Life between predators and a small space: substrate selection of an interstitial space-dwelling stream salamander

S. D. Martin, B. A. Harris, J. R. Collums & R. M. Bonett

Department of Biological Science, The University of Tulsa, Tulsa, OK, USA

Abstract

Physical space is a fundamental habitat constraint for interstitial space-dwelling organisms; however, few studies have examined how physical space variation structures predator/prey interactions within such communities. Streambeds in the western Ozark Plateau are composed of Silurian/Ordovician chert gravel and contain a rich assemblage of interstitial space-dwelling species, including macroinvertebrates, fishes and aquatic salamanders. Here, we used field and lab experiments to examine interstitial space size preference and structure of an Ozark streambed community, with emphasis on the Oklahoma salamander, *Eurycea tynerensis*, and its predators (crayfish) and prey (isopods and amphipods). In the field, we implemented a three by six grid of enclosures containing three distinct size classes of well-sorted substrate (*small*, *medium* and *large*) that represented three categories of interstitial space size. Isopods and amphipods were the most abundant macroinvertebrates at the site, and the average size of isopods increased with substrate size. Salamanders were significantly more abundant in *medium*-sized substrate enclosures, whereas isopods of consumable size and amphipods were more abundant in *small* and *medium* substrate, and crayfish were found exclusively in *large* substrate enclosures. Pairwise choice experiments in the lab showed that salamanders always preferred the largest gravel size (i.e. *large* or *medium* to *small*, and *large* to *medium*). A subsequent experiment performed with uniformly *large* gravel demonstrated that salamanders avoided positions near and adjacent to crayfish. We suggest that the finite interstitial space distribution of Oklahoma salamanders is limited by physical constraints of small, prey-rich spaces, and avoidance of predators and prey scarcity in the interstitial spaces among large substrates. This study demonstrates the strong influence of interstitial space size on community structure and predator/prey interactions in chert-bottomed Ozark streams.

Introduction

Food availability and predator avoidance are often two of the most critical factors that influence habitat selection (Diaz & Carrascal, 1991; Persson & Eklöv, 1995; Levin, Petrik & Malone, 1997). Physical space may also be a significant, but often overlooked, habitat requirement for organisms living in structurally complex environments (Rees, 1972; Miller & Buikema, 1977; Gee, 1982; Orth, Heck, & van Montfrans, 1984; Williams & Moore, 1986). When space requirements vary with body size, a growing individual may select spaces of different sizes (Gee, 1982; Pringle, 1982; Douglas, 1984; Williams & Moore, 1986). Furthermore, ontogenetic variation in prey size selection by predators of varying sizes can strongly influence trophic interactions (Elton, 1927; Woodward *et al.*,

2005; Petchey *et al.*, 2008). However, few studies have examined predator/prey interactions in space structured communities. The benthos of lotic environments is often host to diverse faunas, and variation in the size and quantity of available spaces is likely to influence those species that forage or seek refuge in substrate (Allen, 1959). Such interstitial space-dwelling communities present ideal systems to test for the interactions and limitations of predators and prey when physical space size varies.

The relationship between substrate characteristics and the distribution of interstitial space-dwelling organisms has been well addressed in benthic meiofaunal and macrofaunal communities (Wieser, 1959; Cummins, 1964; Jansson, 1967; Hynes, 1970; Schwinghamer, 1981; Minshall, 1984). Substrate exerts direct physical constraints on organisms, and the

amount of space between particles can determine species distributions (Schwinghamer, 1981). Substrate can also indirectly affect faunal distributions and abundances because its size, sorting and stability affects how much silt or organic matter accumulates (for detritivores), whether algae and plants can grow (for grazers), and the abundance of prey taxa (for predators). Some studies have demonstrated a relationship between organism size and particle size (interstitial space size) when comparing different taxonomic groups (Williams, 1972; Bourassa & Morin, 1995), although organisms that burrow through the substrate are subject to different size constraints (Wieser, 1959; Schwinghamer, 1981). In contrast, only a few studies (based mostly on freshwater amphipods) have demonstrated a positive scaling relationship between body size and particle size (or space size) across the ontogeny of a single species (Pringle, 1982; Williams & Moore, 1986). Freshwater amphipods and isopods can be extremely abundant, and are often an important food source for aquatic vertebrates. However, the structure and dynamics of predator/prey interactions due to ontogenetic changes in body size and space size preference of microcrustaceans (or other organisms) has not been well studied. In fact, there is a paucity of studies on space limitations of interstitial space-dwelling vertebrate predators, and none that examine predatory amphibians in this context.

Many spring-fed streams in the western Ozark Plateau of east-central North America contain deep, homogenous beds of Silurian/Ordovician chert gravel. Chert is a very hard microcrystalline sedimentary rock that breaks by fracturing. Consequently, it deposits in streambeds as coarse, loosely associated substrate with many interstitial spaces. Ozark stream communities are rich with macroinvertebrates, fishes and salamanders that dwell in the interstitial spaces and can even use the porous streambed to seasonally follow the water table below the surface (Hynes, 1970; Tumlison, Cline & Zwank, 1990a; Tumlison & Cline, 2003; Bonett & Chippindale, 2006). This habitat contains some unique and regionally endemic species, but interactions among members of this community are understudied.

