The Effects of Experimental Habitat Fragmentation on the Generalist Predator, the Funnel-web Spider, *Atrax sutherlandi.*

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Abstract

Habitat loss and fragmentation is the leading cause of biodiversity loss. Metacommunity theory predicts that habitat fragmentation should reduce food-chain length and food-web complexity. Generalist predators' diets integrated over time should reflect these changes to food-webs. I investigated how habitat fragmentation affects the abundance and distribution, trophic position, and stable isotopic niche of the generalist predator, the funnel-web spider *Atrax sutherlandi.* I focused my study at a large-scale, long-term habitat fragmentation experiment, the Wog Wog Habitat Fragmentation Experiment, in southeastern NSW, Australia. I collected *A. sutherlandi* specimens from pitfall traps and analyzed individuals for the stable isotopes, $\delta^{13}C$ and $\delta^{15}N$. I predicted habitat fragmentation would decrease abundance, reduce trophic position, and reduce the breadth of the stable isotopic niche area in populations of *A. sutherlandi*. However, spiders were significantly more abundant in small fragments, particularly edges of small fragment, when compared to the controlled, continuous forest. Habitat fragmentation did not reduce trophic position (via $\delta^{15}N$) of *A. sutherlandi*. Individuals in the matrix and fragments were on average 1.6 ‰ and 0.62‰ higher in $\delta^{15}N$, respectively, than individuals in controls. Stable isotopic niche area was largest in the matrix (2.86 $‰^2$), followed by fragments (1.60 $‰^2$), and smallest in the continuous forest (1.25$‰^2$). Contrary to predictions from metacommunity theory, habitat fragmentation appeared to increase the trophic position and stable isotopic niche breadth of *A. sutherlandi*. I speculate that these changes may instead be driven by variation in leaf litter depth or alterations in funnel-web behavior due to habitat fragmentation.

Background

Habitat fragmentation, the act of dividing once continuous habitat into isolated patches, is a significant threat to biodiversity and has been shown to alter the trophic structure of communities in some ecosystems (c.f. Layman et al. 2007; Resasco et al. 2017). Both spatial food-web and metacommunity theories predict that habitat connectivity will promote food-web complexity, suggesting that fragmenting habitats will reduce food-web complexity and the trophic level at which generalist predators, like the funnel-web spider, feed (Holt 2002; Layman et al. 2007; Martinson and Fagan 2014; Haddad et al. 2015; Resasco et al. 2017). It is important to test these theories with empirical data to give the theories validity. Any discrepancies between empirical data and theory might suggest potential flaws in the ecological theory.

Changes in the trophic position of generalist predators in fragmented landscapes, compared to continuous habitat, can provide insight into how habitat fragmentation alters food-web structure. Trophic position is a useful predictor of an organism's sensitivity to habitat fragmentation (Henle et al. 2004). By using stable isotope analyses of a generalist predator tissue, we can understand how habitat fragmentation affects the food-web structure of communities (Layman et al. 2012). Stable isotopes, in particular ^{15}N and ^{13}C, are indicators of environmental and resource requirements in a species (Fry 2006; Araújo et al. 2007). To quantify trophic position, we can analyze the stable isotope, ^{15}N. Trophic position is directly proportional to $\delta^{15}N$ ($\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000$, where R is $^{15}N/^{14}N$) (Fry 2006). We observe this positive relationship because levels of ^{15}N are enriched as trophic level increases (Vanderklift and Ponsard 2003; Fry 2006). Using this analysis, we can test the effect of habitat fragmentation on the funnel-web spider's trophic position. Changes in the trophic position of an organism can suggest shifts in food-web structure and/or diet.

In addition to using stable isotopes to quantify trophic position, stable isotopes can be used to create isotopic niches (Layman et al. 2012). The size and position of stable isotopic niches can tell us about how a disturbance affects both trophic structure and the abiotic environment (Layman et al. 2012). Ecological niches can be defined as a hypervolume of n dimensions (Pianka 2011). Each dimension, or axis, represents an environmental or resource requirement of a species (Pianka 2011). Habitat fragmentation is predicted affect both abiotic and biotic dimensions of a species' niche. Our isotopic niche has two axes: one represents what the species eats, $\delta^{15}N$, and the other represents environment factors, $\delta^{13}C$. In addition to the $\delta^{15}N$ axis described above that measures trophic position, $\delta^{13}C$ ($\delta^{13}C=[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is $^{13}C/^{12}C$) of an organism's tissue provides information on abiotic factors in the environment, including temperature and moisture (DeNiro and Epstein 1978). Since a species' isotopic niche encompasses both resource and environmental factors, it can strengthen our evaluation of a community's response to habitat fragmentation. As described above, theory predicts that habitat fragmentation will reduce food-web complexity, and thus we predict that fragmentation will reduce the resource dimension ($\delta^{15}N$) of a species' niche. It is difficult to predict how fragmentation will affect the environmental dimension ($\delta^{13}C$) of a species niche from theory.

We used stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) to examine the trophic position and niche of the funnel-web spider*, Atrax sutherlandi*, in a large-scale habitat fragmentation experiment. *A. sutherlandi* is an appropriate species for stable isotope analyses because it is a generalist predator that consumes a range of different food sources. Additionally, *A. sutherlandi* is a ground dwelling species that burrows in natural crevices in the soil (Gray 2010) and is thus abundant in pitfall traps. Spiders were collected within the habitat fragmentation experiment to determine

how habitat fragmentation, fragment size, and edges impact the trophic structure of communities on fragments, after first determining how fragmentation affected funnel-web spider abundance.

