered within mesocosms and predators were restricted

to the same taxon (i.e., blue crabs). In the first mesocosm experiment (hereafter, “mesocosm-orientation”), 60-mm shell mimics of the same shape were arranged into one of two orientations (0° or 45° ; Fig. 1a, b). Given the results from the field-orientation experiment (see *Results*), only the extreme angles were used in the mesocosm trials. In the second mesocosm experiment (hereafter, “mesocosm-shape”), 80-mm shell mimics with contrasting internal shapes (crevice or channel) were kept at the same orientation (0° ; Fig. 1c, d).

All experiments were conducted outdoors at the University of Tampa Marine Science Center in covered mesocosms (151.4 L; $0.9 \times 0.5 \times 0.4$ m [L \times W \times H]), with sides surrounded by a black plastic tarpaulin to reduce visual disturbance. Wire mesh (6.45-cm² opening) was placed on tops of mesocosms to prevent predator escape and aerators provided oxygen and water circulation. Mesocosms were filled with unfiltered seawater pumped directly from Tampa Bay and held at a constant depth of approximately 35 cm to simulate the shallow-water conditions where oysters are found in the study area.

As only five mesocosms were available, trial replicates of each experiment were conducted in a block design to minimize potential effects of temporal heterogeneity. Treatments and levels (orientation [0° , $n = 10$; 45° , $n = 13$]; shape [crevice, $n = 16$; channel, $n = 10$]) were randomly assigned to one mesocosm and the position of each mesocosm haphazardly rearranged at the start of each trial. “No-predator” controls ($n = 16$) were conducted during trials to estimate background mortality and verify predator consumption of prey. Each mesocosm trial was considered a replicate and received 15 shell mimics of the assigned spatial arrangement treatment and level, which were attached to a plexiglass insert using epoxy putty. The plexiglass sheet was then attached to the bottom of a mesocosm using non-toxic silicone. Shell mimics were spaced at least 5 cm apart to allow for equal predator access and the location of shells relative to one another and direction within tanks were constant across all experiments. A fine layer of filtered sand approximately 0.25 cm deep was added over each plexiglass base.

Mud crabs (9.5–16.0 mm CW; see Appendix S1) were collected by hand before each experiment from the same intertidal oyster reef from which *E. depressus* was collected for use in the field tethering experiments. All mud crabs were held in 37.9-L aquaria and fed shrimp pellets until use in trials. Twelve mud crabs were haphazardly selected and placed into each mesocosm approximately 15 min prior to the start of a trial, allowing prey to access shelter before being exposed to predators. Prey density used in these mesocosm experiments aligns with estimates of mud crab abundance on oyster reefs in southwest Florida (~20–40 crabs/m²; Tolley and Volety 2005). Blue crabs, caught in baited traps from the Tampa Bay vicinity by commercial fishermen, served as focal predators. As blue crab size influences the number of prey items consumed in a given trial (Hill and Weissburg 2013), we did not attempt to manipulate predator size. Only adult blue

crabs >100 mm CW were used and we kept predators as close in size as possible. Blue crabs were stored in 18.9-L buckets with an aerator and starved for 72 h before each trial. The carapace width (cm) of each predator was recorded and only one blue crab was placed in each mesocosm. Once introduced to mesocosms, predators were allowed to forage for 24 h, encompassing the full diurnal period. Individual predators and prey were only used once across all experimental trials.

At the conclusion of each trial, the remaining prey in each mesocosm were counted by checking both structure and sand for mud crabs among the trial replicates. If predators died, molted, or were visibly ailing and refused to eat, the trial was excluded from analysis. The ability of blue crabs to consume their mud crab prey (i.e., predator foraging success) was measured by prey survivorship, or the number of surviving mud crabs after 24 h divided by the initial number of mud crabs present (12) in each mesocosm. However, mud crabs were able to retreat up the corners of each mesocosm, presumably reducing predator access to prey items. Thus, prey survivorship is reported as a corrected proportion by eliminating any mud crabs found above the mesocosm base at the end of a trial from both the total number of mud crabs recovered and those initially present. No differences existed between the outcomes of the uncorrected and corrected statistical analyses (Appendix S2).