The Oklahoma salamander (*Eurycea tynerensis*) is a relatively small species endemic to the Ozarks and is a member of the highly diverse family Plethodontidae (Bonett, 2005). In the western Ozarks, multiple lineages of *E. tynerensis* have independently evolved a permanently aquatic (paedomorphic) life history in association with chert gravel streams (Bonett & Chippindale, 2006; Emel & Bonett, 2011). Although substrate type is important for paedomorphic *E. tynerensis* (Rudolph, 1978; Tumlison *et al.*, 1990a; Tumlison & Cline, 2003; Bonett & Chippindale, 2006) and they avoid embedded substrates (Tumlison *et al.*, 1990a), their microhabitat preferences within streams (especially preferred gravel size) have not been well quantified. *Eurycea tynerensis* feeds on aquatic invertebrates (including amphipods and isopods; Rudolph, 1978; Tumlison *et al.*, 1990a,b); but because they have relatively small mouths, they are gape-limited, prohibiting consumption of larger prey. Size limitations for consuming isopod prey have been shown for other salamanders (Sparkes, 1996). Small aquatic salamanders and larvae are preyed upon by crayfish (Resetarits, 1991; Gamradt, Kats & Anzalone, 1997) and many fishes

(Rudolph, 1978; Petranka, 1983). The behavior and abundance of small aquatic salamanders is known to be influenced by these predators (Petranka, 1983; Resetarits, 1991; Whitham & Mathis, 2000; Bar & Babbit, 2002, 2007; Hickman, Stone & Mathis, 2004; Mathis & Unger, 2012).

Here we examined how an interstitial space-dwelling Ozark stream community is structured with respect to the space size between substrate particles. Although interstitial space is difficult to quantify precisely (Crisp & Williams, 1971), when substrates are well-sorted, the size of substrate particles is a reasonable proxy for the size of interstitial spaces (Wieser, 1959; Jansson, 1967; Crisp & Williams, 1971; Williams, 1972). The relationship between particle and pore size is constant at any scale (Bruce, 1928). Accordingly, we used field enclosures of well-sorted *small*, *medium*, and *large* chert gravel to test how *E. tynerensis* were distributed with respect to interstitial space size, consumable prey, and potential predators. We used laboratory experiments to determine which substrates *E. tynerensis* preferred in the absence of other variables, and how they distributed with respect to potential predators (crayfish). Habitat selection theory predicts that organisms' distributions reflect active selection of patches with available resources (e.g. food or refuge), as opposed to an initially random distribution modified by differential mortality among microhabitats (Fretwell & Lucas, 1970; Brown, 1988; Rosenzweig, 1991). Therefore, we predicted that *E. tynerensis* would select space sizes that maximized spatial overlap with potential prey and minimized interaction with potential predators. We offer insight on how interstitial space habitat structures a multi-tier predator/prey system. Our study is also the first test of space preference in this context for an interstitial space-dwelling amphibian.