Hypotheses and Predictions

In this study, we tested whether habitat fragmentation, in the context of the Wog Wog Habitat Fragmentation Experiment, resulted in (1) decreases the abundance and distribution of the funnel-web spider, *Atrax sutherlandi,* (2) decreases in the trophic position of the funnel-web spider, and (3) changes the isotopic niche space of the funnel-web spider, a generalist predator. We predicted that habitat fragmentation will reduce the abundance of the funnel-web spider because theory predicts that small, isolated populations are at risk of stochastic extinction (Caughley 1994). In addition, species at the top end of food-chains are at greater risk of extinction (Holt 2002). We predicted that fragmentation will reduce the trophic position of funnel-web spiders because theory and empirical studies predict that fragmentation decreases presence of potential prey of higher trophic rank (Layman et al. 2007), and therefore, trophic position of *A. sutherlandi* will be highest in the continuous forest, followed by large fragments, medium fragments, small fragments, and pine matrix, respectively. This means that we predicted that the concentration of ^{15}N, or $\delta^{15}N$, will be highest in continuous forest, followed by large fragments, medium fragments, small fragments, and pine matrix, respectively. With more area, we predicted that there will be higher biodiversity, and therefore more species of higher-trophic rank for the spider to prey on. Lastly, we predicted that fragmentation will reduce the breadth of the stable isotopic niche space of the funnel-web spider, *A. sutherlandi*. We predicted that the stable isotopic niche space will be smallest in the matrix, followed by the fragments, and lastly, the stable isotopic niche space will be largest and least variable in the continuous forest. In continuous forest, food-webs will be more complex, and therefore the stable isotopic niche space will be larger and foster more variation in $\delta^{15}N$ and $\delta^{13}C$.

Methods

Study system

We test these predictions in a large-scale, long-term habitat fragmentation experiment at Wog Wog for the generalist predator, the funnel-web spider, *Atrax sutherlandi*. The Wog Wog Habitat Fragmentation Experiment in southeastern NSW, Australia provides an ideal location for studying the effects of fragmentation on trophic structure. The experiment is a long-term (28 years), large-scale fragmentation experiment (Margules 1992). In 1985, two years prior to fragmentation, six replicates were established in native, old growth Eucalyptus forest. Each replicate contains three square patches of varying size treatments (0.25, 0.875, and 3.062 ha). In 1987, the commercial timber industry cleared a portion of the native *Eucalyptus* forest, establishing a pine plantation surrounding four of the six replicates. In this experiment the pine plantation acts as the matrix and is a foreign ecosystem for the organisms within this *Eucalyptus* forest. Two replicates were left within the continuous Eucalyptus forest, acting as controls for the experiment. These replicates mimic the spatial scale of the fragments, although they are within continuous forest.

To monitor invertebrate populations, pitfall traps were set up within each replicate and in the matrix. The experiment consists of 188 pitfall trap sites located in (i) experimentally fragmented eucalyptus forest, (ii) continuous, undisturbed Eucalyptus forest, and (iii) non-native *Pinus radiata* matrix that was planted around each fragment to eventually be harvested (Fig. 1). A pitfall trap is constructed of a plastic cup that is placed in hard plastic sleeve that is buried in the ground. Within the cup contains 50 ml of propylene glycol, to preserve the invertebrate specimens, and a metal cage prevent the capture of other wildlife, including skinks. A 60 cm metal fence is used to guide invertebrates to the pitfall trap and increase catch, and 20 cm metal

roof is set up above the open trap for protection against flooding. Each fragment or continuous patch has eight sample sights that have two pitfall traps each. The eight sample sights are classified by the distance to the edge of the patch and geographical terrain (slopes/drainages). This creates four site classes per plot: edge-slope trap sites, interior-slope trap sites, edge-drainage trap sites, and interior-drainage trap sites. The remaining 22 sample sites, 44 pitfall traps, are located in the matrix.

