SHORT COMMUNICATION

Decreases in the size of riparian orb webs along an urbanization gradient

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Abstract. Urbanization is associated with a variety of anthropogenic impacts that alter aquatic ecosystems and could affect riparian web-spinning spiders. The objective of this study was to evaluate how changes in web structural features and body condition of a horizontal orb-weaver are associated with surrounding levels of urbanization. Along an urban watershed in Puerto Rico, we found a significant negative relationship between the capture area of webs and in the body condition of spiders with increasing levels of surrounding impervious surface. We propose that these changes in web structure and body condition are associated with variations in the diversity and quality of prey, as well as the loss of riparian substrate in more heavily urban areas.

Keywords: Capture area, Puerto Rico, Río Piedras watershed, Tetragnatha, web structure

Increasing levels of urbanization and their associated environmental stressors can have strong effects on the diversity, morphology, and behavior of organisms (Miyashita et al. 1998; Prosser et al. 2006; McKinney 2008). Web-spinning spiders are commonly found along the riparian areas of streams where they can take advantage of emerging aquatic insects (Burdon & Harding 2008; Greenwood & McIntosh 2008; Lambeets et al. 2008; Chan et al. 2009; Akamatsu & Toda 2011). Common impacts of human activities within urban watersheds include the loss of riparian vegetation and changes in aquatic insect diversity (Ramírez et al. 2012). Changes in substrate availability (Vollrath et al. 1997; Laeser et al. 2005; Chan et al. 2009) and changes in the diversity and quality of prey (Sherman 1994; Schneider & Vollrath 1998; Mayntz & Toft 2001; Davis et al. 2011) have both been shown to affect the web-spinning behaviors and overall fitness of spiders. Body condition indices have been found to be a useful method in monitoring changes in nutrition and growth and are associated with an individual's overall fitness (Jakob et al. 1996). Changes in web-spinning behaviors have been analyzed by measuring variations in the structures of orb web produced under different conditions (Zschokke & Vollrath 1995; Vollrath et al. 1997; Schneider & Vollrath 1998; Hesselberg & Vollrath 2004).

Despite limited web variation within a species, some individual spider characteristics (e.g., size, sex, and weight) (Heiling & Herberstein 2000), along with a number of abiotic (e.g., temperature, humidity and wind) (Vollrath et al. 1997) and biotic (e.g., prey diversity and abundance) (Sherman 1994; Blackledge & Zevenbergen 2006) factors are known to contribute to variability in the structural features of orb webs. Web design patterns are a behavioral blueprint or phenotype, which gets expressed daily (Vollrath et al. 1997; Toscani et al. 2012) and represents a major energetic output for spiders (Sherman 1994; Heiling & Herberstein 1999). A typical orb web is a spiral of sticky silk overlaying a radial array of threads in a frame with lines attached to a type of substrate (Vollrath 1988). Previous studies that have investigated the effects of urbanization on spider taxa have generally only focused on changes in diversity or abundance (Laeser et al. 2005; Prieto-Benítez & Méndez 2011; Horvath et al. 2014), but with practically no information on changes in web structures or body condition.

We focused on a species of horizontal orb-weaving spider, *Tetragnatha boydi* O. Pickard-Cambridge, 1898 (Araneae: Tetragnathidae), which is distributed throughout Africa, South Asia, Central and South America and the Caribbean (Okuma 1992). Being an

aquatic ecosystem specialist and because of its high abundance along the Río Piedras watershed, T. boydi makes an ideal species for this study. The objective of this study was to assess if urbanization has an effect on T. boydi resulting in changes in their web structures and body condition. Along the urban gradient of the Río Piedras watershed, increasing levels of urbanization results in the loss of riparian vegetation and a decrease in aquatic insect richness, with the loss of sensitive taxa (e.g. Ephemeroptera and Trichoptera) but a large increase in the abundance of resilient taxa (e.g. Chironomidae) (de Jesús-Crespo & Ramírez 2011). Small chironomid midges feed primarily on fine particulate organic matter (FPOM), which has been shown to be a low quality food source limited in phosphorus and nitrogen when compared to periphyton (Cross et al. 2003), which is consumed by larger ephemeropterans in less urbanized parts of the watershed. Due to the strong effects that substrate and prey availability have on web-spinning behaviors (Sherman 1994; Vollrath et al. 1997; Zschokke 1997; Schneider &Vollrath 1998), we predicted that the size of the web would decrease in more urban areas where there is less riparian vegetation and only small, low quality chironomids available as prey.