Materials and methods

Field experiments

To quantify the substrate size (space) preferences of *E. tynerensis* and other benthic stream species, including potential prey and predators, we conducted a substrate enclosure experiment in a small Ozark stream (Council Hollow, Ottowa County, OK, USA) from October 21 to November 6, 2010. This stream has stable water temperatures (~15°C during the study) and hosts dense populations of paedomorphic *E. tynerensis*, prey such as isopods and amphipods (identification methods in Supporting Information Appendix S1), and potential salamander predators, crayfish (Cambaridae). We prepared 18 enclosures from ~10-L buckets (23.5 cm deep, 22.8 cm diameter), which we filled to the top with gravel from dry gravel bars in one of three sizes: *small*: fit between 4 and 12 mm sieve (average volume 0.2 cm^3); *medium*: slightly larger than 12-mm sieve (average volume 1.7 cm^3); *large*: 'slightly smaller than two inches' (average volume 13.6 cm^3). *Medium* gravel was 8.5 times larger than *small*, and *large* was eight times larger than *medium*. This totaled six buckets filled with each gravel size (*small*, *medium* or *large*). We sank these flush into a relatively uniform section of streambed with weak
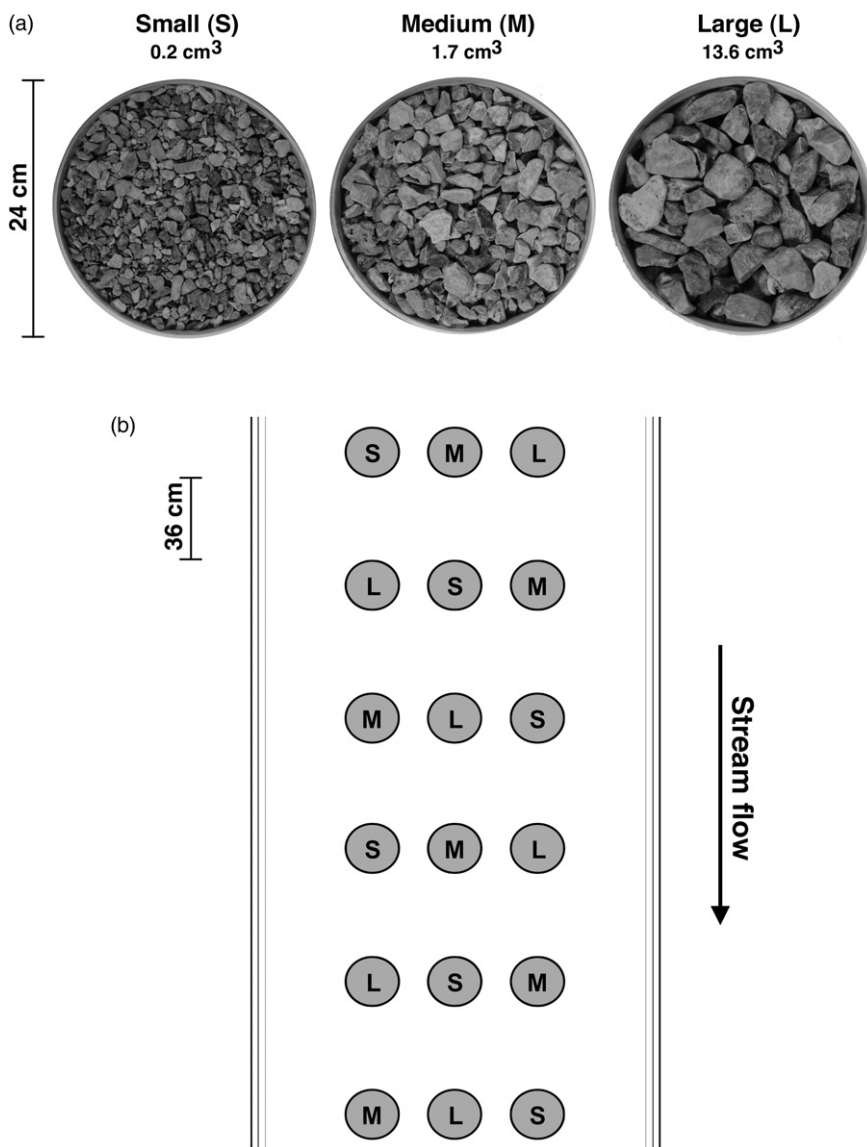
Figure 1 Substrate enclosures and stream experiment layout. (a) Three substrate size classes (*small*, *medium* and *large*) in ~10-L bucket enclosures used in this study (scale 24 cm). The average volume of particles in each size class is shown above the enclosures. (b) Layout of enclosures (gray circles) in the streambed (approximately to scale), with size of substrate listed in each circle. Vertical rows of parallel black lines represent the stream margins, and arrow indicates direction of current in the stream.

current and low embeddedness (Fig. 1); for more details (Supporting Information see Appendix S2).

After 16 days, we removed the enclosures one row at a time (three buckets simultaneously by three researchers), starting downstream and working up to avoid disturbing enclosures downstream. Enclosures were immediately placed into larger (~20 L) buckets to prevent escapes. Invertebrates were removed and preserved in 70% ethanol. Salamanders found in enclosures and additional collected individuals were maintained alive for subsequent laboratory experiments; all vertebrates were handled according to Institutional Animal Care and Use Committee procedures (TU-0029 and TU-0037). We also collected live isopods and amphipods to determine maximum consumable size by *E. tynerensis* (see later). We used one-way analyses of variance (ANOVAs) with Fischer's least significant differences (LSDs) to test for significant dif-

ferences in the average number of individuals recovered from each substrate size class. These substrate preference analyses were performed for *E. tynerensis*; for their potential predators, crayfish (Cambaridae: *Orconectes neglectus*); and for potential prey, isopods (Asellidae: *Caecidotea*) of edible size and amphipods (Gammaridae: *Gammarus*).