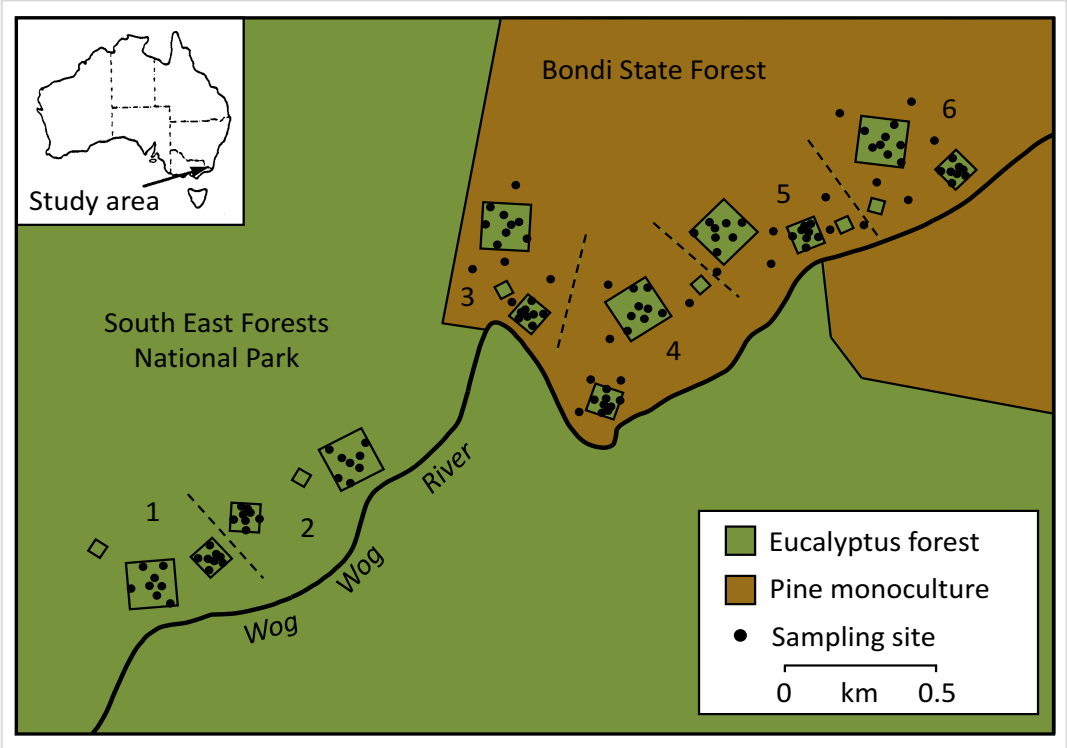

Figure 1: *Map of Wog Wog Habitat Fragmentation Experiment. Green color represents native Eucalyptus forest and brown represents the pine monoculture matrix. Four fragmented replicates are surrounded by pine matrix, and two control replicates are surrounded by continuous eucalyptus forest. Each replicate contains three square patches of varying size treatments (0.25, 0.875, and 3.062 ha). Within each patch, there are eight sampling sites, and there are also sampling sites within the matrix (represented by dots). Map adapted from (Resasco et al. 2017).*

Although this study has been going on for over 30 years, the funnel-web specimens will be retrieved from 14 sampling periods between 2009-2015, in either February, May, or

November. Trapping at these times allow us to control for the natural seasonal cycle (summer, fall, spring, respectively). The trap contents were sent to the Davies Lab in University of Colorado at Boulder. In the Davies Lab, the trap contents are sorted into invertebrate groups, and the funnel-web spiders were identified to species, as well as to sex, approximate age group, and recorded body length. These specimens are stored in a 75% ethanol solution at the University of Colorado at Boulder. Ethanol preserves the specimens but does not significantly affect the $\delta^{15}N$ and $\delta^{13}C$ in the muscle tissue of the specimens (Hobson et al. 1997; Halley et al. 2008). Because there is no significant effect of ethanol preservation on these isotopic values and every specimen was stored in the same solution, we eliminate any bias in the experiment regarding the ethanol solution and stable isotopic values (Halley et al. 2008).

Stable isotope analysis

The funnel-web spider, *Atrax sutherlandi,* is a generalist predator that preys on other ground dwelling invertebrates. Because of the spider's digestive mechanisms, we cannot know exactly what the spiders are eating without observational data. Following sorting the funnel-web specimens, they were prepared for isotope analysis. Only adult specimens were used for isotope analysis (>11 mm in body length). This is to control for other confounding variables, such as diet preferences in different age groups, as well as to meet the minimum size for stable isotope analysis (.02 mg). First, four legs on each spider were cut at the coxa (Fig. 2) and removed as a tissue sample for isotope analysis. Two hind legs on each side of the body are used to avoid removing legs or other appendages that are useful for identification. The pedipalp is useful for identification of males, because the palpal bulb, a specialized part of the pedipalp, transfers sperm in order to reproduce. In the subfamily Atracinae, many males have an apophysis, a cone-

shaped protuberance, on the tibia of their second leg, which makes the second leg important for species identification (Gray 2010).