This study was conducted in the Río Piedras watershed, in the metropolitan area of San Juan, Puerto Rico. The drainage area is about 49 km² and it originates at around 150 m asl and runs for 16 km (Ramírez et al. 2014). The watershed is highly urbanized with heavily modified channels and has been classified by the U.S. Environmental Protection Agency (EPA) as highly polluted (Lugo et al. 2011). Six sites, representing six subwatersheds, were chosen for this study (Appendix 1). These six sampling sites are part of the San Juan Urban Long Term Research Area (ULTRA) project since 2009 and land use data are available for each subwatershed (Ramírez et al. 2014). Using the percent of impervious surface for each subwatershed, we established a gradient from Site 1, the least urbanized with 12.9 percent impervious surface, to Site 6, the most urbanized with 77.2 percent impervious surface (Appendix 1). The percent of impervious surface is strongly correlated with changes in water physicochemistry in the Río Piedras watershed (e.g., increasing concentrations of chloride, sulfate, sodium, magnesium and higher levels of specific conductivity) (Ramírez et al. 2014). These physicochemical variables are generally associated with increasing levels of urbanization (Walsh et al. 2005; Lugo et al. 2011; Ramírez et al. 2014), which makes the gradient ideal for assessing possible effects of urbanization.

A 100 meter transect was established at each of the six sites along the watershed. Transects were surveyed at night (\sim 1900–2000) for

	capture area	free zone	mesh size	radii length	number of radii	spiral length
free zone	0.49*	With more both more		-	enable le care	-
mesh size	0.49*	0.43*		**	-	-
radii length	0.88*	0.50*	0.45*	-		-
number of radii	-0.09	-0.09	-0.21	0.29*	-	-
spiral length	0.94*	0.45*	0.35*	0.01	0.01	10001
number of spirals	0.69*	0.24	0	0.24	0.24	0.80*

Table 1.—Pair-wise comparisons of Pearson correlation coefficients for the seven web parameters. A total of 56 webs was analyzed (df=54). Significant differences of $p \le 0.05$ indicated by (*).

webs of adult female T. boydi and around ten individual webs were sampled at each site (Appendix 2). Spider size and ontogeny have been shown to have an effect on web-spinning behaviors and, therefore, we controlled for this factor by utilizing only adult females. Webs that were incomplete, had large gaps or holes, and those in the middle of construction were not utilized. Webs were powdered with cornstarch to make them visible and a photo was taken of the web and a centimeter grid paper (for the purpose of scale; Eberhard 1976) using a digital camera (Pentax Optio WG-3). Individuals corresponding to each web were collected and the body condition of each spider was determined by calculating a ratio index using the equation: mass/ cephalothorax width³ (Jakob et al. 1996). Cephalothorax width was chosen to represent body size due to previous studies finding this to be the most accurate estimator of overall body mass in spiders (Hagstrum 1971; Marshall & Gittleman 1994; Jakob et al. 1996) and by raising cephalothorax width to the third power we take into consideration that linear measurements generally increase isometrically with mass (Jakob et al. 1996). Cephalothorax width was measured by photographing each spider and using image processing software Image J (Abramoff et al. 2004). After being photographed, spiders were placed in a drying oven at 70°C for a minimum of 48 hours in order to obtain their dry mass.

Various web features are known to reflect neurological and environmental impacts on spiders (Samu & Vollrath 1992; Sherman 1994; Vollrath et al. 1997; Hesselberg & Vollrath 2004; Wyman et al. 2011). We measured: number of radii and radii length, number of spirals and spiral length, capture area, mesh size, and free zone area. Mesh size was calculated using the formula suggested by Herberstein and Tso (2000):

mesh size
$$= \frac{1}{2} \left(\frac{r_u - Hr_u}{s_u - 1} + \frac{r_l - Hr_l}{s_l - 1} \right)$$