Statistical analyses

For all experiments, prey survivorship was identified as a binomial response in the form of x successes (i.e., alive) out of number of initial prey. The effect of 3D interstitial space produced by different spatial arrangements on prey survivorship was analyzed using separate generalized linear models (GLM) with either a binomial or a beta-binomial error distribution and subsequent likelihood ratio tests. To select the appropriate error distribution, each response variable was fitted using both distributions and the resulting models were compared using Akaike information criterion corrected for sample size (AIC_c) scores. The model with the lowest AIC_c score was then selected.

For field tethering experiments and the mesocosm-orientation experiment, a binomial error distribution was used to model prey survivorship as a function of either shell orientation or shell shape. In the mesocosm-shape experiment, a beta-binomial error distribution was selected to model prey survivorship as a function of internal shell shape. In both mesocosm experiments, trial was initially included as a predictor in each model, but the blocking factor was removed after being nonsignificant and data reanalyzed. Predator size is well understood to influence foraging success in structured habitats (e.g., Hill and Weissburg 2013) so blue crab size (i.e., carapace width) was analyzed as an additional predictor in both mesocosm experimental models. Furthermore, the interaction between spatial arrangement and predator

size was also included in both mesocosm experimental models. All analyses were conducted in the R statistical computing environment (R Development Core Team 2015, version 3.1.3).

RESULTS

Field tethering experiments

In the field, predator foraging success varied significantly when the 3D interstitial shape of shell mimics was modified by orientation (Fig. 2a; $df = 2$, $\chi^2 = 54.00$, $P < 0.001$). Specifically, survivorship of tethered prey decreased as interstices increased in 3D size and the shape of that volume became increasingly shallow (i.e., length decreased) and more open (i.e., height increased; Table 1), but not in a linear way. A 0.76 increase in proportional survivorship was recorded when 0° and 45° orientations were compared, but only a 0.13 increase was observed between 0° and 22.5° orientations.

In the field-shape experiment, no significant difference in predator foraging success was detected when the 3D interstitial shape of shell mimics was modified by internal shell shape (Fig. 2b; $df = 1$, $\chi^2 = 1.29$, $P = 0.255$). Proportional survivorship of tethered prey approached

≥ 0.90 for both crevice and channel interstices, despite marked differences in the internal shape of each mimic, particularly length (Table 1). It is noteworthy that the foraging success observed from experiments with internal shell shape treatments, in which mimics were both fixed at 0° orientation, approximated the survivorship outcomes for the 0° orientation shells in the field-orientation experiment (Fig. 2a, b).

The inclusion of mud crabs tethered to shell mimics with 45° orientation in the December field-shape experiment provided evidence of reduced predator foraging relative to the September field-orientation experiment. In December, proportional survivorship of tethered mud crabs with shells of 45° orientation was 0.27 after 24 h ($n = 15$), compared to 0.0 for mud crabs in September (Fig. 2a). Three tiles of 45° orientation in the December field-shape experiment were overturned and removed from consideration. Across all field experiments, no tethers were lost and only one to three prey were found to be tangled upon recapture.

Mesocosm experiments

The ability of blue crab predators to consume mud crab prey differed when the 3D interstitial space was