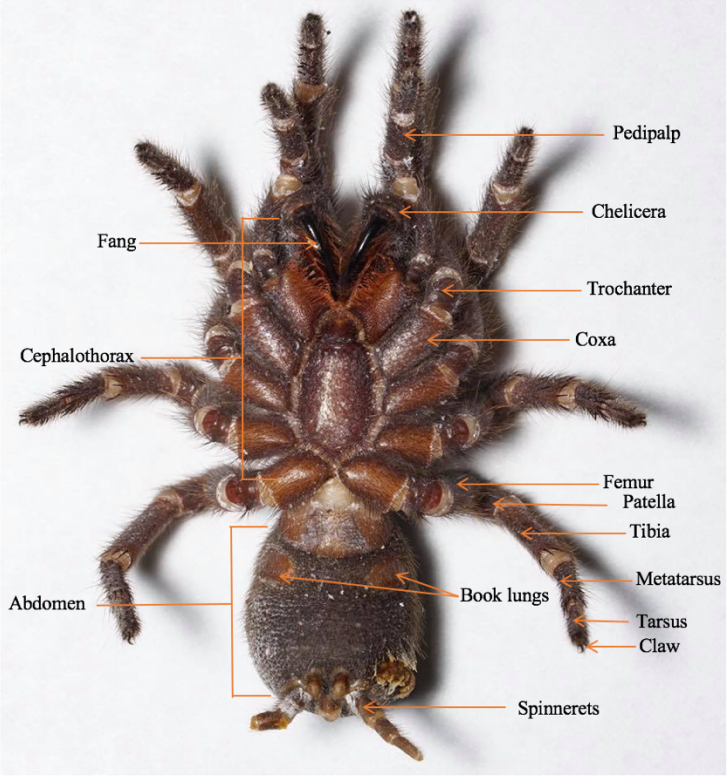

Figure 2: *Diagram of the morphology of a female funnel-web spider, Atrax sutherlandi. Pedipalp, tibia II, chelicera, and other traits are important for identification and sexing of the funnel-web spider. Atrax sutherlandi males have a cone-shaped apophysis on their Tibia II. For isotope analysis, four hind legs were cut at coxa, freeze dried, and homogenized.*

Photography: Andy Better

These samples were labeled and thoroughly dried so that they were viable for analysis. All specimens have been preserved in a solution of 75% Ethanol since each collection period (2009-2015). To dry the specimens, they went in the oven for 24-72 hours depending on their size. After the samples were oven dried, they were submerged in liquid nitrogen and crushed using a mortar and pestle that was thoroughly cleaned between each sample to avoid contamination. We crushed the samples to homogenize the tissue fibers. Different tissues can have different $\delta^{15}N$ and $\delta^{13}C$, as well as different turn-over rates, so it is important to homogenize the sample to control for this variation.

The prepared samples were then sent to the University of New Mexico Center for Stable Isotopes in Albuquerque, NM. Stable isotope specialists used a Costech 4010 or CarloErba NC2500 elemental analyzer along with a Thermo Scientific Delta V mass spectrometer to calculate Nitrogen and Carbon stable isotope ratios ($\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R is $^{15}N/^{14}N$ and $\delta^{13}C=[(S_{sample}/S_{standard}) - 1] \times 1000$, where S is $^{13}C/^{12}C$). These ratios indicate the trophic position of each specimen because ^{15}N is enriched in the specimen tissues relative to its prey (Cabana and Rasmussen 1994). Because of this, $\delta^{15}N$ is correlated to the specimen's trophic position (Post 2002).

Data analysis

We analyzed the funnel-web spider's response to habitat fragmentation, as well as the effect of fragment size, edge effect, and habitat. Five explanatory variables were defined by (i) *Fragmentation* (fragments and controls), (ii) *Treatment* (forest fragments, continuous forest, and pine plantation), (iii) *Fragment Size* (small, medium, and large), (iv) *Edge* (edge sites and interior sites), and (v) *Topography* (slopes and drains). By combining fragmentation, size, and edge, we create a sixth variable, *Fragment Size Edge*, that represents the interactions between each individual explanatory variable. We use two random effects, *Replicate* and *Plot,* to eliminate unintentional variation. Using generalized linear mixed models (GLMMs), we fit models using the R package lme4, assuming Poisson distribution for abundance and normal distribution for isotopic data. We examined the difference in $\delta^{15}N$ and $\delta^{13}C$ between the continuous forest, large fragments, medium fragments, small fragments, and pine matrix using GLMMs, the R package lme4, and the R package SIAR. Stable Isotope Analysis in R (SIAR) quantifies isotopic niche space, using $\delta^{15}N$ and $\delta^{13}C$ data from adult spider specimens. Since our sample size was 156, this metric adequately calculates the isotopic niche. Using this we can

examine how habitat fragmentation affects the trophic niche space of *A. sutherlandi* at Wog Wog.

Results

The funnel-web spider data from 2009-2015 that were used for this analysis are summarized in Table I.

Summary	Funnel-Web Spider, *A. sutherlandi*
Total number of individuals	436
Range per sampling site	0 - 12
Mean per sampling site	2.319
Total in fragments	269
Total in controls	56
Total in matrix	111
Total Adults	156
Total Mature Males	32

Table 1: *A summary of funnel-web spider data from 2009-2015.*

Response in abundance and distribution

Habitat fragmentation altered the abundance and distribution of the funnel-web spider, *A. sutherlandi.* Figure 3 shows the effect of habitat fragmentation on the funnel-web spider, *A. sutherlandi,* using an effect size plot so show significance. *A. sutherlandi* abundance increased in the small fragments compared to the controls (Figure. 3). In the small inner sites, funnel-web spider abundance was 2.7 times higher than in controls (Figure 3). In the small outer sites, funnel-web spider abundance was 4.4 times higher than in controls (Figure 3). Funnel-web spiders did not show an edge effect (Table 2). Lastly, funnel-web spiders were not more or less abundant in the pine-plantation matrix than in controls (Table 2).