Isopods and amphipods are important prey for *E. tynerensis* (Tumlison *et al.*, 1990a,b) and were the most common invertebrates in our study. However, many microcrustaceans collected from our enclosures appeared larger than *E. tynerensis* could consume, so we determined the upper consumable size limit using feeding trials. We photographed and digitally measured the total and pleotelson (most posterior segment) lengths of a size series of 30 live isopods using Motic Images 2.0 (Xiamen, China). There was a strong correlation between isopod pleotelson length and total body length ($R = 0.981$).
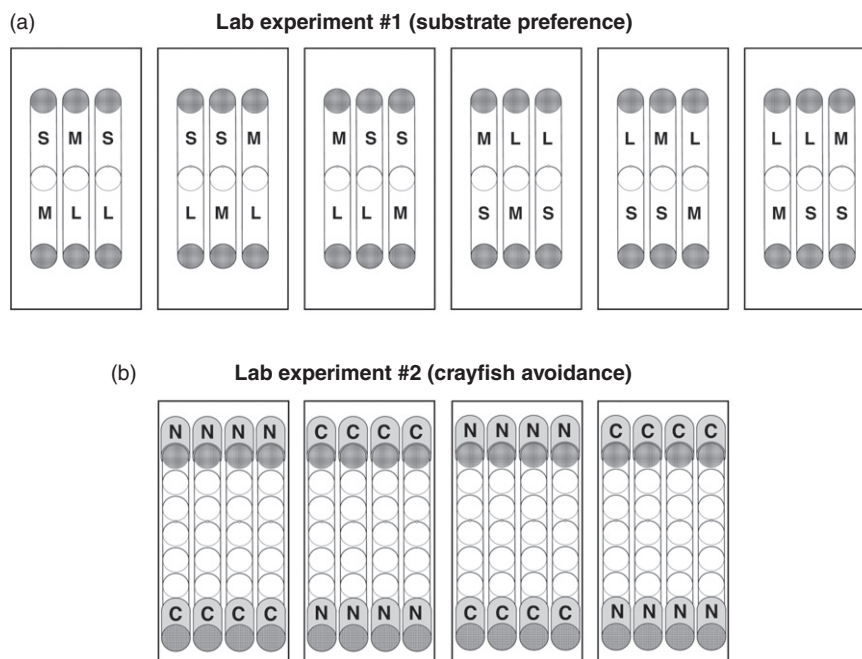
(a)

Lab experiment #1 (substrate preference)

(b)

Lab experiment #2 (crayfish avoidance)

Figure 2 Layout of test chambers used in two lab experiments, from a top-down view. Black rectangles represent ~40-L (10 gal) aquaria; cylinders and aquaria are shown approximately to scale. The cylinders were 1 ft (30.5 cm) in length, and 2 in. inner diameter (51 mm). Ends were sealed with nylon screen. (a) Layout of experiment #1, used to test salamander substrate size preferences. Sizes of substrate in each side of each cylinder are indicated by the letters S, M and L, which correspond to *small*, *medium* and *large* substrates, respectively. A white plastic grid with 15-mm square holes inserted into the cylinder's center kept the two substrates distinct. Each aquarium contained one of each pairwise substrate size comparison, with a total of six cylinders for each comparison, and the direction and order of the three comparisons were alternated. (b) Layout used to test whether salamanders avoided crayfish predators. Sixteen cylinders were marked into six equal sections of 51 mm, and filled with uniformly *large* gravel. Light gray sections on ends of the cylinders are 10.2 cm lengths of polyvinyl chloride, sealed on the outside by nylon screening and separated from the main cylinders by screen. These enclosures contained crayfish (C = crayfish) at one end of each aquaria and were left empty at the other (N = none). Location of crayfish was alternated among aquaria and black circles along each cylinder allowed us to record salamander positions.

Therefore, we used pleotelson length as our metric for isopod body size. Similarly, for amphipods, head lengths can serve as a proxy for total lengths (Pickard & Benke, 1996). To verify this, we photographed and digitally measured the total lengths (body straightened out) and head lengths of a size series of amphipods. There was a strong correlation between amphipod body and head length ($R = 0.955$). We measured the pleotelsons of 720 isopods and the heads of 280 amphipods collected from the enclosures, and used one-way ANOVAs to test for differences in average body size (based on pleotelson or head length) between each substrate size class. Lastly, we placed a live isopod or amphipod in an 11.4-cm square container filled with ~150 mL water and one adult (~27 mm) salamander from the study stream. Salamanders were not fed for a week prior to the trials and were randomly assigned an isopod or amphipod from a range of sizes. Containers were checked daily for 3 days for uneaten microcrustaceans. Of 30 isopods tested, none with pleotelsons ≥ 2.2 mm in length were consumed, establishing a maximum isopod prey size for *E. tynerensis* in this population. Thirty of 32 amphipods were eaten, including some of the largest individuals, so all sizes of amphipods were considered edible.

Lab experiments

To address salamander substrate preferences directly, we conducted behavioral trials using 18 clear plastic cylinders submerged in ~40 L (10 gal) glass aquaria (Fig. 2a; Supporting Information Appendix S3). Each cylinder was filled with two of the three gravel sizes (*small* and *medium*, *small* and *large*, or *medium* and *large* gravel), for three pairwise comparisons. The experiment was performed under a 12-h dark/light schedule and at ~21°C. Salamanders were introduced into the center of the cylinders, and after a 24-h acclimation period, their positions (with respect to the gravel size) were recorded every two light hours for 2 days. Because there were equal numbers of observations for each pairwise comparison (72 total observations), each individual had equal weight, so we pooled the data for greater statistical power. We performed chi-squared analyses to test for frequency differences in salamander preference for each gravel size (considering each of the three comparisons separately).

