Top predator removals have consistent effects on large species despite high environmental variability

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Top predator losses affect a wide array of ecological processes, and there is growing evidence that top predators are disproportionately vulnerable to environmental changes. Despite increasing recognition of the fundamental role that top predators play in structuring communities and ecosystems, it remains challenging to predict the consequences of predator extinctions in highly variable environments. Both biotic and abiotic drivers determine community structure, and manipulative experiments are necessary to disentangle the effects of predator loss from other co-occurring environmental changes. To explore the consistency of top predator effects in ecological communities that experience high local environmental variability, we experimentally removed top predators from arid-land stream pool mesocosms in southeastern Arizona, USA, and measured natural background environmental conditions. We inoculated mesocosms with aquatic invertebrates from local streams, removed the top predator *Abedus herberti* (Hemiptera: Belostomatidae) from half of the mesocosms as a treatment, and measured community divergence at the end of the summer dry season. We repeated the experiment in two consecutive years, which represented two very different biotic and abiotic environments. We found that some of the effects of top predator removal were consistent despite significant differences in environmental conditions, community composition, and colonist sources between years. As in other studies, top predator removal did not affect overall species richness or abundance in either year, and we observed inconsistent effects on community and trophic structure. However, top predator removal consistently affected large-bodied species (those in the top 1% of the community body size distribution) in both years, increasing the abundance of mesopredators and decreasing the abundance of detritivores, even though the identity of these species varied between years. Our findings highlight the vulnerability of large taxa to top predator extirpations and suggest that the consistency of observed ecological patterns may be as important as their magnitude.

The importance of top predators in structuring ecological communities is widely appreciated (Terborgh et al. 2001, Duffy 2003, Estes et al. 2011). Their importance, however, does not make them immune to environmental perturbations; there is growing evidence that organisms at higher trophic levels are disproportionately vulnerable to disturbance (Ledger et al. 2013). The combined influences of anthropogenic stressors such as habitat degradation and climate change have negatively impacted top predator populations worldwide (Duffy 2003). Thanks to a rich history of field observations and predator manipulation experiments, we can identify many pathways by which top predator extinctions may impact fundamental community processes such as food web dynamics (Hairston et al. 1960, Thebault et al. 2007) and community assembly (Chase et al. 2009, Vonesh et al. 2009, Wesner et al. 2012). Most top predators are large-bodied relative to the rest of the food web and have correspondingly high resource requirements (Woodward and Hildrew 2002). Small reductions

in top predator abundance can trigger secondary extinctions and modify biotic interactions at lower trophic levels (Borrvall and Ebenman 2006, Säterberg et al. 2013). Reductions in top predator populations are frequently associated with increases in the diversity and abundance of secondary predators (Soulé et al. 1988). This 'mesopredator release' has been documented in terrestrial, freshwater, and marine ecosystems (Baum and Worm 2009, Elmhagen et al. 2010, Ritchie et al. 2012) and is a likely mechanism by which top predator extinctions generate trophic cascades (Prugh et al. 2009).

While the role of predators in community structure and food web dynamics is well-studied, little is known about the consistency of these patterns against a backdrop of high variability in local environmental conditions. It is widely accepted that community structure is determined by a combination of biotic and abiotic factors (Menge and Sutherland 1987, Wellborn et al. 1996). Manipulative experiments have demonstrated that both top predators and

environmental extremes can effectively 'filter' species from the regional species pool into a smaller subset that can survive local conditions (Chase 2007, Chase et al. 2009), thus modifying trophic dynamics (Greig et al. 2012, Ledger et al. 2013). Due to these concurrent biotic and abiotic influences, the effects of top predator extirpations are difficult to predict and become even more obscure when local environments oscillate between environmental extremes. Predation is generally assumed to exert a stronger influence on ecological communities in benign environments than in extreme environments (Peckarsky 1983, Callaway et al. 2002), and the effects of an extreme abiotic environment may obscure patterns generated by top predator extinctions (Wellborn et al. 1996).

Given predictions of increasing environmental variability (Christensen et al. 2007) and anthropogenically-induced predator extinctions (Duffy 2003) in the near future, it is imperative that we understand the effects of top predator extinctions on ecological communities across a range of environmental conditions. Studies examining the relationship between top down effects and environmental conditions demonstrate little consistency in the sign and strength of community responses to predator loss (Borer et al. 2005, Kurle and Cardinale 2011). Ecosystems that exhibit high seasonal and interannual environmental variability can be useful models for examining the consistency of the effects of top predator extirpations, because the regional species pool may remain relatively constant while background conditions naturally vary at a single location.

Arid-land streams are ideal systems to examine the ecological consequences of top predator loss under variable environmental conditions because they occur in regions with naturally high environmental variability (Grimm et al. 1997) and are currently experiencing top predator extinctions due to extreme climatic events (Bogan and Lytle 2011). Climate variability is predicted to increase in North America over the next century, including the frequency, severity and duration of extreme weather events (Christensen et al. 2007). In particular, there is wide consensus among climate change models that drought severity and duration will increase in the southwestern United States (Seager et al. 2007, Balling and Goodrich 2010). These changing drought patterns will intensify the fragmentation of aquatic habitats and degradation of abiotic conditions (e.g. increased water temperature, decreased dissolved oxygen levels) that already occur in these streams on a seasonal basis (Boulton 2003, Bogan and Lytle 2007).