Variable	d.f.	Deviance	*P*-value
Replicate stratum			
Fragmentation	1	2.568	0.109
Plot stratum			
Fragmentation by Size	2	9.609	**0.008**
Site stratum			
Fragmentation by Edge	1	0.178	0.673
Fragmentation by Topography	1	1.427	0.225
Frag. By Size + Frag. by Edge	1	0.178	0.673
Frag. by Size by Edge interaction	2	3.560	**0.014**

Matrix

Variable	d.f.	Deviance	*P*-value
Replicate stratum			
Fragmentation	2	4.068	0.131

Table 2: *This table shows the effects of habitat fragmentation, fragment size and edges and their interactions on spider abundance from generalized linear mixed model analyses. Significance of each variable was determined by the change in deviance associated with adding that variable. P values < 0.05 are considered significant.*

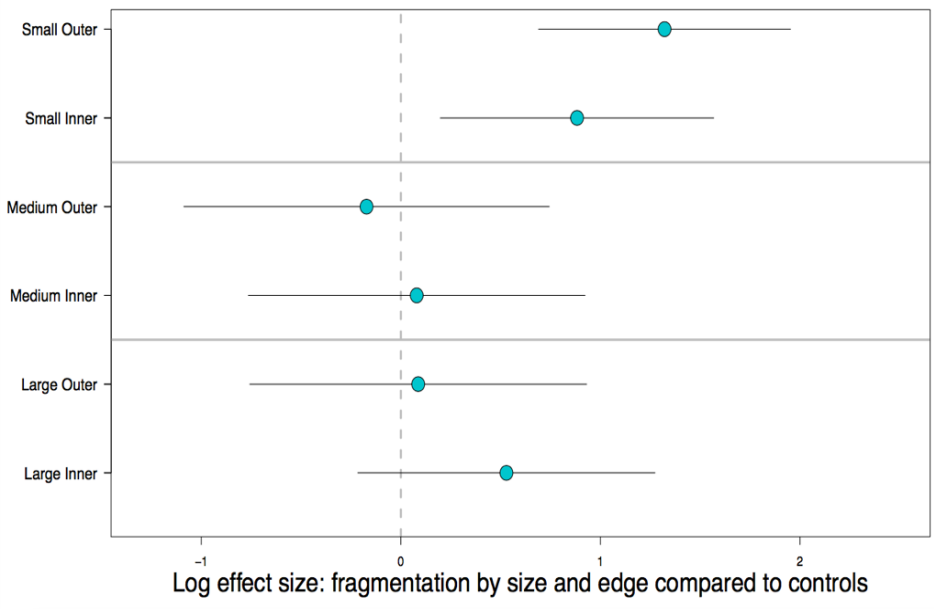

Figure 3: *This figure shows the log effect of habitat fragmentation, fragment size and edge on funnel-web spider abundance compared to controls. There are significantly more funnel-web spiders in small outer and small inner fragments compared to controls. Log translation: In the small inner sites, funnel-web spider abundance was 2.7 times higher than in controls. In the small outer sites, funnel-web spider abundance was 4.4 times higher than in controls.*