To address whether salamanders innately avoided large gravel or whether it was a more proximate predator-avoidance response, we conducted another experiment using

16 of the cylinders described earlier. Adult crayfish (*O. neglectus*) of both sexes and uniform sizes were collected from the study site, kept in the lab for 3 days, and fed 1 day prior to the experiment. One crayfish was introduced to each of the polyvinyl chloride compartments at one end of the cylinders in each tank (Fig. 2b; Supporting Information Appendix S3). This provided a gradient with predator compartments at one end and control compartments at the other. Salamanders were introduced into the center of the cylinders and given a 24-h acclimation period with crayfish predators present. Salamanders were monitored for 2 days (bi-hourly, during daylight hours) to determine whether they would be found at lower frequencies in positions near and adjacent to crayfish, for 192 total observations. Their frequencies at each position in the cylinders (1–6) were compared using a chi-squared.

Results

Substrate preferences in the field

Isopods of different sizes colonized *small*, *medium* and *large* gravel ($F_{2, 717} = 62.76$, $P < 0.001$; Fischer's LSDs $P < 0.001$ for all comparisons, Fig. 3a). Sixty-seven per cent of isopods were small enough to be consumed by salamanders (pleotelson length <2.2 mm, Fig. 3b), and these were non-randomly distributed ($F_{2, 15} = 4.57$, $P = 0.028$; Fig. 3c). There were fewer isopods of edible sizes in *large* gravel compared with *small* ($P = 0.018$) and *medium* ($P = 0.021$), but no difference between *small* and *medium* ($P < 0.953$). Based on head length, there was no difference in the size of amphipods that colonized *small*, *medium* or *large* gravel ($F_{2, 264} = 0.29$, $P < 0.749$; Fig. 3d). All amphipods were of edible size, and only a low percentage (<2%) were very large (>1-mm head length; Fig. 3e). Amphipods were also non-randomly distributed among gravel sizes ($F_{2, 15} = 22.35$, $P < 0.001$; Fig. 3f). More amphipods colonized *medium* gravel than *small* ($P = 0.009$) or *large* ($P < 0.001$) gravel, and more amphipods colonized *small* than *large* gravel ($P = 0.002$).

Salamanders and crayfish each primarily colonized a single size class of gravel, and there was little size variation among individuals, preventing us from testing for relationships between body size and space size. Salamander snout-vent lengths averaged 27.75 ± 3.73 mm, and all individuals were large larvae or non-breeding adults. The distribution of salamanders was non-random among substrate sizes ($F_{2, 15} = 14.05$, $P < 0.001$), with a significant preference for *medium* gravel over *small* and *large* ($P < 0.001$ and $P = 0.001$, respectively), and no difference between *small* and *large* gravel ($P = 0.298$; Fig. 4a). Crayfish were juveniles (average carapace length 10.8 ± 2.3 mm) and found exclusively in *large* gravel, and this pattern was also significant ($F_{2, 15} = 10.97$, $P < 0.001$ compared with both *small* and *medium*; Fig. 4b). Two species of darters (*Etheostoma flabellare* and *E. spectabile*) colonized medium and large gravel, but this pattern was not significant ($F_{2, 15} = 1.24$, $P = 0.317$; Fig. 4c).

Salamander substrate choice and crayfish avoidance in the laboratory

In the lab, salamanders demonstrated a preference for the larger gravel size in each pairwise comparison. Salamanders preferred *medium* ($n = 57$) to *small* ($n = 15$) gravel ($\chi^2_1 = 24.5$, $P < 0.001$); *large* ($n = 56$) to *small* ($n = 16$) gravel ($\chi^2_1 = 22.2$, $P < 0.001$), and *large* ($n = 47$) to *medium* ($n = 25$) gravel ($\chi^2_1 = 6.72$, $P < 0.01$). Preference for *large* gravel was stronger when *small* gravel was one of the available choices; salamanders were never observed to actually move among small gravel particles. We found identical patterns of size preference in an earlier pilot experiment using a slightly different design. Salamander distributions in cylinders of uniformly *large* gravel were non-random in the presence of crayfish predators ($\chi^2_1 = 20.3$, $P < 0.001$). They were rarely found in the position closest to crayfish predators, and more frequently at other positions (Fig. 5).

Discussion

Interstitial space and community structure

Physical space exerts strong constraints on the distributions of species (Schwinghamer, 1981) and can be an important parameter for structuring communities. In Ozark chert gravel streams, we found that interstitial space size exerted physical constraints on benthic fauna, and its effects varied among species. The most abundant animals recovered collectively showed a general trend toward increasing space size (substrate size) with body size. Larger organisms (crayfish) were primarily found in *large* gravel, and are probably physically excluded from small spaces; intermediate-sized animals (salamanders) were found in all sizes but primarily in *medium* gravel. Smaller organisms (isopods and amphipods) were most abundant in *small* and *medium* gravel, and average isopod body size increased with increasing interstitial space size. The natural streambed is more heterogeneous than our artificially discrete substrate size categories. However, it is also a matrix with well-sorted areas that vary in average particle sizes, and the trends observed here likely occur along many gradients throughout stream benthos.

