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Resolving spatio-temporal uncertainty in rare resource acquisition: smell the shell

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Abstract Complex environments present substantial spatio-temporal uncertainty in where and when rare ecological resources become available. How animals navigate this uncertainty to turn the seemingly unpredictable into the predictable is a fundamental question in evolutionary ecology. Here we use subtidal hermit crabs (*Pagurus acadianus*) as a model system to experimentally test in the field how animals resolve spatio-temporal uncertainty in resource availability. Quadrat sampling within the subtidal zone revealed that hermit crabs face an extreme ecological challenge, based on the rarity of empty shells across space and time. We show how this spatio-temporal uncertainty is ultimately resolved using long-distance chemical cues, which are associated with non-destructive shell predation on living gastropods, the original source of shells. By experimentally releasing cues that simulated the chemical by-products of predation, we reveal that certain flesh cues provide fine-grained information about the precise spatial and temporal window of new shell availability. These cues were most attractive to individuals with the greatest existing resource needs, and in the absence of this information individuals were highly constrained in their ability to discover newly available resources. Broadly, these experiments reveal that exploiting simple cues from heterospecific predators can provide a solution to the general ecological challenge of finding resources that are rare in space and time.

Keywords Cues · Information · Spatio-temporal uncertainty · Resource acquisition · Predation

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Introduction

Many animals face complex spatial environments in which resources are patchily and heterogeneously distributed (Levin 1992). Such spatially complex environments present major challenges for locating and acquiring resources. Temporal variability can further exacerbate these spatial uncertainties by changing resource distribution within patches over time, thereby producing fluctuating and stochastic patterns in where and when resources become available. Within such environments, animals experience strong natural selection pressures to evolve specialized sensory systems (Stevens 2013), which are capable of resolving spatio-temporal uncertainty and enabling the acquisition of rare resources. A major goal of evolutionary ecology is to elucidate the information that animals use to navigate these challenging spatio-temporal landscapes (Dall et al. 2005), ultimately allowing animals to turn the seemingly unpredictable into the predictable.

Hermit crabs provide an ideal system to explore spatio-temporal uncertainty because they are reliant upon rare resources that are persistently in short supply and only opportunistically become available in space and time (Laidre 2011). These resources, shells, which derive from gastropods, are vital for survival, growth, and reproductive success (Hazlett 1981), and individuals are highly vulnerable without them (Laidre 2007). While in theory conspecifics that already occupy shells could provide a rich source of these resources, only terrestrial hermit crabs are uniquely dependent upon socially-derived shells (Laidre 2012a, 2014). Marine hermit crabs, in contrast, specialize on and prefer brand new shells, deriving directly from living gastropods and never before having been occupied by a conspecific (Laidre and Trinh 2014). Critically, in marine environments, gastropods are preyed upon by a variety of predators (Alcaraz and Arce 2017) and, paradoxically, some of this predation may create new resources. This is because not all predation on gastropods results in destruction of the shell (Vermeij 1993). Some predators (e.g. Brachyuran crabs) break the shell to access the flesh inside, but others (e.g. starfish and predatory whelks) are able to externally secrete enzymes that directly degrade all the gastropod flesh while leaving the shell itself intact. Importantly, destructive shell predation releases bits of ripped off gastropod flesh for possible scavenging, but by cracking the shell it destroys this potential home (Vermeij 1993). In contrast, non-destructive shell predation offers little or no opportunity for scavenging flesh (which is completely cleaned from the shell and fully digested by the predator). What non-destructive predation does offer is a highly valuable opportunity for hermit crabs to immediately acquire intact, newly emptied shells (McLean 1974). However, to take advantage of such an opportunity, in which a living gastropod is directly converted to an empty shell, necessitates precise cues to alert individuals to the specific location and timing of predatory events.

While several sensory modalities could potentially provide such cues (Laidre 2010), the habitat complexity of marine hermit crabs in the subtidal allows only certain modalities to be effective, while placing severe constraints on others (Stevens 2013). Specifically, visual, tactile, and auditory modalities appear to be largely inoperable in this context. For instance, visual cues are constrained by limited light (Cronin et al. 2014), and this constraint is intensified by the density of seaweed and other physical barriers, which obscure crabs' view (Video S1). Moreover, using visual or tactile cues to differentiate available from unavailable shells would likely be impossible, given the sheer abundance of living gastropods in many environments and the similarity of their shell exteriors to those of newly available empty shells (Vermeij 1993; Laidre 2011). Finally, auditory cues appear to be non-existent among gastropods (Vermeij 2010). In contrast to these other modalities,

chemical cues provide a uniquely effective sensory solution: they operate over large spatial scales (Breithaupt and Thiel 2011); they are highly time-sensitive due to rapid spread and dissipation of their active space in subtidal environments (Gherardi and Tricarico 2011); they can elicit stark reactions (Hanley et al. 2011; Tran 2015) that can enhance fitness (Asahina et al. 2008); they are often exploited by crustaceans (Harzsch and Krieger 2018); and, most critically, chemical cues can be highly specific to predatory events, based on the enzymes that non-destructive shell predators use to degrade gastropod flesh (Wilber and Herrnkind 1984). Interestingly, prior work has established that marine hermit crabs are highly attracted to gastropod flesh (Laidre 2007, 2011; Laidre and Elwood 2008; Laidre and Greggor 2015; Greggor and Laidre 2016). This attraction does not appear to be simply food-motivation, given how insignificant gastropod flesh is as part of hermit crabs' highly omnivorous diet (Hazlett 1981). Instead, there is an indication that predatory chemical cues emanating from gastropod flesh may act as informative cues to shell opportunities, which follow imminent gastropod death (Rittschof 1980; Wilber and Herrnkind 1984). Prior studies, however, have rarely controlled or experimentally tested the many complex ecological parameters that are crucial for revealing whether chemical cues are both necessary and sufficient for reducing spatio-temporal uncertainty about shell availability.