The top predator in most arid-land headwater streams in the southwestern United States is *Abedus herberti* (Hemiptera: Belostomatidae), a large, flightless aquatic insect that is well-adapted to seasonal habitat fragmentation and extreme environmental conditions but cannot survive complete stream drying and has limited dispersal capacity. As a result, *A. herberti* is vulnerable to climate-induced extinction, and local extinctions have recently been recorded in two southeastern Arizona streams, along with widespread changes to local aquatic communities (Bogan and Lytle 2011). To explore the consistency of the effects of top predator extinctions on arid-land stream communities, we experimentally manipulated *A. herberti* presence/absence in replicate mesocosm communities in two years with very

different background environmental conditions. Both manipulations were conducted during the harsh dry season, however the two years represented two environmental extremes as reflected by differences in stream flow, canopy cover, and the composition of the aquatic community. We used these manipulative experiments to test the classic ecological hypotheses that top predator extinctions 1) generate cascading effects on lower trophic levels and 2) increase the richness and abundance of mesopredators (mesopredator release). We predicted that these patterns would be consistent despite strong environmental differences between the two years.

Methods

Study area and species

Our study was conducted in the Chiricahua Mountains of southeastern Arizona, USA, during the dry seasons (May–July) of 2010 and 2011. During these months, streams naturally fragment to a series of small bedrock pools, often separated from one other by dry reaches, and abiotic conditions intensify. The food web in these fragmented pools is numerically dominated by a diverse collection of beetle, dragonfly, damselfly, dobsonfly, and true bug predators, and cannibalism rates are high (Bogan and Lytle 2007). A less diverse group of grazing caddisflies and mayflies make up the herbivore community, and the detritivore class is mostly comprised of small fly larvae, with a few large-bodied taxa consuming leaf litter and other coarse particulate organic matter (Bogan and Lytle 2007). The top predator in these pools, *Abedus herberti*, is a flightless, long-lived (up to 3 years), and large (~3 cm length) true bug that can reach densities of up to 50 ind. m^{-2} in stream pools. Raptorial forelimbs and piercing mouthparts make *A. herberti* a voracious top predator, capable of consuming both invertebrates and vertebrates (Velasco and Millan 1998, Supplemental material Appendix 1 Fig. A1). Recent studies suggest *A. herberti* in adjacent streams are genetically segregated, exhibiting high site fidelity and severe dispersal limitation (Finn et al. 2007, Phillipsen and Lytle 2012).

Mesocosm experiments

We conducted predator manipulation mesocosm experiments in 2010 and 2011 at the American Museum of Natural History's Southwestern Research Station in Portal, AZ, USA. We used 60-l plastic tanks (hereafter referred to as 'mesocosms') to experimentally replicate fragmented bedrock stream pools. We fitted each mesocosm with aluminum flashing to prevent the escape of *A. herberti* and added two cinder blocks per mesocosm to provide aquatic invertebrates with a perch and site for emergence. Mesocosms were filled with well water and arranged in a grid, 25 cm apart (Supplemental material Appendix 1 Fig. A2), approximately 100 m from Cave Creek.

One week prior to the beginning of the experiment in each year, we sampled aquatic invertebrates from Cave Creek, East Turkey Creek, and North Fork Cave Creek (Cochise Co., AZ, USA) using a 500 μm mesh D-frame net,

taking care to sample representative microhabitats (see Bogan and Lytle 2007 for full sampling description) and collect sediment and detritus in each stream. We combined these samples in a 200-l tank to create a diverse inoculum with which to seed the mesocosm communities. In 2010, the inoculum was distributed across 19 containers – 16 were added to the mesocosms and three were preserved in 70% ethanol as initial samples. In 2011, the inoculum was distributed across 24 containers – 20 added to mesocosms and four initial samples. After a one-week acclimation period, we randomly applied control and *A. herberti* removal treatments to half of the mesocosms in each year, yielding eight mesocosms per treatment group in 2010 and ten in 2011. *A. herberti* were removed by hand with small aquarium nets, as they are large and easily targeted. We ensured the effectiveness of our removals by repeating the removal procedure on three consecutive days, although all *A. herberti* were successfully removed with the first attempt. Control mesocosms were standardized to contain 12 adult *A. herberti* each, which mimicked the typical dry season in-stream densities (Boersma unpubl.). The experiments were conducted for the duration of the summer dry season (from stream fragmentation to first monsoon storm), 1 June – 14 July 2010 (six weeks) and 6 June – 8 July 2011 (four weeks). The start of the 2011 experiment was delayed due to a large wildfire in the Chiricahua Mountains, but results from this study and a previous mesocosm study in the same location (Bogan and Boersma 2012) suggest that four weeks was a sufficient duration to allow dry season community composition to stabilize. At the conclusion of each experiment, the contents of each mesocosm were preserved in 70% ethanol and identified to the lowest practical taxonomic level given available keys (Merritt et al. 2008). We measured temperature, dissolved oxygen, and pH for each mesocosm at the end of each experiment.

Environmental conditions

The winter seasons preceding each experiment created very different background stream conditions in 2010 and 2011. In 2010, total Jan–Apr precipitation at the Southwestern Research Station was 114.9 mm (29.4%) above the long-term (1990–2011) Jan–Apr mean, while in 2011 precipitation was 54.4 mm (38.7%) below the mean (long-term mean = 88.8 ± 66.3 mm; Fig. 1). As a result, the source streams had over 10 times greater stream flow in 2010 than in 2011 (e.g. East Turkey Creek: June 2010 = 11 l s^{-1}, June 2011 < 1 l s^{-1}). Despite the dramatic difference in winter precipitation preceding the two experiments and subsequent changes to stream drying trajectories, mean daily rainfall during the experiments did not differ between years (total precipitation: 2010 = 0.58 mm, 2011 = 1.14 mm; Welch's t-test, t = −0.825, DF = 38.356, p = 0.415).