Body size has undergone dynamic changes in the evolution of tetrapod diversity (Laurin, 2004), and while many diminutive lineages have evolved, few taxa inhabit small interstitial spaces between substrate particles. To our knowledge, no other studies have examined biotic interactions and space limitations of interstitial space-dwelling tetrapods. Some paedomorphic populations of *E. tynerensis* are among the smallest and most elongate *Eurycea* (Bonett, unpubl. data). Paedomorphic populations of *E. tynerensis* are known to be closely associated with chert gravel streams (Tumlison & Cline, 2003; Bonett & Chippindale, 2006; Emel & Bonett, 2011). Field surveys within and among these streams found salamanders present in sites with 'larger' substrate particles, and absent from habitats that are highly embedded with fine particles (Tumlison *et al.*, 1990a). Our experiments showed that adult *E. tynerensis* rarely occupied small substrates in the field or the
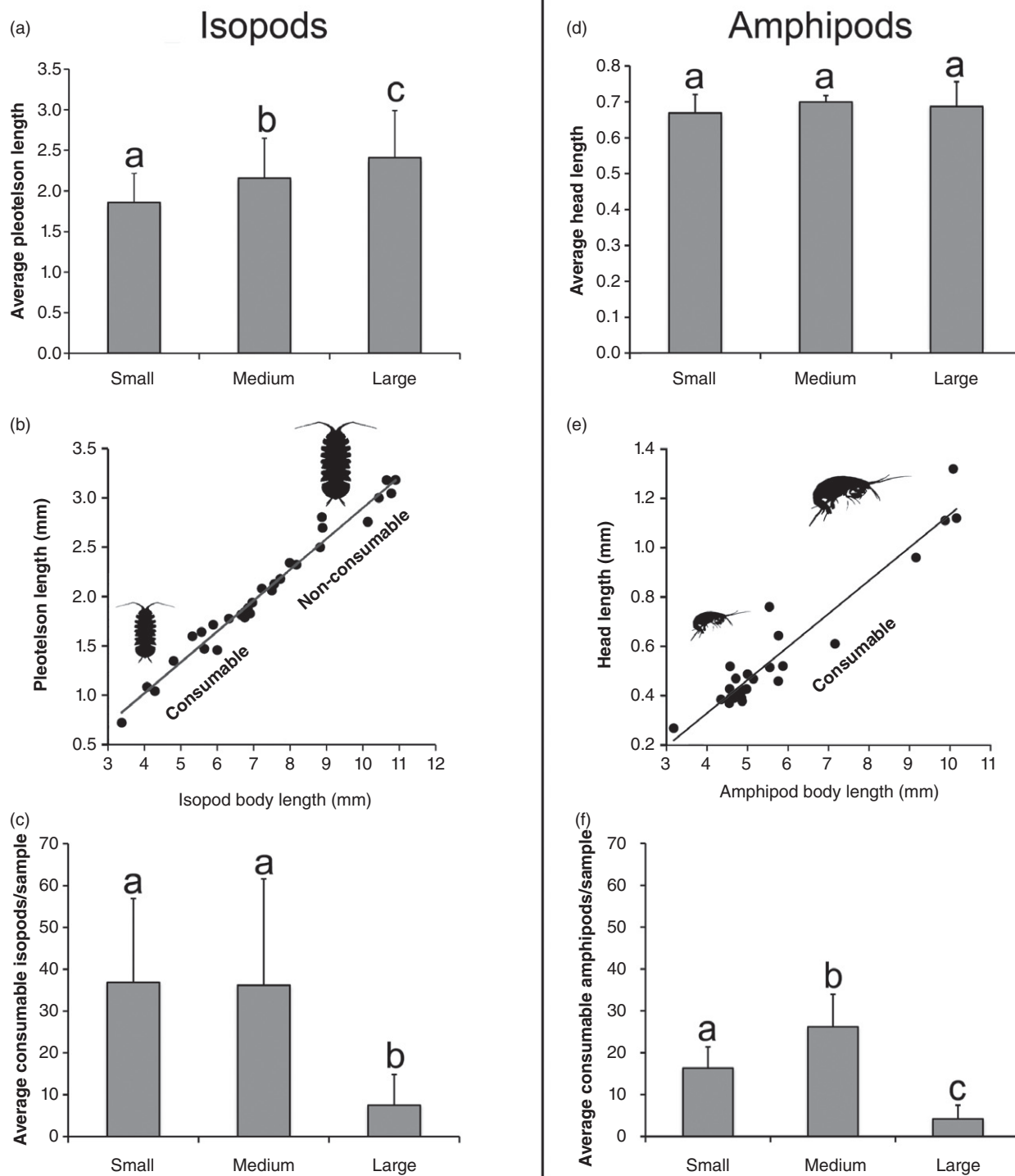
Figure 3 Distributions of microcrustaceans recovered from enclosures and body sizes limiting consumption by salamanders (isopods on left, and amphipods on right). (a) and (d) Average lengths of isopods' pleotelsons and amphipods' heads among individuals recovered from each of the three classes. (b) and (e) Scaling relationship between body size and length of the pleotelson (for isopods) or head (for amphipods). Shaded gray area in each graph indicates which individuals were small enough to be consumed by salamanders during feeding trials. (c) and (f) Distribution of consumable microcrustaceans; average number of individuals small enough to be consumed by salamanders found in each enclosure of a given substrate size class. Data were analyzed by analysis of variance and unique lower-case letters (a, b or c) indicate statistically significant group means based on Fisher's least significant differences ($P < 0.05$).
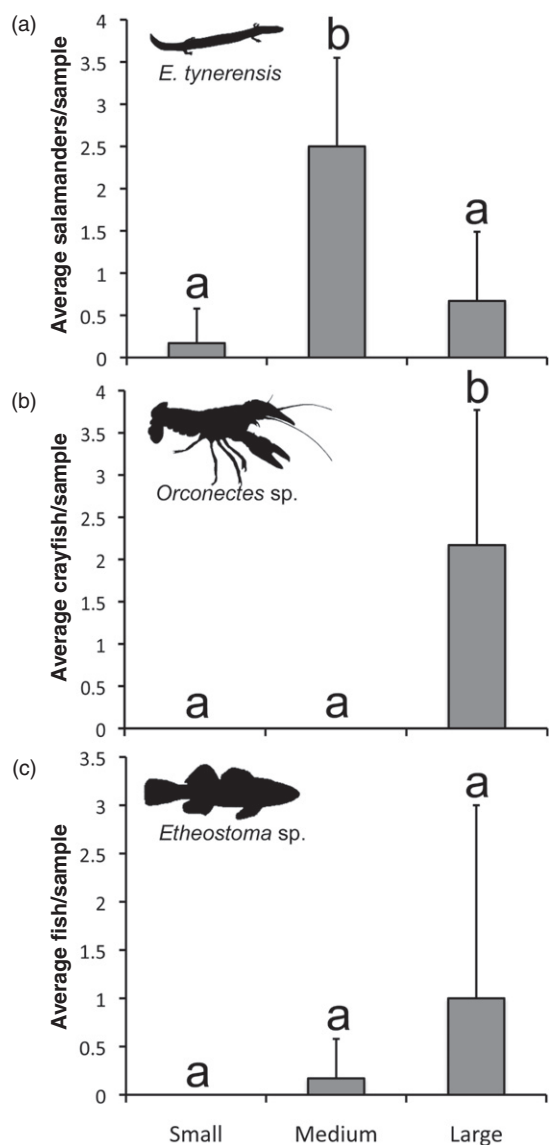
Figure 4 Distributions of vertebrates and crayfish recovered from enclosures containing three substrate sizes. Average number of salamanders (a), crayfish (b) and darters (c) found in each enclosure containing a given substrate size class. Data were analyzed by analysis of variance and unique lower-case letters (a, b or c) indicate statistically significant group means based on Fisher's least significant differences ($P < 0.05$).