Here we use hermit crabs (*Pagurus acadianus*) to experimentally test in the field how animals resolve spatio-temporal uncertainty in resource availability. We first quantify the extreme ecological challenge these animals face, based on the rarity of empty shells in space and time. We then test if this spatio-temporal uncertainty can be resolved using long-distance chemical cues, which are associated with non-destructive predation on living gastropods. We examine how effective these cues are in providing fine-grained information about the precise spatial and temporal window of resource availability, and we determine whether this information benefits individuals in the population that have the greatest existing resource needs. Finally, we test whether in the absence of chemical cues of predation, individuals across the population are constrained in their ability to locate newly available resources.

Materials and methods

Study site and ecological community

We conducted this study at the Shoals Marine Laboratory on Appledore Island (Fig. S1), which is located approximately 6 miles (10 km) off the coast within the Isles of Shoals in the Gulf of Maine. ML undertook preliminary field observations on Appledore during September 2016, and ML and LV conducted pilot field experiments during May 2017. All the experiments reported herein were then conducted during June, July, and August 2017 on the west side of the island in the area located beyond the 'Great Tidepool'. Experiments were performed in the subtidal during daylight hours (between 0500 and 2030 hours) within 2.5 h of low tide and in water that was 0.5–1.5 m deep.

This study site is characterized by an abundance of Acadian hermit crabs (*Pagurus acadianus*) (Grant 1963), the focal species of our experiments, as well as live gastropods, including common periwinkle snails (*Littorina littorea*), flat periwinkle snails (*Littorina obtusata*), and dog whelks (*Nucella lapillus*). Another hermit crab species (*Pagurus pubescens*) also occurs in the Isles of Shoals, but is found deeper in the subtidal, so was not part of our experiments. Our focal species, *P. acadianus*, was most often observed inhabiting *L.*

littorea shells, was occasionally found in *L. obtusata* and *N. lapillus* shells, and was rarely found in moon snail shells (*Euspira heros*). Predators of living gastropods also occur at this site (Moody and Steneck 1993), including (1) brachyuran crabs (the Jonah crab *Cancer borealis*, the Atlantic rock crab *Cancer irroratus*, the European green crab *Carcinus maenus,* and the Asian shore crab *Hemigraspus sanguineus*); (2) the American lobster (*Homarus americanus*); (3) sea stars (*Asterias forbesi* and *Asterias vulgarus*); and (4) large deep-sea predatory gastropods (*Buccinum undatum, Euspira heros*, and *Colus* spp.). Brachyuran crabs and lobsters perform destructive shell predation, using shell-peeling and shell-crushing techniques to access gastropod flesh, thereby permanently damaging the shell and making it uninhabitable for hermit crabs (Vermeij 1993). In contrast, sea stars and predatory gastropods perform non-destructive shell predation, consuming snail flesh without inflicting damage upon the shell, thus leaving the shell intact and potentially habitable for hermit crabs (Vermeij 1993; though see Carriker 1981 on shell-boring). In addition to gastropod prey, other shelled mollusk prey at this site include: mussels (*Mytilus edulis*), oysters (*Ostrea edulis*), clams (*Spisula solidissima* and *Mya arenaria*), limpets (*Puncturella noachina*), slippershells (*Crepidula spp.*), and barnacles (*Semibalanus balanoides* and *Balanus* spp.).

Quantifying spatio-temporal uncertainty in availability of empty shells

Random quadrat sampling

Random quadrat sampling was performed to quantify the spatial variability in the empty-shell supply and in the species distribution. A metal quadrat (27 × 27 cm) was thrown into the subtidal, with N = 5 random tosses performed each day across 20 days, for a total sample of N = 100 replicates. Within each quadrat, the numbers of shell predators (destructive and non-destructive), empty shells, hermit crabs, and live gastropods were recorded, including what shell species each hermit crab was inhabiting and the species of any empty shells.

Stationary quadrat sampling

Stationary quadrat sampling was performed to complement the random quadrat sampling by assessing the day-to-day temporal variability for the same quadrats resampled across time. Three permanent, stationary quadrat locations were created and were positioned at least 1 m apart from one another. The same categories used for the random quadrat samples were used to take counts of the empty shells and species present within the three stationary quadrats. The N = 3 stationary quadrats were sampled across 20 days (nearly all consecutive, with at most a gap of 1 day between sample days), for a total sample of N = 60 replicates.

Experiment 1: attraction to chemical cues

General experimental design

This experiment measured the attraction of hermit crabs to chemical cues from simulated gastropod predation sites (destructive and non-destructive). Prior to each experiment, a quadrat (27 × 27 cm) was placed on the substrate at a location containing at least one and

no more than five hermit crabs. The number of hermit crabs inside the quadrat was visually counted immediately before the start of the experiment. The experiment began when a randomly chosen bottle (containing one of seven chemical cue conditions, see below) was placed on the substrate in the center of the quadrat and opened, allowing its chemical contents to emanate. After 5 min, the number of hermit crabs inside the quadrat was then counted again, providing a before-and-after measure of the number of hermit crabs attracted to each chemical cue condition (Fig. 1). Any additional macroscopic fauna within the quadrat were also recorded. All seven conditions were tested, in randomized order, within the same tidal cycle during the same day, and the quadrat was always placed at least 3 m away from any previously tested locations. Experiments were carried out across twenty separate days, for a total of N = 140 experiments (N = 20 replicates for each of the seven conditions).