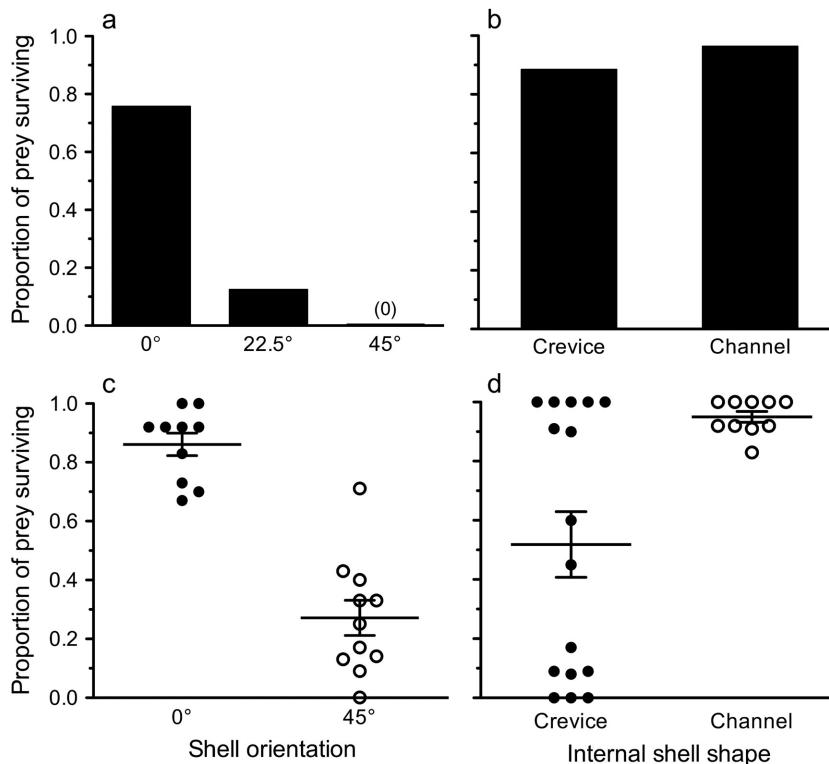


FIG. 2. Proportional survivorship after 24 h of tethered mud crabs (*Eurypanopeus depressus*) placed on an oyster reef in Tampa Bay, Florida, USA with 3D-printed shell mimics of (a) 0° ($n = 33$), 22.5° ($n = 32$), or 45° ($n = 27$) orientation or (b) crevice ($n = 26$) and channel ($n = 28$) internal shape. Mean (\pm SE) proportional survivorship of mud crab prey after 24 h in mesocosms with a blue crab (*Callinectes sapidus*) predator and 3D-printed shell mimics of (c) 0° ($n = 10$) and 45° ($n = 11$) orientation or (d) crevice ($n = 16$) and channel ($n = 10$) internal shape. Individual points show the proportional survivorship of each replicate. See Table 1 for metrics describing relationship between spatial arrangement and 3D interstitial space.

modified in spatial arrangements of either orientation or shape (Fig. 2c, d). In the mesocosm-orientation experiment, a significant effect of shell orientation on mud crab survivorship was observed (Fig. 2c; $df = 1$, $\chi^2 = 79.29$, $P < 0.001$). Mean (\pm SE) proportional survivorship was higher for mud crabs in shell mimics of 0° orientation, with 0.86 ± 0.04 of prey surviving, compared to only 0.27 ± 0.06 of prey surviving in shell mimics with 45° orientation. A similar pattern was observed with results from field-orientation trials; specifically, prey survivorship decreased as interstices became larger in 3D volume and the shape of the space became increasingly shallow (i.e., length decreased) and more open (i.e., height increased; see Table 1). In the mesocosm-shape experiment, a significant effect of internal shell shape on mud crab survivorship was also observed as mean proportional survivorship of mud crabs was 0.43 lower in the crevice interstice compared to shells containing a channel shaped space (Fig. 2d; $df = 1$, $\chi^2 = 17.81$, $P < 0.001$). Interestingly, prey survivorship responded solely to differences in 3D shape of interstices, in this case width and length, not volume (Table 1). Consumption was highly variable for crevice shapes, with the majority of values for survivorship clustering either near 0.0 or 1.0 (Fig. 2d).

Neither predator size nor the interaction between spatial arrangement and predator size influenced the ability of blue crabs to consume their mud crab prey in either mesocosm experiment. Width of blue crab carapaces ranged between 114.6 and 197.1 mm for the mesocosm-orientation experiment and 126.4 and 164.5 mm for the mesocosm-shape experiment, but was a nonsignificant predictor of predator foraging success in both models ($df = 1$, $\chi^2 = 0.16$, $P = 0.690$ and $df = 1$, $\chi^2 = 2.63$, $P = 0.105$, respectively). Furthermore, the interaction terms were nonsignificant in the mesocosm-orientation and mesocosm-shape experiments ($df = 1$, $\chi^2 = 3.13$, $P = 0.077$ and $df = 1$, $\chi^2 < 0.01$, $P = 0.981$, respectively). Only one mud crab died during the control trials ($n = 16$), suggesting that blue crab consumption was responsible for prey loss.