All mesocosms were covered by 60–100% canopy, although the nature of this canopy differed between years. In 2010 mesocosms were located under a natural oak canopy, while in 2011 we constructed artificial shade structures to standardize the canopy across all mesocosms. The artificial canopy consisted of 12×0.9 m strips of opaque shade cloth suspended 1 m above each row of mesocosms. Each strip was separated by 0.3 m to block direct sunlight
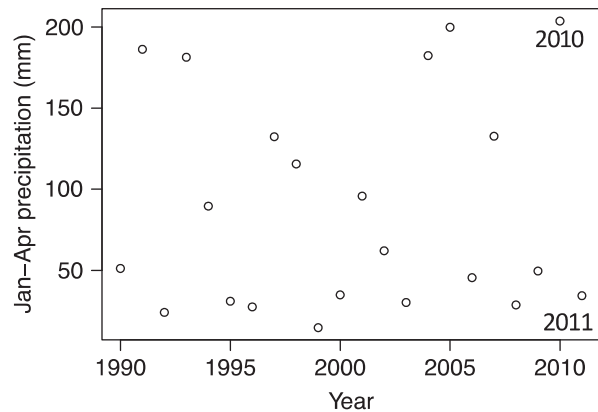

Figure 1. January–April precipitation from 1990–2011. The two years of this study are labeled.

but allow indirect light to reach the surface of the water. Shade cloths extended beyond the mesocosm array on all sides to ensure that both edge and interior mesocosms received approximately 85% canopy cover.

Analysis

Univariate analyses

We compared abiotic conditions (e.g. temperature, dissolved oxygen) between years or treatments using t-tests or their non-parametric equivalents. We compared species richness and abundance between treatments using a Hotelling's T2 test to correct for the potential for type 1 error associated with multiple tests. Variables were transformed prior to comparison when required to meet statistical assumptions. Supplemental material Appendix 2 Table A2.1 provides details on transformations and tests. We used generalized linear models (GLMs) with a Poisson distribution to compare mesopredator species richness between treatments and years because low richness values are Poisson-distributed (Bolker et al. 2009, Zuur et al. 2009), although in all cases GLM inferences were the same as those obtained from Welch's t-tests. Due to the strong environmental differences between years, we compared treatment effects within years only.

Multivariate analyses

We used multi-response permutation procedures (MRPP) to test for differences in community composition and nonmetric multidimensional scaling (NMDS) ordinations to visualize these differences (McCune and Grace 2002). Except where noted, we applied a Wisconsin transformation to the species matrices before ordinating, which first relativizes by species maxima (dividing the abundance of each species in a mesocosm by that species' total abundance across all mesocosms) and then applies a square root transformation to reduce the influence of highly abundant taxa (Legendre and Gallagher 2001). We present results from both two and three-dimensional ordinations, determined to be the best fit in each case based on stress values and convergence. Both MRPP and NMDS employed the Sørensen distance measure (Sørensen 1948). We used indicator species analysis (ISA; Dufrene and

Legendre 1997) to identify representative species for control and top predator removal treatments. Species were considered significant indicators if they had indicator values > 60 and ISA permutation test p < 0.05.

Analyses of initial communities

To quantify initial community composition, we destructively sampled several mesocosms at the beginning of each experiment (2010: n = 3; 2011: n = 4). We used two-sample tests and GLMs to compare initial species richness between years (Supplemental material Appendix 2 Table A2.1) and MRPP to compare initial community composition between years. Small sample sizes limited our power to detect differences between initial communities.

Analyses of colonization patterns

Colonization by aerially dispersing insects is an important driver of community structure in fragmented arid-land streams during the dry season (Bogan and Boersma 2012). One way to identify taxa that colonized mesocosms during the course of the experiment is to compare initial and final invertebrate communities within each year. However, this method cannot differentiate between colonizing taxa and those developing from egg masses present in the initial inoculations (i.e. selective oviposition vs species sorting). We used information from a separate mesocosm study that restricted dispersal and colonization (Boersma et al. in press) and another that recorded colonization of un-inoculated mesocosms (Bogan and Boersma 2012) to create a list of likely colonists for use in this analysis. An additional challenge was that our small number of initial communities limited our ability to detect colonists to only abundant taxa. We defined colonist taxa as those that had abundances of 0 in initial samples and > 10 in final samples, and have been observed as dry-season colonists in other studies (Bogan and Boersma 2012, Boersma et al. in press). Because of our low power to detect differences, we avoided the use of inferential statistics to compare colonists between years and instead examined the identity of colonist taxa. We used NMDS to visualize coarse differences in the composition of colonist taxa between years and treatments.

Effects of top predator removal

To test our hypothesis that top predator removal would generate cascading effects on lower trophic levels, we compared aquatic invertebrate community composition between control and removal treatments within each year using MRPP. We also examined the relationship between experimental treatment and trophic trait composition of mesocosm communities. We created a functional feeding group (FFG) matrix that placed each taxon in a trophic category based on a combination of diet and primary feeding mode (Merritt et al. 2008). FFGs are commonly used to describe aquatic insect trophic niches and facilitate comparisons of community composition among sites with different species (Hauer and Lamberti 1996). We multiplied the transposed FFG matrix (FFG categories × species) by each species matrix (species × mesocosms) to generate abundance-weighted trophic trait matrices (mesocosms × trophic trait prevalence). We used NMDS to visualize the effects of predator removals on community and trophic trait composition

and ISA to determine which species or trophic traits were representative of the treatments.