Chemical cue conditions

All chemical cue conditions were prepared 20–60 min before the start of the experiment. Each condition was placed inside a 20 ml opaque plastic bottle (Fig. 1), which was filled with 2 ml of distilled water. A screw-top lid was kept on the bottle and only taken off at the start of the experiment. Fine plastic mesh, placed over the mouth of the bottle and held with a rubber band, confined the bottle's contents while allowing chemical cues to freely emanate. A 6 oz. (i.e., 170 g) fishing weight was also attached to each bottle to keep the bottle weighted to the substrate. In our experiments, we used the digestive enzyme trypsin, which is found within many non-destructive shell predators that prey upon living gastropods, externally digesting the gastropod's flesh while leaving the shell intact (Vermeij 1993). Treating flesh with trypsin thus allowed us to produce chemical cues similar to those released during non-destructive shell predation. Conversely, providing pure flesh (that was not treated with trypsin) allowed us to produce chemical cues similar to those released during destructive shell predation, in which the flesh is torn apart once the shell is crushed, but with the flesh then digested internally rather than externally. Given that trypsin is highly conserved evolutionarily and is widely distributed across invertebrates and vertebrates (Mukhin et al. 2007), we acquired trypsin commercially (Sigma-Aldrich # T8003; trypsin derived from bovine pancreas; Type I, ~ 10,000 BAEE units/mg protein: http://www.sigmaaldrich.com/catalog/product/sigma/t8003?lang=en&region=US).

We tested a total of seven chemical cue conditions, comprising two control conditions, four experimental flesh conditions, and one experimental non-flesh (shell) condition:

1. Trypsin (control): 1.2 mg of trypsin, nothing else added.
2. Live gastropod (control): a live *Littorina littorea* gastropod (1–3 g).
3. Pure gastropod flesh: 1 g of flesh extracted from a freshly smashed live *Littorina littorea* gastropod and separated from its shell. This condition therefore simulated the pure flesh chemical cues that are associated with destructive shell predation on gastropods.
4. Trypsin-treated gastropod flesh: this condition was identical to the pure gastropod flesh condition, except 1 g of gastropod flesh was treated with trypsin (1.2 mg). This condition therefore simulated the digested flesh chemical cues that are associated with non-destructive shell predation on gastropods.
5. Pure mussel flesh: 1 g of flesh extracted from a freshly smashed live *Mytilus edulis* mussel and separated from its shell. This condition therefore simulated the pure flesh

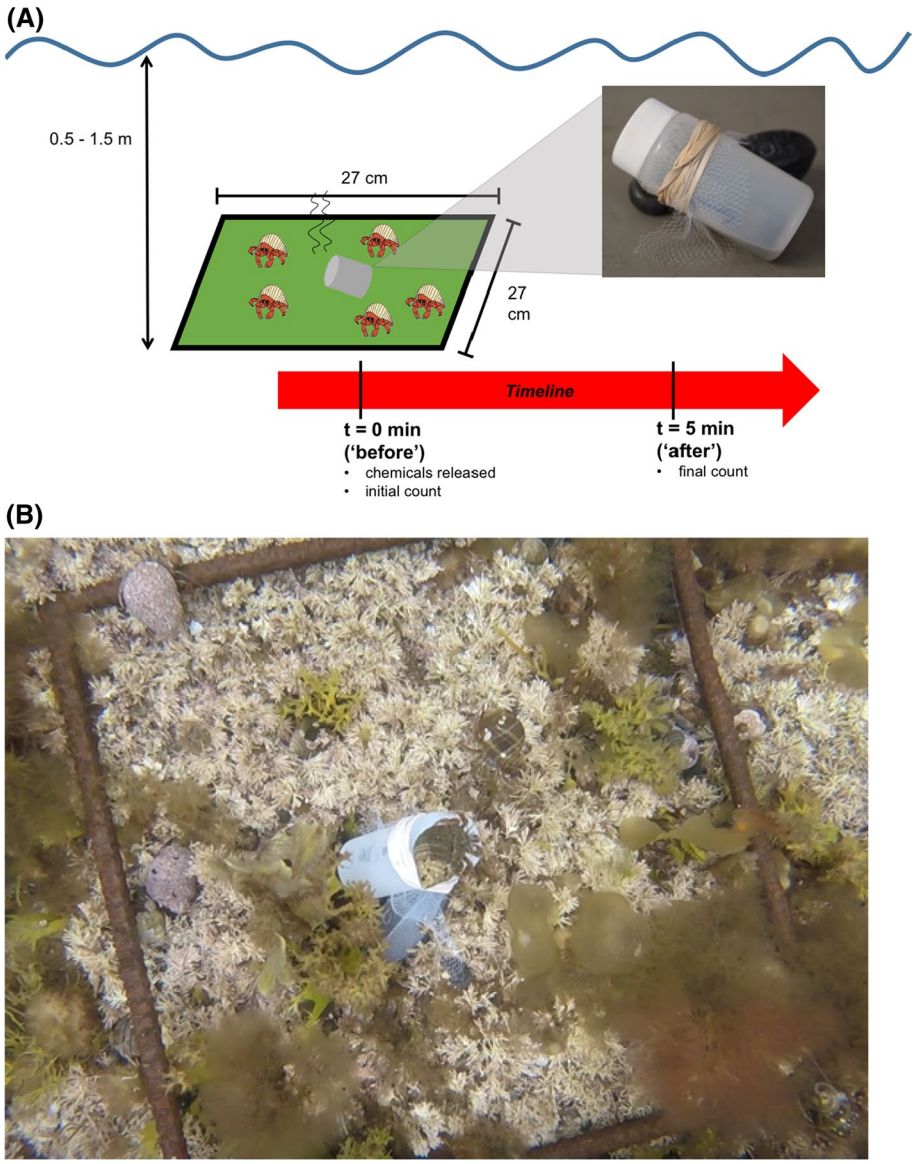

Fig. 1 Experimental design. **a** Quadrat containing stimuli, including bottle weighted to substrate, with mesh covering to allow chemical cues to emanate. Timeline of experiments: chemical stimuli were released at time zero and the number of hermit crabs (and other macroscopic fauna) within the quadrat were counted both 'before' (at time zero) and 'after' (5 min later). **b** Picture from above of quadrat in the subtidal, with hermit crabs (*Pagurus acadianus*) attracted

chemical cues that are associated with destructive shell predation on a non-gastropod mollusk.