DISCUSSION

Identifying and quantifying the relevant properties of habitat structure that mediate predator-prey interactions remains a persistent challenge across ecological systems, especially given that few studies investigate the effects of spatial arrangement and most restrict to a one- or two-dimensional view of an otherwise three-dimensional habitat. Using a novel technology (i.e., 3D printing), our study demonstrates that alteration of the 3D interstitial space provided by shell structural components strongly influences the ability of reef-associated predators to consume their space-utilizing prey when spatial arrangement differed with respect to orientation or internal shape. Our new findings expand upon earlier work that has primarily focused upon trophic interactions and density of structural components (e.g.,

Gotecitas and Colgan 1989, Andruskiw et al. 2008, Nanjo et al. 2014, Carroll et al. 2015) and reveal strong effects of spatial attributes on predator foraging success that are independent of density.

Evidence to support the importance of 3D interstitial space as refugia from predators was generally consistent across the two spatial arrangements investigated in both field and mesocosm experiments. Predators consumed more mud crab prey as angle of oyster shell orientation increased from 0 to 45° (Fig. 2a, c). As angle increased, interstices became larger in 3D size while the 3D arrangement of that volume became increasingly shallow (i.e., length decreased) and more open (i.e., height increased; Table 1). The larger and more vertical arrangement of this space likely resulted in a greater ability of blue crabs and other reef associated predators to encounter, detect, and/or access mud crabs utilizing these interstices.

Predator foraging success was also impacted when the 3D interstitial space of structural components varied with respect to internal shell shape. Prey survivorship was extremely high when mud crabs were offered channel mimics in both field and mesocosm trials, but significantly lower when blue crabs tried to capture mud crabs utilizing crevice mimics in mesocosms (Fig. 2b, d). Blue crab foraging success generally increased as interstices became shallower (i.e., length decreased) and wider in 3D shape. Interestingly, differences in interstice shape, but not volume, were sufficient to influence blue crab consumption (Table 1). The change in physical accessibility of prey, likely due to their differential use of internal shell shape, presumably inhibited blue crab foraging success via modifying mud crab detection and access (Fig. 2d). However, the latter result stands in contrast to higher survivorship of mud crabs observed in the December field-shape experiment when predators were presented prey utilizing crevice mimics.

Changes in predator guild composition and/or seasonal differences in predation (e.g., abundance, activity) may explain the comparatively lower predator foraging success recorded for crevice mimics in the December field-shape experiment compared to prey consumption by blue crabs recorded with the same mimics in mesocosms (Fig. 2b, d). Survivorship of tethered crabs on tiles (i.e., shells at the 45° orientation) placed into the field simultaneously with the crevice and channel shells indicated that predation pressure was lower in the December (0.27) compared to the September (0.0) tethering experiment. Blue crab abundance in Tampa Bay declines in winter months (Flaherty and Guenther 2011) and prey consumption in structured habitats has been reported to depend on predator identity (e.g., O'Connor et al. 2008, Klecka and Boukal 2014, Carroll et al. 2015). Thus, differences in predator feeding intensity or guild composition may explain at least some of the inconsistency in survivorship between experiments.

While the overall results from the mesocosm-shape experiment indicated that blue crab predators consumed

significantly more mud crabs in crevice- vs. channel-shaped interstices, the majority of predators either consumed nearly all mud crabs or none at all in mesocosm trials containing crevice-shaped interstitial spaces (Fig. 2d). The striking dichotomy of responses observed contrasts with the unimodal patterns observed for blue crab foraging success in all other spatial arrangement levels (Fig. 2c, d). Given that trial, predator size, and interaction term were not significant predictors in this study, predators and prey were used once, and predators were starved for the same duration, the differences in foraging are likely due to some unmeasured individual variation within predators or prey that caused intraspecific differences in blue crab foraging success. Repeatable differences in “personality” between individuals have been shown to influence predator–prey outcomes via consumptive pathways (Royauté and Pruitt 2015). Irrespective of the mechanism, this small subset of results suggests that specific 3D attributes of interstitial space may interact with variable traits displayed by individual predators and/or prey to produce unexpected patterns of foraging success.