Figure 5 Salamander distributions in response to predator presence. Graph indicates the frequency that individual salamanders were located at a given position (1–6), with position 1 being closest to the end of the cylinder containing a crayfish predator, and position 6 farthest from crayfish. Probability is from chi-squared test with 1 degree of freedom.

lab. In our field enclosures, salamanders were found primarily in *medium* gravel, although they preferred larger substrates in lab experiments (*medium* to *small*, and *large* to *medium* and *small*). This is evidence that adult salamanders may be physically excluded from small spaces (based on field and lab experiments), but some other factor caused them to be less abundant in *large* gravel in the field in mid-fall (discussed later).

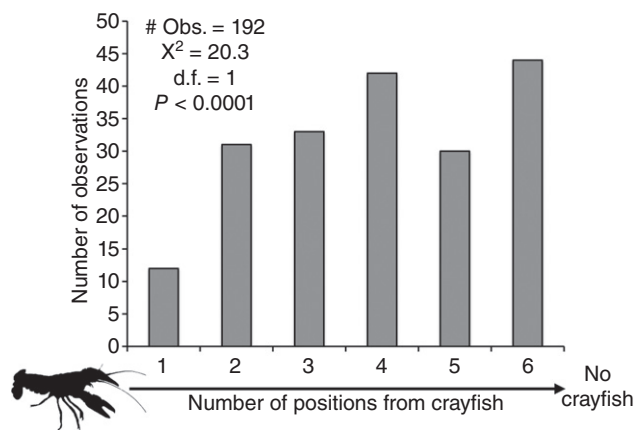The few studies that have examined the interstitial space preferences and limitations of vertebrates have focused on benthic stream fishes. Distributions of such fishes may reflect the availability of coarse substrates (Vives, 1987; Greenberg & Stiles, 1993). Many darters use substrate for refuge and foraging, and therefore prefer coarser substrates with larger interstitial spaces to match their body size (Chipps, Perry & Perry, 1994; Seilheimer & Fisher, 2010). Darters primarily colonized the largest spaces in our field enclosures, but this pattern was not significant, and the individuals were too small to prey on salamanders.