6. Trypsin-treated mussel flesh: this condition was identical to the pure mussel flesh condition, except 1 g of mussel flesh was treated with trypsin (1.2 mg). This condition there-

fore simulated the digested flesh chemical cues that are associated with non-destructive shell predation (but on a mollusk whose leftover shell is never usable by hermit crabs, even when the shell is left intact).

7. Shell fragments: live *Littorina littorea* gastropods were boiled, so that their cooked flesh could be completely removed from their shells, and then each empty shell was cleaned and sun-bleached before being shattered to create 1 g of shell fragments. This condition therefore simulated the pure shell-fragment chemical cues that are associated with destructive shell predation, but without any associated flesh chemical cues.

Experiment 2: extended time

These experiments assessed the temporal window in which chemical cues were attractive to hermit crabs. The same general procedure as in Experiment 1 and the same chemical cue conditions were used, but the time frame during which the quadrat was monitored was extended 12 times its original length, from 5 to 60 min. Counts of the number of hermit crabs within the quadrat were taken visually every 5 min during the entire hour of the experiment. We completed a total of N = 21 experiments (N = 3 replicates for each of the seven chemical cue conditions), with one to three of these extended time experiments being completed each tidal cycle. These experiments allowed us to determine qualitatively when peak numbers of hermit crabs accumulated in the quadrat and whether this occurred immediately or later in the experiment. If chemical cues are initially highly attractive, but rapidly dissipate over longer time intervals, then the numbers of hermit crabs should peak early on.

Experiment 3: quantifying shell inadequacy of attracted hermit crabs

Field collection

This experiment tested whether hermit crabs that were attracted to trypsin-treated gastropod flesh were more likely to have inadequate shells, and hence were more motivated for shell-acquisition, compared to (1) hermit crabs that were attracted to pure gastropod flesh and (2) control hermit crabs (which were present in the same general vicinity but had not been attracted to either gastropod flesh condition). Experiments followed the same design as Experiment 1 and were carried out using trypsin-treated gastropod flesh and pure gastropod flesh, with all attracted hermit crabs collected after each experiment. Every day, two experiments were conducted, one for each of the gastropod flesh conditions, with the order randomized across days. After these two experiments were completed, we used random quadrat sampling on that same day to collect an equivalent number of control hermit crabs within the same area that the experiments were conducted. A total of twenty experiments were carried out, ten for each of the two gastropod flesh conditions, which resulted in a net collection of N = 67 hermit crabs for the trypsin-treated gastropod flesh condition, N = 44 hermit crabs for the pure gastropod flesh condition, and N = 82 control hermit crabs. The collected hermit crabs (both controls and those attracted to the two gastropod flesh conditions) were brought back to the laboratory, where we assessed the relative inadequacy of their shells and then returned them to the field on the same day at high tide.

Laboratory measurements

Each collected hermit crab was induced to vacate its shell by applying heat to the apex of the shell using a soldering iron. The few hermit crabs that failed to exit their shells after several minutes of heat application were removed via gently cracking their shells in a bench vice, which did not harm the crabs. For each shell, we measured shell weight to 0.01 g using an electronic balance, and shell diameter and wall thickness to 0.01 mm using electronic calipers (see Laidre 2012b). We also recorded the presence of any holes or breakages in the shell, as well as any sessile organisms living on or within the shell. For each corresponding hermit crab, we recorded the crab's body weight to 0.01 g, its shield length to 0.01 mm, and noted whether it was missing any appendages or was carrying eggs. 'Shell inadequacy' was defined as the fit of the shell relative to the hermit crab occupant's body, and was quantified as the ratio of crab body weight to shell weight. More inadequate, poorer quality shells weigh proportionately less relative to the crab's weight. Such shells are inferior for marine hermit crabs because they offer less protection (Bertness 1981; Vermeij 1993).

Experiment 4: time until discovery of empty shells without chemical cues

This experiment tested how easily hermit crabs are able to locate empty shells in the absence of any associated flesh chemical cues. Brand new empty gastropod shells (N = 21; with diameters that were medium-sized relative to the population distribution) were acquired by collecting live gastropods from the study site, boiling them to solidify their flesh, and removing each gastropod body in a single coagulated unit, which could be readily pulled out from the shell. Empty shells were then rinsed thoroughly in freshwater and allowed to dry. For each trial, a quadrat was placed at a location in the subtidal using the same criteria as in Experiment 1. Then a randomly chosen empty shell was placed aperture up in the center of the quadrat and left for 1 h. We recorded the number of hermit crabs present in the quadrat at time-zero and after 5 and 60 min. We also continuously filmed each experiment using a GoPro (HERO4 Session) placed on a tripod outside the quadrat. The following data were extracted from the video footage: (1) the time at which the first hermit crab made contact with the shell; (2) whether a hermit crab ever entered the empty shell (an initial 'shell swap'); and (3) if a hermit crab did enter the empty shell, then any additional shell swaps (in which later arriving hermit crabs entered shells left behind by prior crabs). We compared the time hermit crabs first made contact with the empty shell (which had no associated flesh chemical cues) to the results of Experiment 1 (which involved flesh chemical cues). In addition, we tallied the total number of shell swaps, thereby determining how likely it was for empty shells (with no associated flesh chemical cues) to generate 'vacancy chains' (Weissburg et al. 1991), in which subsequent crabs move into shells left behind after other crabs have already swapped.