To test our hypothesis that top predator removal would increase the richness and abundance of mesopredators, we created a subset of the full species matrix that contained only medium- and large-bodied secondary predators ('mesopredators': all non-*A. herberti* predators > 5 mm length, a total of 17 taxa; Supplemental material Appendix 3 Table A3.1). We used this matrix to compare mesopredator richness and abundance between treatments and examine treatment differences in mesopredator assemblage composition using MRPP and NMDS.

To examine consistency in top predator removal effects between the two years, we first compared abiotic conditions, initial samples and colonization between years. Dramatic differences in year and background conditions led us to analyze treatment effects within each year separately; inter-annual comparisons of coarse patterns are presented alongside each year's results below. All statistical analyses were conducted in R with the perm (Fay and Shaw 2010), ICSNP (Nordhausen et al. 2012) and vegan (Oksanen et al. 2012) packages.

Results

Mesocosm water quality

Mesocosm dissolved oxygen and pH at the conclusion of the experiments did not differ between treatments, but mean mesocosm water temperature was significantly higher in 2011 than in 2010 (two-sample permutation test, p < 0.001, Supplemental material Appendix 4 Table A4.1).

Initial inoculations

Mean species richness in the initial samples did not differ between years (Welch's t-test, t = 1.315, DF = 3.732, p = 0.264). Taxonomic composition in these initial samples varied significantly between years (MRPP; A = 0.703, p = 0.030), with twenty taxa unique to 2010 initial samples and nineteen taxa unique to 2011. The 2010 specialists were mostly cold-water, lotic taxa, while the 2011 specialists were warm-water, lentic taxa (Supplemental material Appendix 5 Table A5.1).

Colonization

We found 22 taxa with abundances > 10 in our final samples that were absent from initial samples and determined to be likely colonists from previous studies (Bogan and Boersma 2012, Boersma et al. in press), suggesting that they colonized mesocosms during the course of the experiments. Of these, only two colonist taxa overlapped between 2010 and 2011. The 14 colonist taxa exclusive to 2010 were a diverse mix of larval dragonflies, mayflies, caddisflies and true flies, and small adult beetles, while the six colonists exclusive to 2011 included only adult beetle and true bug species (Supplemental material Appendix 6 Table A6.1). Mesopredators (Supplemental material Appendix 3 Table A3.1) comprised 41% of the colonizing
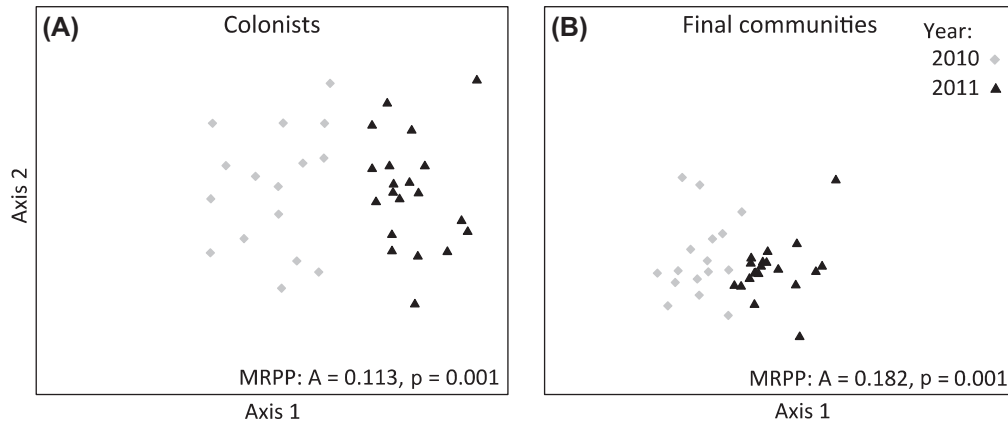
Figure 2. Nonmetric multidimensional scaling ordinations of interannual differences in (A) taxa colonizing during the experiments (square-root transformation, k = 2, Stress = 0.154, p = 0.020, R^2 = 0.877), and (B) overall community composition (Singleton taxa removed, k = 2, Stress = 0.186, p = 0.02, R^2 = 0.829). MRPP tests for interannual differences in community composition.

taxa (as compared to 25% of the overall taxonomic pool) and the identities of these mesopredators also differed between years. The three mesopredator colonists unique to 2010 were soft-bodied dobsonfly and dragonfly predator-engulfers, while the four unique to 2011 were all hard-bodied adult beetle and true bug predator-piercers (Supplemental material Appendix 3 Table 3.1). Despite the small initial sample sizes, MRPP and ordinations confirm that there was little overlap in colonist community composition between years (MRPP: A = 0.113, p = 0.001; NMDS: k = 3 axes, R^2 = 0.872, Stress = 0.134, p = 0.039; Fig. 2A).

Final community samples

We identified 91 invertebrate taxa overall, including initial samples: 74 in 2010 and 57 in 2011. We identified a total of 64 taxa in the final samples taken at the end of the experiments (53 in 2010 and 39 in 2011). On average, final mesocosm samples in 2011 contained fewer species than mesocosms in 2010 (mean richness: 2010 = 20.84, 2011 = 13.21; Welch's t-test, t = 10.203, DF = 26.924, p < 0.001). Final mesocosm community composition also differed between years (MRPP: A = 0.181, p = 0.001; NMDS: R^2 = 0.837, Stress = 0.183, p = 0.020; Fig. 2B). The significant differences between 2010 and 2011 led us to conduct the analyses of top predator removal effects on each year separately.