Our ability to identify relationships between predator foraging success and structure benefited when the three-dimensional nature of interstitial space was quantified. In contrast to three-dimensional metrics, use of common one-dimensional or two-dimensional metrics would have limited our ability to distinguish differences among the interstices of shell structures. For example, the Sp/Pr Index, often utilized to predict foraging success in structured habitats, considers only one linear aspect of space opening (either width or height), but the aspect selected for measurement is unspecified (Bartholomew et al. 2000) and can even vary within a single study (e.g., Humphries et al. 2011). In our study, strikingly different outcomes for prey survivorship were recorded in experiments when shell mimics varied in their orientation (Fig. 2a, c), but measurement of the linear width of these structures alone would have indicated interstices were nearly of identical magnitude (Table 1). Additionally, interstices of different internal shape (crevice vs. channel) possessed approximately equal heights, yet marked differences in blue crab foraging success were noted between the two shapes in mesocosm trials (Table 1, Fig. 2d). If both length and width of space openings had been considered simultaneously (e.g., Wong 2013), the substantial changes in interstice depth (i.e., length) that occurred in both the orientation and internal shape spatial arrangements would not have been detected (Table 1), which may have been of strong importance underlying prey capture. Here, we provide evidence that consideration of multiple parameters simultaneously to formulate a three-dimensional description of interstitial space offers more complete insight into the structural features that provide effective prey refuge. Simply, the inclusion of 3D measures should provide more information than that collected by one or two-dimensional perspectives, and this additional information may be essential, in some cases, to predict foraging success.

Although our study does not provide a new measure of refuge potential, the results here can assist in guiding future metric development. We demonstrated that interstitial space size, or the amount of space and shape, or the arrangement of space, provide relevant aspects of 3D structure, which could be incorporated into existing metrics, such as the Sp/Pr Index, to make them three-dimensional. By doing so, an individual-based model could then be developed that considers 3D aspects of predator and prey morphology, as well as that of the structural interstices, to determine predation risk for a given structure (e.g., Schindler et al. 1994). Inclusion of three-dimensionality in investigations of predator–prey interactions can be facilitated by the use of high-resolution, digitized images of structures obtained by either non-destructive X-ray computed tomography (CT) or laser scanning, as these methods can assist in the rapid quantification of interstitial space in more complex structures or over larger spatial scales (e.g., Rodriguez et al. 2014, Orland et al. 2016).

CONCLUSION

Our findings highlight the importance of spatial arrangement in mediating consumptive pathways in hard-structured habitats. Specifically, results from both field and mesocosm experiments suggest that 3D aspects of interstitial space, which have direct relevance to prey refuge quality, are key determinants of predator foraging success in the settings explored here. Other studies have emphasized that density of structural elements can influence predator–prey interactions, but our findings reveal that the three-dimensional size and shape of interstitial space capture aspects of habitat structure that have been missing from empirical studies of trophic interactions and structural complexity.

Viewing spatial attributes of habitat structure in three dimensions, as argued here, should be applicable to a myriad of habitats formed by hard physical structure, including biogenic reefs, rock outcrops, and/or woody vegetation in which structure-generated crevices, holes, or living space might be a source of refugia. Moreover, our findings provide a basis for predicting that as habitats become increasingly simplified overtime (e.g., Rothschild et al. 1994, Alvarez-Filip et al. 2009), reduction of a habitat’s three-dimensionality might have direct implications for trophic interactions if the effectiveness of prey refugia is altered. Accordingly, 3D aspects of spatial arrangement, specifically interstitial space, merit attention when living or artificial structures are designed, given that “living space” can be of critical importance for maintaining trophic links.

ACKNOWLEDGMENTS

We thank S. Rice and The University of Tampa for use of facilities and the University of South Florida’s Advanced Visualization Center for 3D printing. We also thank G. Herbert, D. Lewis, D. Smee, K. Walters, and two anonymous reviewers

for helpful comments on the manuscript. J. Garlock and E. Kiskaddon assisted with fieldwork. This research was supported in part by the Porter Family Foundation. All specimens in this study were collected with permission from Florida Fish and Wildlife Conservation Commission (SAL-14-1633-SR).

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