Predictions and statistical analyses

If hermit crabs face uncertainty in locating empty shells in space, then empty shells should be rare across quadrats. If hermit crabs also face uncertainty in locating empty shells in time, then there should be a superabundance of living gastropods and a rarity of non-destructive shell predators, such that only when these species manage to intersect in space

and time will new empty shells be generated. We hypothesized that hermit crabs would use chemical cues associated with non-destructive predation on live gastropods to reduce their spatio-temporal uncertainty and orient directly to newly available empty shells. Based on this logic, we made a series of predictions:

1. Neither controls (trypsin or live gastropod) nor shell fragments will attract hermit crabs, since controls provide no information and shell fragments only indicate structurally damaged shells.
2. Flesh will attract hermit crabs, especially gastropod flesh compared to mussel flesh, since gastropod flesh derives from sources of useable shells.
3. Trypsin-treated flesh will attract significantly more hermit crabs than pure flesh, since it is indicative of non-destructive shell predation rather than destructive shell predation. However, this effect will only occur if the flesh derives from sources of useable shells (i.e., gastropods and not mussel).
4. Chemical cues that attract hermit crabs to quadrats will operate within temporally narrow time windows due to their efficient diffusion and dissipation, thus providing rapid and reliable information for targeting newly available shells in space and time.
5. The shell inadequacy of hermit crabs attracted to trypsin-treated gastropod flesh will be higher, since these hermit crabs are more motivated for shell acquisition than hermit crab that are attracted to pure gastropod flesh or that are not attracted at all.
6. In the absence of flesh chemical cues associated with predation on gastropods, hermit crabs will have substantially greater difficulty locating empty available shells and will take longer to find these shells, since chemical cues provide the key information source for reducing uncertainty.

To test these predictions, we first performed One-way ANOVAs across all conditions within each experiment. We then compared the before-to-after change in the number of hermit crabs for each condition using paired t-tests. We also used orthogonal contrast tests to (*i*) compare the number of hermit crabs that were attracted across different conditions and (*ii*) compare the relative shell inadequacy of hermit crabs that were attracted or not attracted to different conditions. All statistics were run in JMP® Pro (version 12.1.0). When undertaking multiple tests on the same data, the Bonferroni method was used to control the overall alpha level at 0.05.

Results

Spatio-temporal uncertainty in availability of empty shells

Random quadrat sampling revealed an extreme skew in the abundance of shell predators, empty shells, hermit crabs, and live gastropods (Fig. 2a; One-way ANOVA: $F_{3,407} = 124.25$, $p < 0.0001$). Empty shells were exceedingly rare, constituting a small fraction of the abundance of hermit crabs (Contrast test: $F_{1,404} = 28.35$, $p < 0.0001$). In contrast, live gastropods were the most abundant, significantly exceeding the abundance of hermit crabs (Contrast test: $F_{1,404} = 6.23$, $p = 0.01$). Notably, the extreme rarity of shell predators implied substantial uncertainty in where and when live gastropods might be converted into useable empty shells for hermit crabs via predation. Indeed, while non-destructive shell predators were observed at the study site, they were nevertheless entirely absent in all quadrat samples,

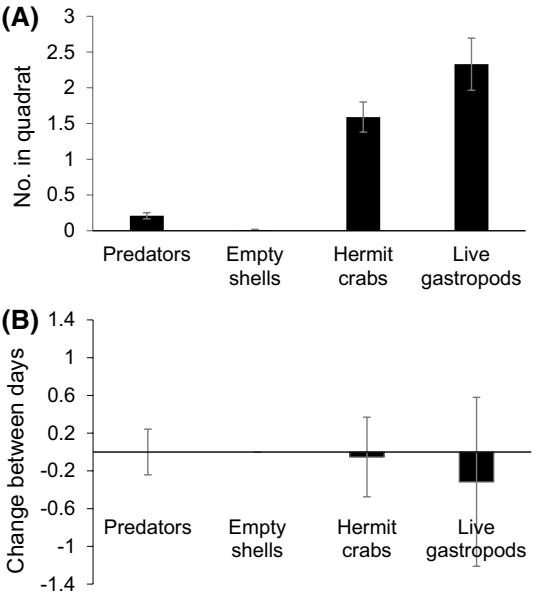

Fig. 2 Spatio-temporal uncertainty in availability of empty shells. **a** Number of predators, empty shells, hermit crabs, and live gastropods (mean ± standard error) found in each quadrat during random quadrat sampling (N = 100 replicates). **b** Change between days in the number of predators, empty shells, hermit crabs, and live gastropods (mean ± standard error) that were found in each quadrat during stationary quadrat sampling, which involved repeatedly sampling the same quadrat across N = 20 days

further underscoring the extreme spatio-temporal uncertainty in where and when new empty shells might become available. Stationary quadrat sampling confirmed these same general patterns as the random quadrat sampling (Fig. S2). In addition, stationary quadrat sampling revealed that despite there being no net change across time in species composition within quadrats, there was nevertheless substantial variability and turnover between days (Fig. 2b and Fig. S3).

Species attracted

Hermit crabs (*Pagurus acadianus*) were by far the most common species attracted to the quadrats. They dominated the scene, with accumulations of up to 47 individuals within the quadrat in a mere 5 min. Hermit crabs immediately approached bottles that contained flesh, climbing onto them and clinging to their mesh-covered openings. In the presence of the gastropod flesh conditions, hermit crabs were also observed performing behaviors used to investigate shells (e.g., "piggybacking" on conspecifics' backs). The sole other species attracted to the quadrats was the green crab *Carcinus maenus*, which only occurred in 5 of the 140 experiments, with at most one green crab attracted in each of those five experiments (one in a pure gastropod flesh experiment, one in each of two pure mussel flesh experiments, and one in each of two trypsin-treated mussel flesh experiments). No other macro-fauna, besides live gastropods, were present within the quadrat during our experiments, hence all further analyses focused exclusively on hermit crabs.