The inflexible exoskeletons of invertebrates may limit interstitial movements among substrate particles; this is particularly true for larger taxa, such as crayfish. Although crayfish can burrow into substrate, the juvenile individuals that colonized our enclosures were found exclusively in the largest gravel size class, and inhabited interstitial spaces between particles larger than themselves. *Orconectes* are known to prefer coarse substrates for shelter (Stein & Magnuson, 1976; Hill & Lodge, 1994; Flinders & Magoulick, 2007).

There was a scaling relationship between space size and isopods recovered from our enclosures; average size of isopods increased in larger spaces, consistent with other reports of ontogenetic shifts in habitat preference among microcrustaceans (Miller & Buikema, 1977; Gee, 1982; Pringle, 1982; Williams & Moore, 1986). Although amphipod size may scale with interstitial space size, we found no difference in the average sizes of amphipods from different substrate sizes, although amphipods were much more abundant in *small* and *medium* substrate size classes. These distributions have implications for the predator/prey interactions within this community.

Predator–prey interactions in interstitial space communities

Food availability and predator avoidance are two of the most important parameters that influence habitat selection (Diaz &

Carrascal, 1991; Persson & Eklöv, 1995; Levin *et al.*, 1997). Because microcrustaceans often represent a significant proportion of stream fauna (Minckley, 1963) and are an important food source for larger animals, their distributions can immediately impact the distributions of higher members in the food chain. Microcrustaceans display a broad range of sizes and have been shown to exhibit a positive scaling relationship between body size and substrate size (this study; Gee, 1982; Pringle, 1982; Williams & Moore, 1986), suggesting they may be subject to changing physical space constraints across ontogeny. As growing individuals move into larger spaces, they will encounter progressively larger potential predators. Here we confirmed an increase in average isopod size with increasing interstitial space size, and as our feeding trials demonstrated, isopods can grow too large for most *E. tynerensis* to easily consume. Isopods of sizes consumable by salamanders were less abundant in *large* gravel. Amphipods were all small enough for salamander consumption, and were also less abundant in *large* gravel.

Eurycea tynerensis feed primarily on the most abundant small invertebrates at any given time, and isopods and amphipods are among their most common prey (Rudolph, 1978; Tumlison *et al.*, 1990b). Larval salamanders in the genus *Eurycea* can detect chemical cues to seek out prey (Petranka, Kats & Sih, 1987), and the distributions of prey among these interstitial spaces may in part predict the presence of *E. tynerensis* (Tumlison *et al.*, 1990a). We found that *E. tynerensis* primarily colonized *medium* gravel in the field; overlapping with the densest concentration of consumable prey. Adult salamanders may have been physically excluded from *small* gravel, where edible prey were also abundant. Although no juvenile salamanders were present during fall, such smaller individuals could potentially access smaller interstices than those in our study. Thus, in fall, prey availability appears to impact habitat selection by *E. tynerensis*, when physical size constraints permit.

In our field experiment, few *E. tynerensis* colonized *large* gravel, where crayfish were most abundant; these are known to prey on larval *Eurycea* (Resetarits, 1991). Tumlison *et al.* (1990a) noted that predatory crayfish were ubiquitous in the interstitial salamander habitats, but did not examine microdistributional patterns. Salamanders may avoid crayfish more locally by using small spaces inaccessible to large crayfish. Salamanders were most abundant in *medium* gravel in the field, even though they preferred the largest gravel in the lab. In the field, *medium* gravel physically excluded crayfish predators. The salamander distributions we observed in the lab may represent predator-avoidance behavior, with significantly lower frequency only in positions immediately adjacent to crayfish. Other larval *Eurycea* are known to modify their habitat selection behavior when predators or predator cues are present (Bar & Babbit, 2002, 2007). *Eurycea tynerensis* avoids habitats where it has encountered predator cues (Mathis & Unger, 2012). Whitham & Mathis (2000) demonstrated that *E. tynerensis* (therein referred to as *E. multiplicata*) reduces foraging activity in the presence of predatory fish cues, but ignores non-predatory fish. Such behavioral changes reduce predation risk for *Eurycea* at the cost of slower growth (Resetarits, 1991). Interstitial life in the highly perforated chert gravel streambeds by paedomorphic *E. tynerensis* may be less impacted by such a trade-off (foraging time vs. predation risk). Instead, finite selection of interstitial space size in this vast benthic network may provide an ideal, prey-rich habitat with minimal predation risk.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Isopod and amphipod identification.
Appendix S2. Field experiment description and images.
Appendix S3. Lab experiment descriptions and images.

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