Response in tropic position

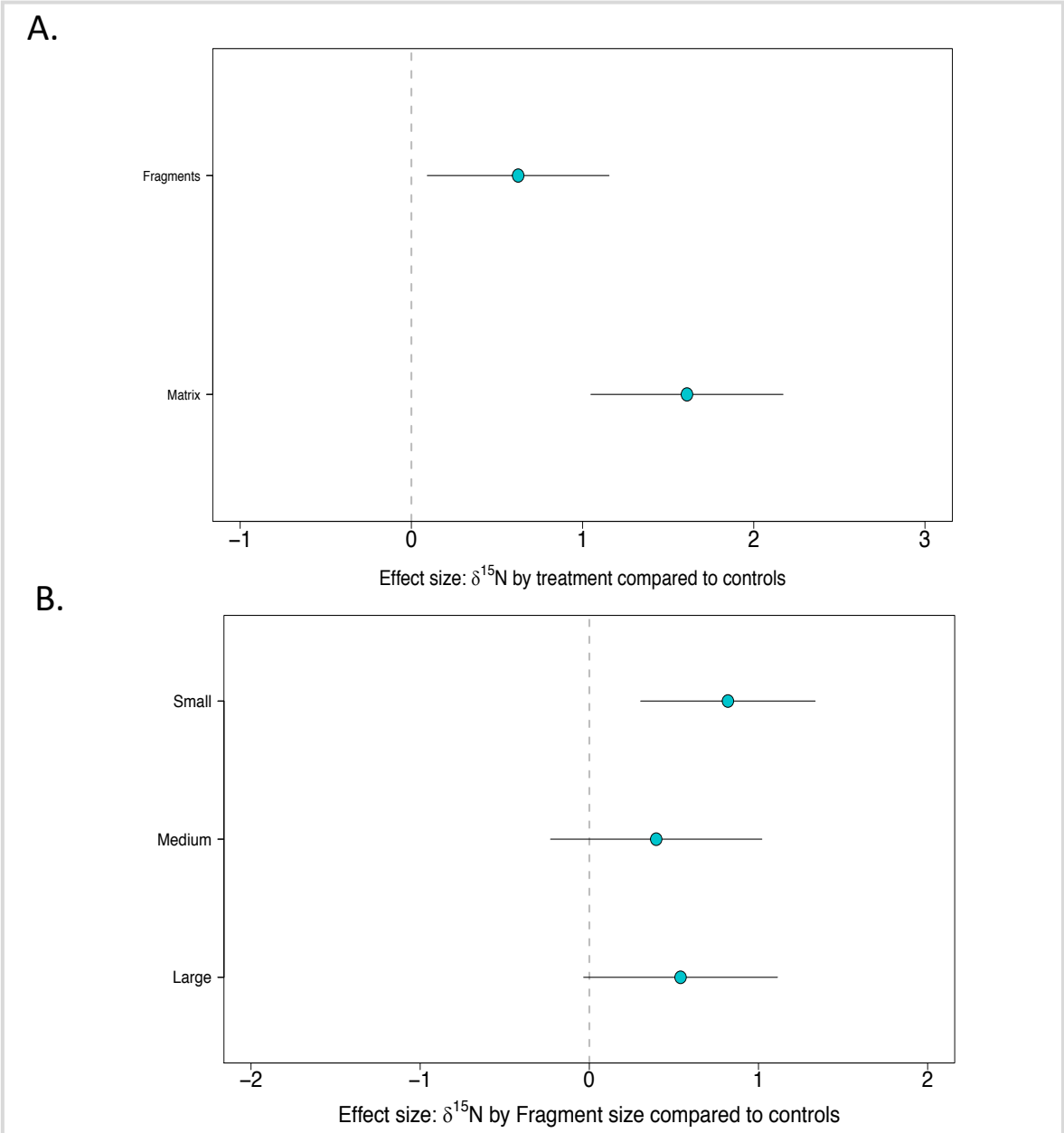

Figure 4: *This figure shows the effects of* ***A.*** *the experiment on the Funnel-web Spider* $\delta^{15}N$ *compared to controls and* ***B.*** *fragment size on Funnel-web Spider* $\delta^{15}N$ *compared to controls.*

Habitat fragmentation also altered $\delta^{15}N$, suggesting a change in trophic position of the funnel-web spider due to habitat fragmentation. Trophic results are summarized in Figure 4 and Table 3. Funnel-webs had higher $\delta^{15}N$ in the fragments by 0.6238 ‰ compared to the controls, on average. The funnel-webs also had higher $\delta^{15}N$ in the matrix compared to the controls by, on

average, 1.6098 ‰. Unlike $\delta^{15}N$, $\delta^{13}C$ did not change in funnel-web tissue in response to habitat fragmentation, fragment size, edge, or topography.

Variable	d.f.	Deviance	*P*-value
Replicate stratum			
$\delta^{15}N$: Fragmentation	1	5.226	**0.022**
Plot stratum			
$\delta^{15}N$: Fragmentation by Size	2	3.493	0.174
Site stratum			
$\delta^{15}N$: Fragmentation by Edge	1	0.229	0.632
$\delta^{15}N$: Fragmentation by Topography	1	2.430	0.119
$\delta^{15}N$: Frag. By Size + Frag. by Edge	1		
$\delta^{15}N$: Frag. by Size by Edge interaction	2		

`Matrix

Variable	d.f.	Deviance	*P*-value
Replicate stratum			
Fragmentation	1	21.442	**<0.001**

Table 3: *Summary of the linear mixed model analysis of the effects of habitat fragmentation, fragment size, edges, and matrix on spider stable isotope. Significance of each variable was determined by the change in deviance associated with adding that variable. P values < 0.05 are considered significant.*

Response in trophic niche space

The trophic niche space of the funnel-web spider was affected by habitat fragmentation. We used Stable Isotope Analysis in R (SIAR) to calculate trophic niche space. SIAR fits a Bayesian model to estimate the funnel-web spider's niche in each treatment (Jackson et al. 2011). Using $\delta^{15}N$ and $\delta^{13}C$ data in SIAR, we were able to create ellipses that represent the spider's stable isotopic niche space in the fragments, controls, and matrix. There were significant differences that separated the ellipses among treatments. Although $\delta^{13}C$ was not significantly

affected by habitat fragmentation, the areas of the ellipses, and therefore the ranges of $\delta^{13}C$ among treatments, were considerably different throughout the experiment. Figure 5 shows the relationship between trophic niche ($\delta^{13}C$ and $\delta^{15}N$) and the experiment. The trophic niche, or standard ellipse area (SEA_c), was largest in the matrix (2.86 ‰^{2}), followed by fragments (1.60 ‰^{2}), and smallest in the continuous forest (1.25‰^{2}).