Number of hermit crabs attracted

Whereas no significant difference existed across conditions in the number of hermit crabs in the before count (One-way ANOVA: $F_{6,133} = 0.86$, $p = 0.53$), there was a significant difference across conditions after just 5 min (Fig. 3; One-way ANOVA: $F_{6,133} = 29.83$,

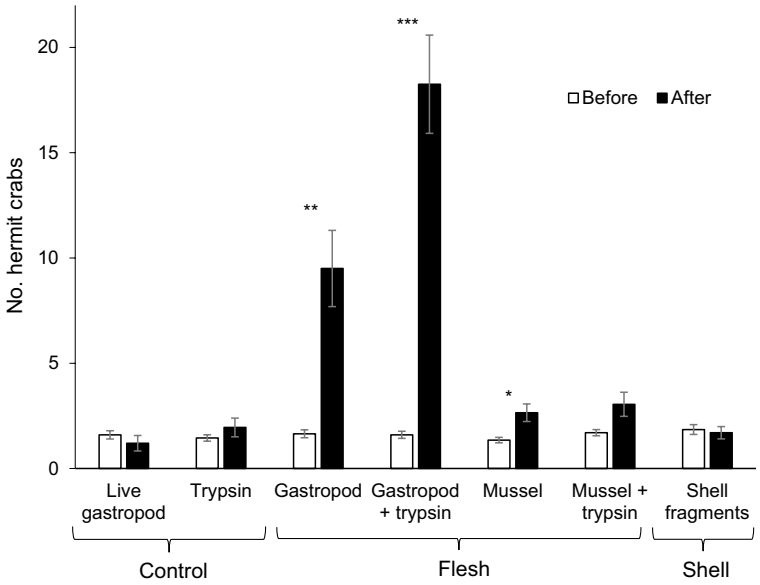

Fig. 3 Attraction to chemical cues of simulated predation. Number of hermit crabs (mean ± standard error) in quadrat before and 5 min after chemical cues were released (seven conditions, each with N = 20 replicates). *$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$

$p < 0.0001$). The number of hermit crabs did not change significantly from before to after for either of the controls or for the shell fragment condition (paired *t* test for trypsin control: t = 1.21, *df* = 19, $p = 0.24$; live gastropod control: t = 1.57, *df* = 19, $p = 0.13$; shell fragments: t = 0.41, *df* = 19, $p = 0.69$). However, three of the four flesh conditions showed significant increases in the number of hermit crabs from before to after (paired t-test for pure gastropod flesh: t = 4.63, *df* = 19, $p = 0.0002$; trypsin-treated gastropod flesh: t = 7.15, *df* = 19, $p < 0.0001$; pure mussel flesh: t = 3.81, *df* = 19, $p = 0.0012$). The only flesh condition that failed to show a significant increase in the number of hermit crabs from before to after was trypsin-treated mussel flesh (paired t test: t = 2.38, *df* = 19, $p = 0.028$; non-significant with Bonferroni correction).

Contrast tests of the number of hermit crabs that accumulated after 5 min revealed the following: (1) The four flesh conditions attracted significantly more hermit crabs compared to the shell fragment condition ($F_{1,133} = 26.68$, $p < 0.0001$). (2) Gastropod flesh (pure and trypsin-treated) attracted significantly more hermit crabs than mussel flesh (pure and trypsin-treated) ($F_{1,133} = 91.32$, $p < 0.0001$). (3) Trypsin-treated mussel flesh failed to attract more hermit crabs than pure mussel flesh ($F_{1,133} = 0.06$, $p = 0.81$). (4) Trypsin-treated gastropod flesh attracted significantly more hermit crabs than pure gastropod flesh ($F_{1,133} = 28.76$, $p < 0.0001$). Finally, (5) trypsin-treated gastropod flesh attracted significantly more hermit crabs than trypsin-treated mussel flesh ($F_{1,133} = 15.72$, $p = 0.0001$).

Extended time

Experiments that extended the timeline from 5 min to 1 h revealed the following (Fig. 4): (1) conditions that failed to attract hermit crabs during the shorter experiments (i.e., both control conditions and the shell fragment condition) qualitatively failed to attract