Due to our small number of initial samples, we consider our estimates of species loss through time to be conservative. Overall species richness did not significantly differ between initial and final samples in 2010 (initial = 24.33, final = 20.125; Welch's t-test, t = −2.205, DF = 2.999, p = 0.115; Supplemental material Appendix 7 Fig. A7.1), nor did the species richness of mesopredators (initial = 1.33, final = 2.19; GLM Poisson: z = −0.938, p = 0.348). In contrast, in 2011 we observed significant declines in species richness (initial = 21.5, final = 11.55; Welch's t-test, t = −8.973, DF = 4.418, p < 0.001; Supplemental material Appendix 7 Fig. A7.1) and mesopredator species richness (initial = 3.5, final = 1; GLM Poisson, z = 3.595, p < 0.001) between initial and final samples, despite the small number

of initial samples. Species that disappeared during the course of the experiments in both years were mostly cold-water, lotic taxa including black flies, stoneflies, mayflies, and caddisflies, however we did not record emergence/consumption so cannot speculate on the mechanism behind their disappearance.

Hypothesis 1: Top predator removal generates cascading effects on lower trophic levels

We found no effect of top predator removal on the univariate metrics of species richness and abundance in either year (Table 1). In 2010 there were no significant differences between control and removal treatments in the composite richness/abundance variable (Hotelling's T^2, T^2 = 0.9255, DF 1 = 2, DF 2 = 13, p = 0.421). Similarly, there were no significant differences between treatments in 2011 (Hotelling's T^2, T^2 = 0.366, DF 1 = 2, DF 2 = 17, p = 0.699).

Despite the lack of a pattern in richness/abundance, top predator removal affected invertebrate community composition, especially for large taxa. Top predator removal caused a statistically significant difference in overall community composition in 2010 (MRPP: A = 0.063, p = 0.020; NMDS: k = 3, Stress = 0.13, p = 0.020, R^2 = 0.835; Fig. 3A) but clustering was only marginally significant in 2011 (MRPP: A = 0.038, p = 0.060; NMDS: k = 3, Stress = 0.148, p = 0.020, R^2 = 0.775; Fig. 3B). The large (> 10 mm) detritivore shredder caddisfly *Phylloicus mexicanus* was an indicator species for the control treatment in both

Table 1. Diversity metrics for mesocosm communities subject to experimental top predator removal.

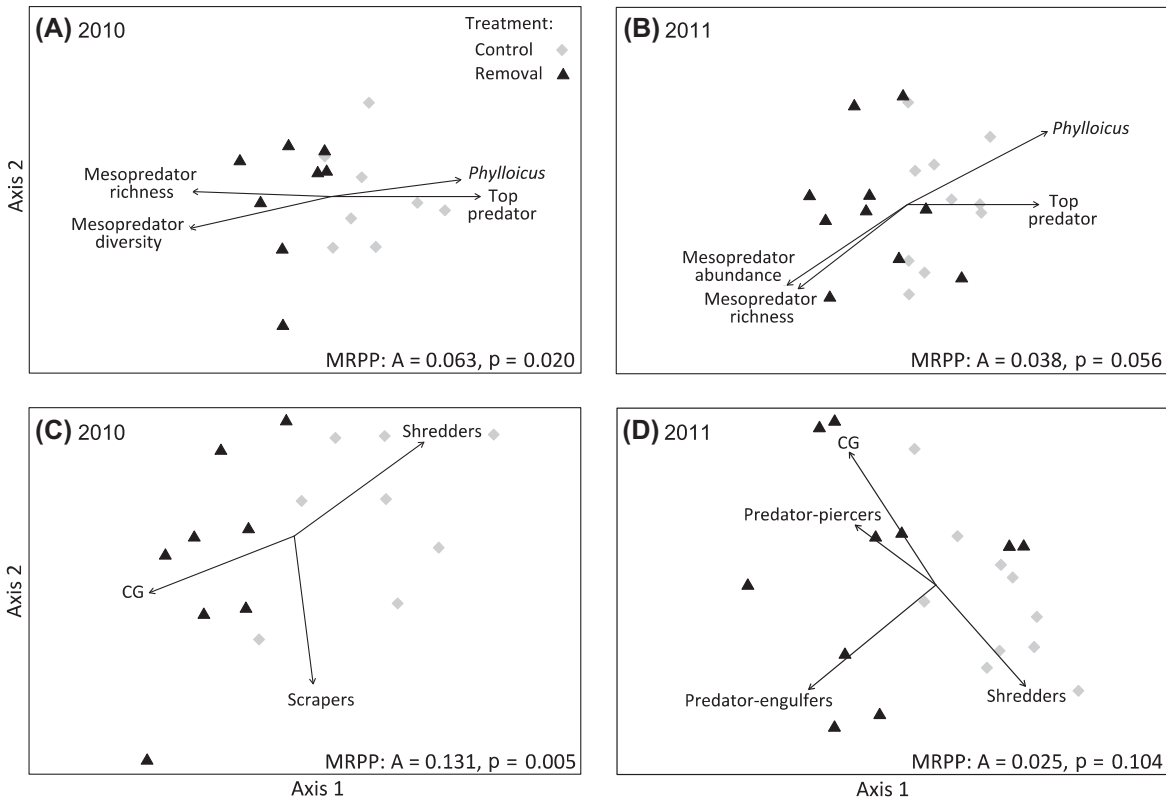| Year	Total species richness	Mean mesocosm species richness	Mean mesocosm abundance
2010	53	20.13	4564
control		19.13	4957
removal		21.13	4170
2011	39	11.55	2368
control		11.2	2392
removal		11.9	2343