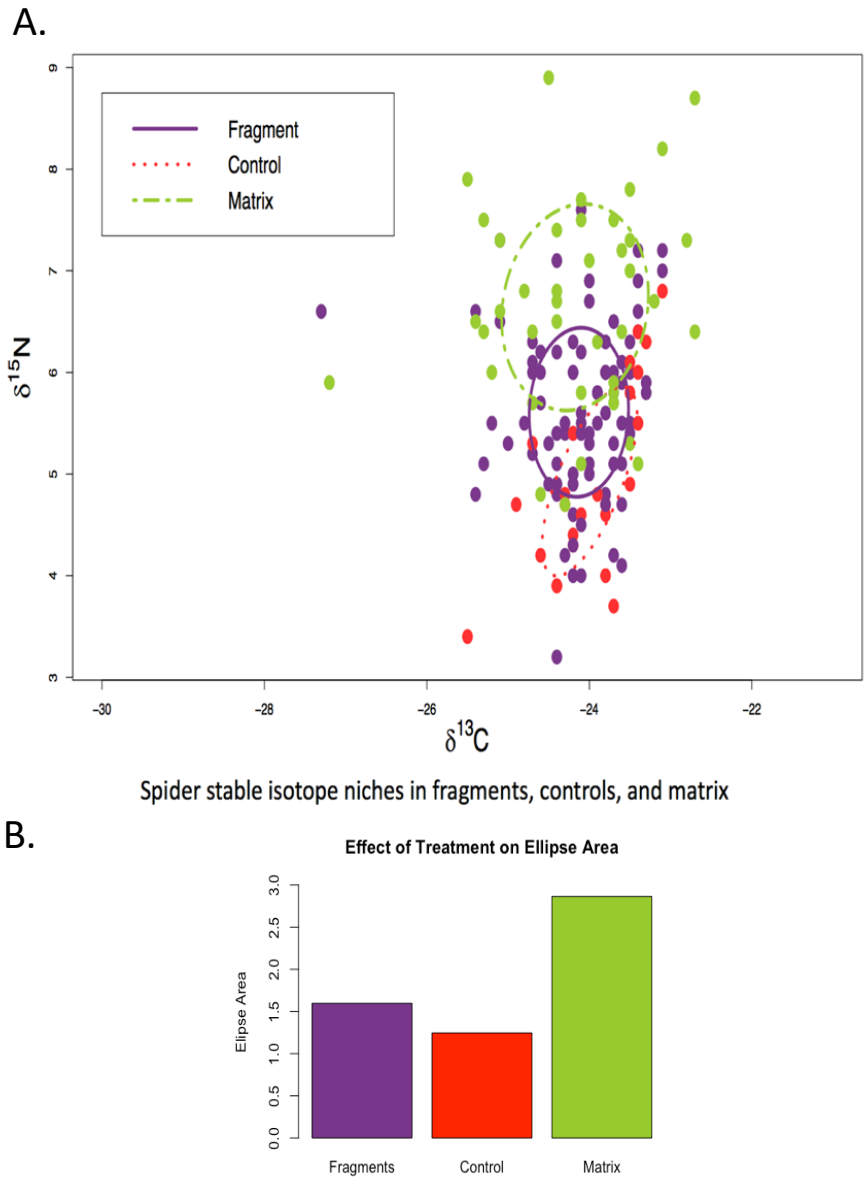

Figure 5: *The effect of habitat fragmentation and the matrix on the trophic niche of A. sutherlandi. Figure 5A shows the stable isotopic niches in the fragments (purple), controls (red), and matrix (green). Figure 5B shows the effect of the experiment on tropic niche ellipse area- ‰^{2} (SEAc).*

Discussion

Habitat fragmentation altered the abundance of the funnel-web spider, *A. sutherlandi*. Contrary to our hypothesis, funnel-web spiders were most abundance in small fragments. Our result contrasts with theory which predicts that organism abundance will increase with habitat area (Caughley 1994). These changes are likely attributed to increased resources in the small fragments. As the result of habitat fragmentation, leaf litter depth is greatest in the small fragments. Because of this abiotic difference and increased resource availability at the base of the food-chain, many arthropods, potential prey for the spiders, are likely to be more abundant in small fragments.

Trophic position ($\delta^{15}N$) of *A. sutherlandi* was also affected by habitat fragmentation. We expected $\delta^{15}N$ levels to be lower in fragments because tropic position is directly correlated with $\delta^{15}N$. Contrary to predictions from metacommunity and spatial food-web theories (Holt 2002; Layman et al. 2007; Martinson and Fagan 2014; Haddad et al. 2015; Resasco et al. 2017), habitat fragmentation increased trophic position ($\delta^{15}N$) of the funnel-web spider.

In addition, stable isotopic niche breadth (from a $\delta^{13}C$-$\delta^{15}N$ biplot) increased with fragmentation, including in the matrix. This means that there is more variation in both $\delta^{13}C$ and $\delta^{15}N$ in spiders in the fragments and matrix compared to the controls. These findings indicate that habitat fragmentation does not always cause the collapse of food-webs, as theory predicts (Holt 2002; Layman et al. 2007; Martinson and Fagan 2014; Haddad et al. 2015; Resasco et al. 2017).

These trophic differences and the deviation from what has been predicted according to theory could be attributed to various factors. The increased leaf litter depth in fragments, which increases resource availability at the bottom of the food-chain, could be increasing food-web

complexity in the smaller fragments. An increase in resource availability at the bottom of the food-chain will support a larger, more complex invertebrate fauna and therefore a greater variety of higher trophic-ranking prey. However, this explanation does not explain the trophic change in the matrix. Another and competing potential explanation for the increase in trophic position is that female funnel web spiders in the fragments and matrix could be exhibiting increasingly vagrant behavior, wandering outside of their web into competitive territory and eating higher trophic ranking prey (Bradley 1993). In Figure 6 in the Appendix A, the effect of the experiment and fragment size is further broken up by sex, showing that females are driving this result while males are highly variable and less significant. This behavior is not common in funnel-web spiders but is seen when the spiders do not have sufficient food resources. Lack of food sources can cause increased forays outside of the web.