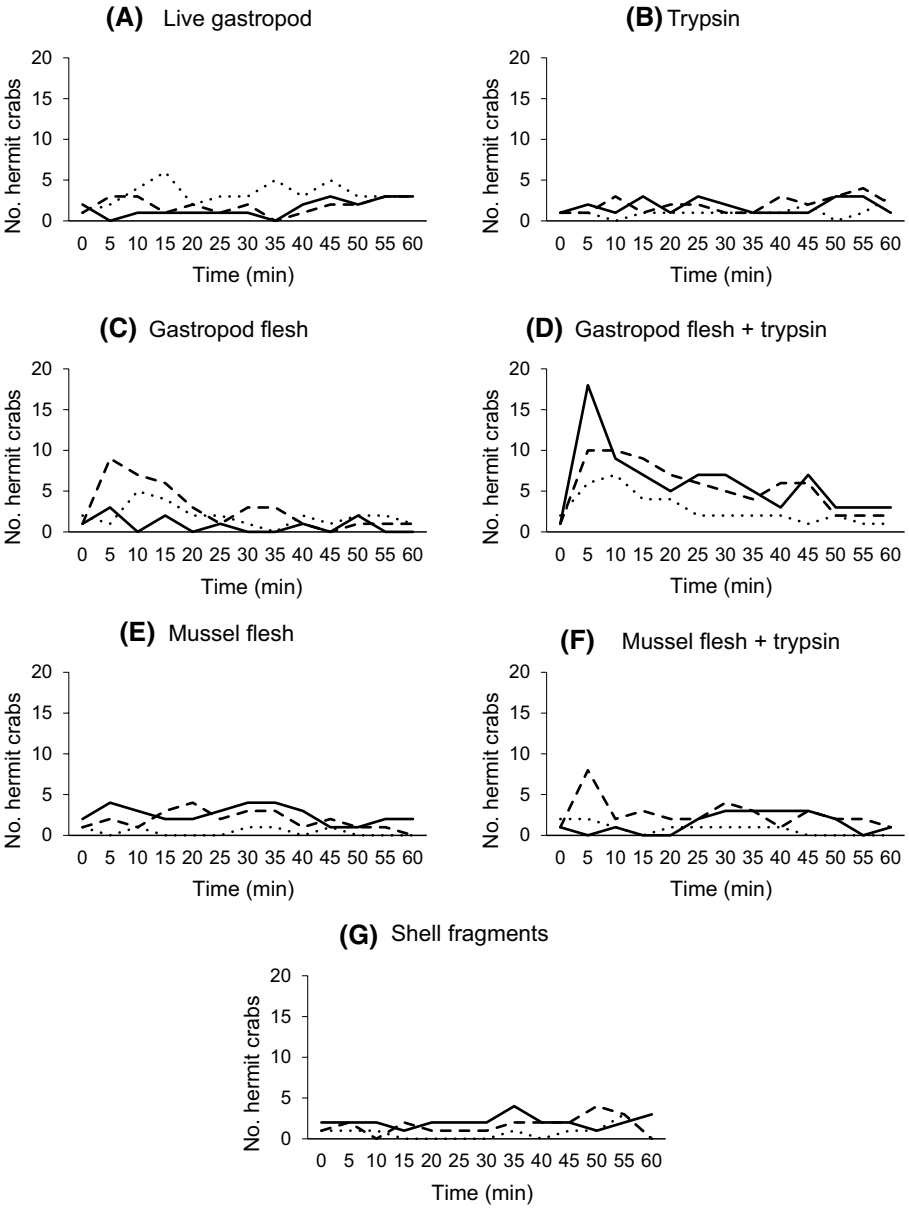

Fig. 4 Extended time experiments. Number of hermit crabs in quadrat across 60 min. Chemical cues were released at time zero and counts were taken every 5 min till the end of the experiment (seven conditions, each with N = 3 replicates designated by a different line)

them during the longer experiments (Fig. 4a, b, g). Furthermore, (2) conditions that did attract hermit crabs during the shorter experiments (i.e., flesh conditions) qualitatively attracted them during the longer experiments, with visible peaks in numbers occurring

shortly after the original experimental length (at approximately 5–10 min), and with these numbers then rapidly declining back to baseline levels thereafter (Fig. 4c-f).

Shell inadequacy of attracted hermit crabs

Hermit crabs that were attracted to trypsin-treated gastropod flesh had inadequate shells, which were smaller relative to their bodies compared to both (1) hermit crabs attracted to pure gastropod flesh and (2) control hermit crabs that were not attracted at all (Fig. 5; Contrast test: $F_{1,190} = 7.80$, $p = 0.0058$). There was no difference in the shell inadequacy of hermit crabs attracted to pure gastropod flesh versus that of control hermit crabs (Contrast test: $F_{1,190} = 0.39$, $p = 0.54$). In contrast, the shell inadequacy of hermit crabs attracted to trypsin-treated gastropod flesh nearly exceeded that of hermit crabs attracted to pure gastropod flesh (Contrast test: $F_{1,190} = 3.67$, $p = 0.0571$) and did exceed that of control hermit crabs (Contrast test: $F_{1,190} = 8.77$, $p = 0.0035$).

Time until discovery of empty shells without chemical cues

When empty intact gastropod shells (not associated with any flesh chemical cues) were experimentally provided, they often were not discovered by hermit crabs. Of N = 21 shells, 19.0% were never contacted over the entire 1 h experiment (the rest of the shells were contacted and were thereafter always entered by a hermit crab, which moved out of its old shell and into the new one). Conservatively assuming contact times of 60 min for the shells that were never contacted within our 1 h experiments, we found that the minimum time till crabs first discovered empty shells was 18.3 ± 4.9 min (mean ± standard error). This minimum was itself several times longer than the relatively short intervals (always < 5 min) for the flesh chemical cue conditions, in which many crabs rapidly oriented to and made contact with bottles containing chemical cues. Notably, the empty shells (which we provided free of chemical cues) failed to generate any accumulation in hermit crabs within the quadrat: there was no significant difference in the number of hermit crabs at time 0, 5, or 60 min (Fig. S4; One-way ANOVA: $F_{2,54} = 0.34$, $p = 0.71$). Finally, the N = 21 empty shells only generated 0.71 ± 0.14 (mean ± standard error) shell swaps per shell.

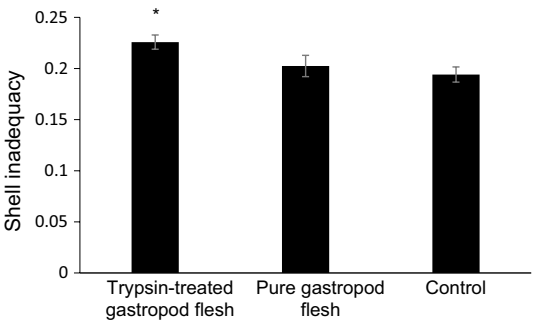

Fig. 5 Shell inadequacy of attracted hermit crabs. Shell inadequacy (ratio of hermit crab body weight to shell weight; mean ± standard error) for individuals attracted to trypsin-treated gastropod flesh (N = 67) or pure gastropod flesh (N = 44), and for control individuals that were not attracted (N = 82). *$p < 0.01$

Discussion

Animals face challenges in locating and acquiring rare but essential resources in space and time (Levin 1992). Resolving this spatio-temporal uncertainty is a crucial determinant of survival and reproductive success (Dall et al. 2005). Our results reveal that in subtidal environments, where empty shell resources are scarce and where visual, tactile, and acoustic modalities are less operable, individuals can exploit chemical cues associated with predation. In particular, chemical cues from non-destructive shell predation help hermit crabs to reduce their spatial and temporal uncertainty, thereby locating one of their most essential resources, new gastropod shells. Individuals possessing inadequate shells were the most attracted to these chemical cues. And critically, these chemical cues not only provide information with spatial specificity, but also have a high degree of temporal specificity, since the attractiveness of these cues rapidly dissipated within minutes. In the absence of such cues, brand new resources, which are both rare and valuable, were more difficult to discover.