Figure 3. Nonmetric multidimensional scaling ordinations of the effects of experimental top predator removal on: (A) community composition in 2010 (k = 3, Stress = 0.13, p = 0.020, R^2 = 0.835), (B) community composition in 2011 (k = 3, Stress = 0.148, p = 0.020, R^2 = 0.775), (C) trophic composition in 2010 (k = 3, Stress = 0.0598, p = 0.020, R^2 = 0.972), and (D) trophic composition in 2011 (k = 3, Stress = 0.089, p = 0.020, R^2 = 0.939). We facilitated interannual comparisons of community composition by rotating each NMDS ordination to align with a vector representing the abundance of *A. herberti* in the final mesocosm samples, as reproduction and natural mortality generated some variability in predator counts. For each three-dimensional ordination, we present the two axes that captured the most variability along the *A. herberti* abundance axis. In panels (A) and (B), vectors represent correlations between axis scores and community statistics (p < 0.05), where 'Mesopredator richness' = no. of predator taxa > 5 mm, 'Mesopredator diversity' = Shannon diversity of predators > 5 mm, '*Phylloicus*' = abundance of *P. mexicanus*, and 'Top predator' = abundance of *A. herberti*. In panels (C) and (D), vectors represent correlations between axis scores and abundance-weighted trophic groups (p < 0.05). The predator groups in panels (C) and (D) represent all predators > 5 mm. Each three-dimensional ordination was rotated so that its first axis was parallel to the top predator abundance vector, and only axes 1 and 2 are presented here. MRPP tests for treatment differences in community composition.

years (2010: I.V. = 73, p = 0.037; 2011: I.V. = 73, p = 0.029), and in 2010 the control treatment was also represented by another shredder *Helichus triangularis* (I.V. = 75, p = 0.043). Two taxa were indicators of the top predator removal treatment in 2010: the large herbivorous collector-gatherer mayfly *Callibaetis* (I.V. = 77, p = 0.017) and predaceous diving beetle *Rhantus atricolor* (I.V. = 63, p = 0.026). There were no significant indicators of the removal treatment in 2011 (Supplemental material Appendix 8 Table A8.1). All of these indicator taxa were larger than 5 mm, suggesting a potential selective impact of top predator removal on large taxa. Interestingly, all of the 16 insect taxa exclusive to the predator removal treatment were also > 5mm, with the exception of two species of flies (Supplemental material Appendix 5 Table A5.1).

Top predator removal caused communities to significantly differ in their trophic trait composition in 2010 (MRPP: A = 0.131, p = 0.005; NMDS: k = 3, Stress = 0.060, p = 0.020, R^2 = 0.972; Fig. 3C) but not in 2011 (MRPP: A = 0.025, p = 0.104; NMDS: k = 3, Stress = 0.089,

p = 0.020, R^2 = 0.939; Fig. 3D). ISA of trophic traits revealed that shredders were associated with the control treatment in both 2010 and 2011 (Supplemental material Appendix 8 Table A8.1). Collector-gatherers, including many of the soft-bodied prey species like mayflies, were associated with top predator removal in 2010 (I.V. = 76.4, p = 0.009), and predators with piercing mouthparts were associated with top predator removal in 2011 (I.V. = 70.5, p = 0.010).

Hypothesis 2: Top predator removal increases the richness and abundance of mesopredators
Top predator removal increased mesopredator abundance in 2010 (Welch's t-test, t = −2.887, DF = 13.763, p = 0.012) and 2011 (Welch's t-test, t = −2.231, DF = 17.686, p = 0.039; Fig. 4B). In 2010, top predator removal increased mesopredator richness (GLM Poisson: z = 2.743, p = 0.006), but in 2011 there was not a statistically significantly difference in treatment means (GLM Poisson: z = 1.736, p = 0.082; Fig. 4A). Mesopredator colonization of the removal treatment mesocosms in
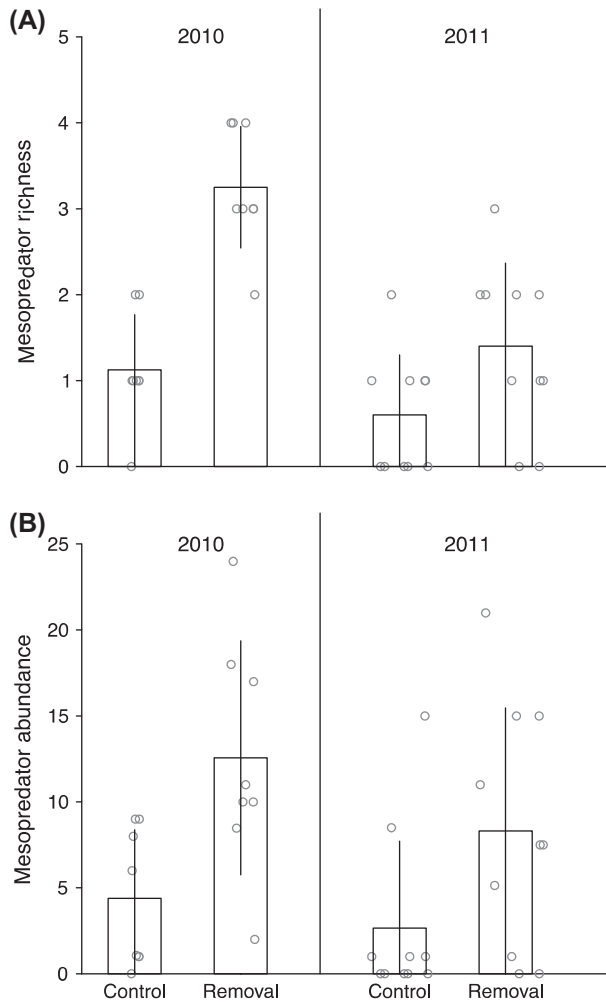
Figure 4. Mesopredator richness and abundance in control and top predator removal treatments in 2010 (left) and 2011 (right). (A) Mesopredator richness in final samples. (B) Mesopredator abundance in final samples. Mesopredators = all predatory taxa >5 mm with the exclusion of the top predator (Supplemental material Appendix 3 Table A3.1). The grey circles represent the jittered values for each mesocosm.