Our findings show how the funnel-web spider, *A. sutherlandi,* specifically reacted to habitat fragmentation, including fragmentation's effect on abundance, distribution, trophic position, and stable isotopic niche of the spider. Shifts in these factors indicate changes in the abiotic and biotic environment due to fragmentation. Establishing empirical relationships between food-web structure and habitat fragmentation may help to more accurately predict the organism's success in fragmented landscapes. The changes we observe in trophic position and niche suggest that habitat fragmentation alters food-web structure of arthropod communities. Our results reject predictions from metacommunity theory about food-webs. Scientists in the field use existing metacommunity theory to make predictions about habitat fragmentation and food-web structure. There are few experimental tests that actually test these predictions, and our study shows that what happens in practice when a landscape is fragmented is much more complex than current theories predict. Since habitat fragmentation does not completely isolate the funnel-web

spider populations, we do not observe decreased abundance or increased risk of extinction for the spider. Using a generalist predator at the top of the food-chain, we can also infer that the spider's prey species are also not reacting to fragmentation in a typical, predicted way that could lead to a collapse in the food-web in habitat fragments. It is important to recognize the discrepancy between empirical data and theory, to assess, and consider modifying theory and ecological models.

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Appendices

Appendix A: extra figures

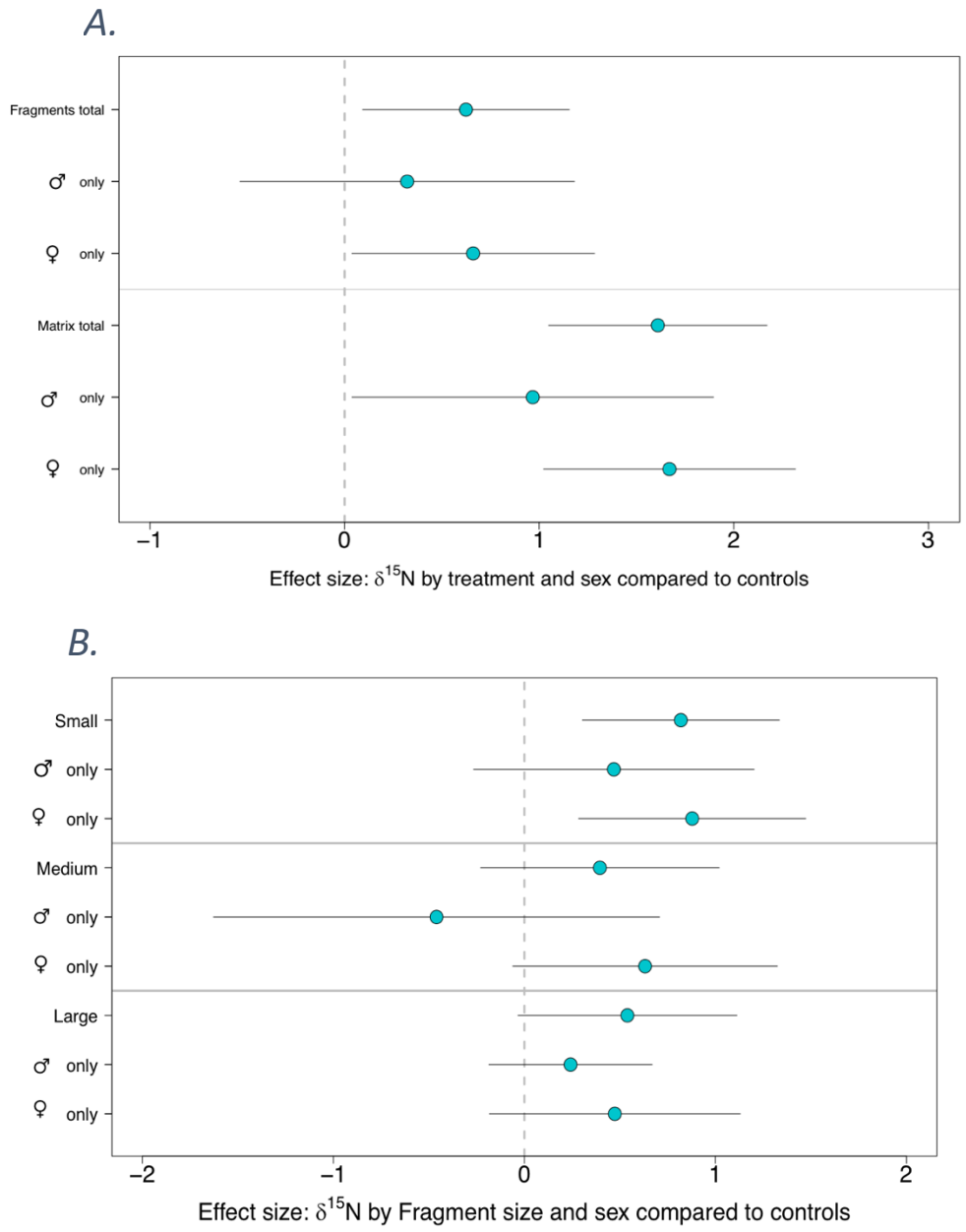

Figure 6: Effect of **A.** the experiment and sex on the Funnel-web Spider $\delta^{15}N$ compared to controls and **B.** Fragment size and sex on Funnel-web Spider $\delta^{15}N$ compared to controls.

Appendix B: equations

A. Equation 1: $\delta^{15}N$ calculation

$$\delta^{15}\mathrm{N} = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000, \text{ where R is } \frac{^{15}N}{^{14}N}$$

B. Equation 2: $\delta^{13}C$ calculation

$$\delta^{14}\mathrm{C} = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000, \text{ where R is } \frac{^{14}C}{^{13}C}$$

Figure 7: Equations used to calculate both **A.** $\delta^{15}N$ and **B.** $\delta^{13}C$.