Long-distance chemical cues operate in many contexts, from sexual attractants used by mating moths to pollination signals from plants to pollinators (Lawson et al. 2017). The use of long-distance chemical cues likely provides advantages over alternate search strategies (Hussey et al. 2015). For instance, sit-and-wait strategies can be highly inefficient, particularly if resources only sporadically become available (Dall et al. 2005). In the case of empty shells, a sit-and-wait strategy could leave individuals waiting indefinitely for a resource that never materializes within their patch. Similarly, constant movement can be energetically wasteful and may result in individuals departing areas that eventually become 'hot spots' of new resource opportunities. In contrast, search strategies integrating long-distance chemical cues can be highly effective (Breithaupt and Thiel 2011), allowing animals to sample larger spatial scales (Levin 1992) and detect resources that might otherwise be overlooked. Other sensory modalities likely interact with chemical cues over various spatial scales. For instance, over large scales, vibration and noise pollution in subtidal marine environments (Roberts and Elliott 2017) might negatively impact animals' ability to orient or to integrate chemical and multi-modal information. And over small spatial scales (e.g., once resources are within close range) modalities like vision and touch may act in concert with chemical cues (Laidre 2010, 2013; Stevens 2013; Cronin et al. 2014), or even override them in relative importance (Stutz et al. 2017).

Despite the effectiveness of long-distance chemical cues, hermit crabs were not indiscriminately attracted to all chemical cues from mollusk death. Experiments simulating non-destructive shell predation on gastropods were most attractive and, uniquely, these events generate empty, intact, useable shells post-predation. Selective attraction makes adaptive sense, given that predators of shelled mollusks can also prey upon secondary occupants of these shells, like hermit crabs (Vermeij 1993; Alcaraz and Arce 2017). Individuals coming to predation sites therefore face risks of themselves becoming targets of predation, which may trade off against potential rewards of resource acquisition (Gherardi and Tricarico 2011). Notably, the only flesh chemical cue condition that failed to yield a significant before-to-after increase (trypsin-treated mussel flesh) is itself indicative of non-destructive shell predation, but on a mollusk whose shell is never useable by hermit crabs, even when left intact. Individuals may thus avoid predation sites if the cost is not offset by any potential benefit of acquiring a new shell. Furthermore, behavioral responses to chemical cues may be plastically influenced by motivation: for individuals already outfitted with an optimal shell, attraction to predation sites may represent a poor decision, as was found with control crabs, which were not attracted to either pure or trypsin-treated gastropod

flesh. In contrast, for individuals in poorly fitting shells, predation sites, while risky, may represent the only opportunity to replace their inferior shell with a superior one. Future research should explore the potential impact of variable ecological landscapes in generating plastic behavior. Depending on whether the surrounding housing market (Laidre and Vermeij 2012) has a dearth or surplus of empty shells, local behavioral responses might vary dramatically, potentially revealing emergent properties that are linked to the supply and demand of 'biological markets' (Noë and Hammerstein 1994).

Long-distance predatory chemical cues involve not only heterospecific predators, but also conspecific competitors (Hazlett 1981). With many individuals independently orienting to sites of non-destructive shell predation, some may overlap and be forced to compete for the same shell. Indeed, given that marine hermit crabs prefer brand new shells acquired directly from predators rather than used shells previously occupied by conspecifics (Laidre and Trinh 2014), strong pressures exist to arrive at predation sites before others. Individuals that are more sensitive to lower concentrations of the relevant chemical cues or that respond faster will have the best chance of 'beating the crowd' and being first to acquire a new predator-generated shell. This 'beat the crowd' strategy of marine hermit crabs thus places strong selection on increasingly acute chemosensory abilities, but only weak selection on social behavior (Gherardi and Tricarico 2011). In contrast, terrestrial hermit crabs are known to experience strong selection for social strategies (Laidre 2014), which require spending prolonged periods around conspecifics (Laidre 2010, 2013). Unlike marine hermit crabs (which prioritize the newest, structurally strongest shells), for terrestrial hermit crabs the most valuable shells are those previously worn and remodeled by conspecifics (Laidre 2012a; Laidre et al. 2012). These conspecific-derived remodeled shells can be acquired only by socializing (Bates and Laidre 2018) and engaging in ritualized vacancy chains, in which shells are exchanged in groups following the eviction or death of fellow conspecifics. These different resource acquisition strategies of marine versus terrestrial hermit crabs provide a stark evolutionary contrast, each shaped by differing ecologies.

Flesh chemical cues are clearly discriminated by marine hermit crabs, but the proximate mechanism is not fully understood (Breithaupt and Thiel 2011; Gherardi and Tricarico 2011). Our experiments used trypsin to digest flesh. The actual proteolytic enzymes responsible for naturally cleaving gastropod flesh are still unknown, but may involve trypsin-like serine proteases (Rittschof and Cohen 2004) that generate one or a suite of relevant peptides as the key cue. Future mechanistic analyses should provide a more complete characterization of the enzymes used by non-destructive shell predators, as well as the precise peptides that are generated, ultimately testing their specificity in the field.

Subtidal hermit crabs offer a model system for understanding how spatio-temporal uncertainty can be resolved through the exploitation of long-distance chemical cues. These animals evolved within a heterogeneous landscape, in which new resources are rare in space and time, and become available unpredictably. However, this unpredictability can be reduced based on incidental chemical by-products from heterospecific predators. These chemical cues provide the basis for transcending smaller spatial scales and sensing new resource availability over much broader areas. Using this information, individuals can unlock a powerful capacity to localize ecological resources that are critical determinants of evolutionary fitness.

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