2010 may explain the significant increase in mesopredator richness in that treatment (Fig. 4A).

Notably, the largest mesopredators (diving beetles and dobsonflies ≥10 mm in length) were only found in the removal treatments in both years. In 2010, these were diving beetles *Dytiscus*, *Rhantus atricolor* and *R. gutticollis gutticollis*, and dobsonfly *Neohermes*, while in 2011 these were diving beetles *R. atricolor* and *Dytiscus*, and the dobsonfly *Corydalus*. Two large dragonfly taxa were also present only in top predator removal mesocosms (Supplemental material Appendix 5 Table A5.1). These species-specific responses to predator removal between years contributed to significant differences in overall mesopredator assemblage composition between years (MRPP: A = 0.044, p = 0.001). Of the 17 mesopredator species identified, only six species were present in both years while the remaining 11 mesopredator species were unique to one year or the other (Supplemental material Appendix 3 Table 3.1).

Discussion

We measured the effects of top predator removal on aquatic community structure in two years with very different background environmental conditions. We found consistent top down effects of top predator loss in both years, especially for large taxa. Top predator removal decreased the abundance of large detritivores, increased the abundance of mesopredators, and generated different colonization patterns between treatments in both years. Trophic cascades vary in strength across studies, scales and ecosystems (Borer et al. 2005), and the top–down effects of predators on ecological communities are highly context dependent (Pace et al. 1999, Holt 2000, Chase et al. 2010, Kurle and Cardinale 2011, Coll and Hargadon 2012). Our results suggest that the effects of top predator extinctions on communities may remain consistent despite significant environmentally-driven variability in community composition, and that body size may be an important determinant of the strength of top–down effects on communities.

H1: Top predator removal generates cascading effects on lower trophic levels

We found consistent effects of top predator removals on large taxa in both years, even though the treatment effect on overall community composition was not consistently strong between years. Body size correlates with many important physiological, behavioral, and life history traits (Woodward et al. 2005) and is known to influence the vulnerability of organisms to disturbances and the stability of food webs (Emmerson and Raffaelli 2004). Large-bodied species exert powerful influences on ecosystem processes in streams (Lecerf and Richardson 2011), and droughts and warming have been documented to disproportionately impact large taxa in aquatic systems (Daufresne et al. 2009, Woodward et al. 2012). Thus it is notable that an effect of top predator removal on large taxa was consistently strong in this arid-land aquatic system in both years despite high environmental variability. In our mesocosms, 99.2% of the individuals were less than 9 mm total body length, yet the indicator species and all of the 16 insect taxa exclusive to the predator removal treatment were >5 mm, with the exception of two species of flies (Supplemental material Appendix 5 Table A5.1). Two detritivores were indicators of the control treatment, and two mesopredators and one herbivore were indicators of the removal treatment; all species >5 mm.

While the two treatments contained different large species, they did not differ in overall species richness or abundance in either year. Researchers have documented both homogenizing and diversifying effects of top predator removal on community structure, depending on the context and the system (Paine 1966, Creed 2006, Chase et al. 2009, Sieben et al. 2011). Our finding of no top predator effect on overall diversity is consistent with observations from nearby fragmented streams with similar community composition. In a study examining the effects of stream drying on aquatic invertebrate community structure, Bogan and Lytle (2011) sampled before and after the local extinction of *Abedus herberti* and found no change in species richness, although they did observe shifts in community

composition similar to those seen in our manipulative experiments: they recorded an increase in the abundance of mesopredators and the disappearance of the detritivore caddisfly *Phylloicus mexicanus* (Bogan and Lytle 2011).

One of the consistent effects of top predator removal was a reduction in the abundance of large-bodied detritivores (>10 mm). Removal of *A. herberti* caused significant decreases in the abundance of the caddisfly *P. mexicanus* and the long-toed water beetle *Helichus triangularis*, two important consumers of coarse particulate organic matter (Merritt et al. 2008). Similar cascading effects of top predator loss on detritivores have been observed in other systems (Ruetz et al. 2002, Wu et al. 2011); these 'apparent trophic cascades' are still relatively understudied despite their importance for food webs (Moore et al. 2004). Reduced abundances of large detritivores may slow decomposition rates and limit the conversion of coarse particulate organic matter into fine particulate organic matter for consumption by lower trophic levels (Ruetz et al. 2002) and ultimately affect food web stability (Moore et al. 2004).

H2: Top predator removal increases the richness and abundance of mesopredators

Mesopredators were more abundant in removal treatments than control treatments in both years, lending support to the hypothesis of mesopredator release (an increase in the density or abundance of secondary predators caused by the removal of apex predators; Prugh et al. 2009, Ritchie and Johnson 2009). Theoretically, mesopredators could fill a trophic niche left vacant by generalist top predators and dampen the effects of top predator removal, however this is rarely seen in natural systems (Chalcraft and Resetarits 2003, Prugh et al. 2009). We observed effects of top predator removal on community structure despite mesopredator release, suggesting that mesopredators and top predators are not functionally equivalent in our system.

Mesopredator abundance reflects only one aspect of mesopredator influence on communities (Byrnes and Stachowicz 2009). Research suggests that predator identity, feeding behavior and assemblage composition may be more important determinants of how predator impact will be transferred through food webs (Schmitz and Suttle 2001, Chalcraft and Resetarits 2003). The significant differences between treatments we observed in the mesopredator community suggest that taxon-specific mesopredator responses to top predator removal may also be important components of the overall community responses. Top predator removal increased the abundance of large, active hunting predators (diving beetles and dobsonflies) in both years, although we only detected increases in numbers of sit-and-wait predators (dragonflies, damselflies, and true bugs) in the 2010 removal treatment. The distinct feeding behaviors of these two groups suggest that they may affect community and trophic structure differently (Schmitz and Suttle 2001). Most large diving beetles and dobsonflies are mobile predators that can hunt in pelagic or benthic habitats and consume both live and dead prey, while dragonflies and damselflies are sit-and-wait predators that capture live prey (Turner and Chislock 2007). The palatability of these two colonist groups to *A. herberti* also differed.

In a series of feeding trials, we observed *A. herberti* feeding on the softer-bodied dragonflies in >50% of trials but never feeding on the harder-bodied diving beetles (n = 24 feeding trials; Boersma unpubl.). These highly edible dragonflies were the primary mesopredator colonists of top predator removal mesocosms in 2010 but were nearly absent in 2011. Other studies have demonstrated that predator feeding behavior determines how predator impacts are transmitted through food webs (Klecka and Boukal 2013). The importance of mesopredator identity in our study suggests that feeding mode may in part determine the strength of trophic cascades and explain the weaker overall community divergence observed in 2011 when compared with 2010. The piercing-and-sucking feeding mode of the top predator may also be an important factor in the strength of top–down effects. This feeding mode allows *A. herberti* to consume prey that are larger than itself and releases it from gape size limitations typical of many predatory species. Similar effects of feeding behavior on trophic cascade strength have been observed in terrestrial and marine systems as well (Schmitz et al. 2004, Bruno and O'Connor 2005).

Mesopredator release and predator feeding behavior are also likely mechanisms behind the reduced abundance of large detritivores in our top predator removal mesocosms. The detritivorous caddisfly *P. mexicanus* is a long-lived univoltine species with a reproductive cycle much longer than the duration of our experiments (Wiggins 1977). Therefore, the treatment differences we observed were due to loss of individuals from the top predator removal mesocosms (via emergence or predation) and not to gain of individuals (via colonization or reproduction) in the control mesocosms. Nislow and Molles (1993) demonstrated that larval caddisflies with cases made of organic matter are regularly consumed by large dragonfly nymphs. Presence of the top predator *A. herberti* may have inhibited dragonfly colonization in our control mesocosms and released *P. mexicanus* from predation. Therefore, local direct and indirect relationships between top predators, mesopredators and detritivores may determine the sign of the effect of top predator removal on detritivores (Wu et al. 2011).

Aquatic invertebrate dispersal abilities vary greatly among arid-land stream species (Bogan and Boersma 2012), and it is likely that this variability also contributed to the differences in mesocosm colonization between years. Mean canopy cover was similar in both years, but the artificial canopy used in 2011 was positioned 1m above the water surface and the natural canopy in 2010 was 2–3 m above the water surface. Many aerially-dispersing aquatic invertebrates use polarized light reflected off of water to find suitable colonization sites (Csabai et al. 2006); this reflective cue may have been visible to dispersing dragonflies when mesocosm canopy was relatively high (2–3 m) in 2010 but not when it was low (1 m) in 2011.

Despite the potentially confounding effect of canopy on colonization and the low power to detect differences in colonizing insects, top predator removal affected mesocosm colonist identity in both years, particularly that of large mesopredators (>10 mm). Large predators can affect prey species both directly (i.e. consumption) and indirectly (i.e. antipredator behavioral changes: Dill 1987, Schmitz and Suttle 2001, Boersma et al. 2008). Several studies have

demonstrated that aquatic invertebrates perceive predator cues and can select oviposition sites to minimize predation risk (Vonesh et al. 2009, Wesner et al. 2012), and it is likely that selective oviposition played a role in colonization processes in our experiments as well. In fact, three species of dragonfly and damselfly larvae and two species of dobsonfly larvae colonized only top predator removal mesocosms, suggesting that they either did not disperse to or could not establish populations in control mesocosms. Our experimental design did not allow us to differentiate between selective oviposition and predation, and further experiments are needed to elucidate the relative influence of these mechanisms on aquatic community structure and ecosystem functioning (Vonesh et al. 2009).

While other aquatic ecologists have replicated top predator removal experiments across environmental gradients (Greig et al. 2012) and examined the effects of extreme abiotic environments on aquatic top predators (Woodward et al. 2012, Ledger et al. 2013), to our knowledge our study is the first to examine the consistency of the effects of top predator removals in the context of extreme natural interannual variability. We demonstrated that top predator removals consistently affected large aquatic taxa of multiple trophic groups despite marked differences in initial community composition and background environmental conditions. Large taxa have strong and often complex influences on ecosystem functioning (Lecerf and Richardson 2011) and are disproportionately susceptible to abiotic changes (Daufresne et al. 2009, Woodward et al. 2012). Our findings highlight the vulnerability of large taxa to biotic changes as well. Finally, we suggest that if global environmental variability increases as climate predictions suggest, the consistency of observed ecological patterns may be equally important to the magnitude of their effects.

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