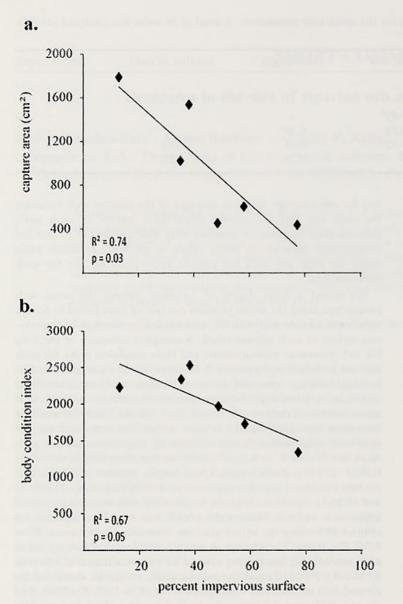
Where r_u and r_l are the distances in centimeters from the center of the web to the outermost capture spiral threads for the upper and lower vertical sectors, Hr_u and Hr_l refer to the distance in centimeters from the center of the web to the innermost capture spiral thread of the upper and lower vertical sector, and s_u and s_l refer to the number of capture spiral threads in the upper and lower sectors (Herberstein & Tso 2000). When the vertical sector had missing parts, large holes, or any other factor that impeded calculating an accurate estimate, the horizontal sectors were considered to the right or left side of the hub. If neither vertical nor horizontal sectors could be used, a sector was arbitrarily chosen and a note was made.

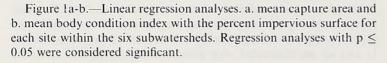
The statistical program R (R Core Team 2012) was used to verify that all data met the statistical requirements for parametric tests, along with all other subsequent statistical analyses. Utilizing calculated pair-wise Pearson correlation coefficients, we tested the seven web variables for collinearity. When a significant correlation ($P \leq 0.05$) and a Pearson correlation coefficient greater than 0.65 was found we selected and kept the variable we determined to most accurately predict changes in web structures (Esselman & Allan 2010). All webs and spiders were pooled together to calculate mean values for each site (Appendix 2). Linear regression analyses were utilized to test for relationships between averages of the selected web variables for each site and the percent impervious surface of each site's subwatershed. Regression analyses were also conducted to test for relationships between the mean values of the body condition ratio index for each site with the percent impervious surface for each subwatershed.

We found a large degree of variation among the seven web parameters along the urban gradient but not all were found to have a significant relationship with the surrounding percentage of impervious surface in each subwatershed. A complete summary of the data for web parameter measurements and body condition index for each site can be found in Appendix 2. Number of radii was more or less constant among sites and linear regression analyses showed no significant relationships between percent impervious surface with mean number of radii (n = 6, $\mathbb{R}^2 = 0.32$, P = 0.24). Mesh size and free zone area decreased slightly in more urban sites but there was no significant relationship between percent of impervious surface with mesh size $(n = 6, \mathbb{R}^2 = 0.26, P = 0.30)$ or free zone area $(n = 6, \mathbb{R}^2 = 0.26, P = 0.30)$ 0.39, P = 0.18). Radii length, spiral length, number of spirals and capture area were significantly correlated with each other (Table 1) and all had a significant negative relationship with increasing levels of impervious surface. Mean radii length was found to decrease by around 48% along the urban gradient from Site 1 to 6 (n = 6, $\mathbb{R}^2 =$ 0.74, P = 0.03). The change in mean spiral length was even more pronounced with Site 6 being around 65% shorter than Site 1 (n = 6, $R^2 = 0.80$, P = 0.02) and the mean number of spirals decreased by around 36% between Site 1 and Site 6 (n=6, $R^2=0.85$, P=0.01). The greatest difference was seen in mean capture area, which decreased by 76% between Site 1 and Site 6 (n = 6, $\mathbb{R}^2 = 0.74$, P = 0.03). As these four variables (radii length, spiral length, number of spirals and capture area) were all significantly correlated with each other (Table 1) and all are associated with general web size, capture area was selected as the variable which best represents overall changes in web structures along the urban gradient (Fig. 1a). Similar to the web parameters mentioned, the mean ratio index representing body condition of the spiders at each site was also found to decrease by around 40% from Site 1 to Site 6 and it had a significant negative relationship with the percent impervious surface (n = 6, $R^2 = 0.67$, P =0.05) (Fig. 1b).

As predicted, the web size and body condition of *T. boydi* had significant negative relationships with increasing levels of urbanization. We attribute the alterations in web structures and body condition to be indirectly associated with increasing levels of anthropogenic impact to the stream ecosystem along the urban gradient. Previous studies have shown that small chironomid midges dominate aquatic insect communities in more urbanized areas of the Río Piedras watershed (de Jesús-Crespo & Ramírez 2011) and this could result in smaller webs due to the reliance on a low quality food source of small prey (Pasquet et al. 1994; Sandoval 1994; Schneider & Vollrath 1998). In addition, there is also less riparian vegetation and substrate available in more urbanized sites which could lead to smaller webs due to changes in microclimate and availability of attachment points for orb-weaving spiders (Vollrath et al. 1997).







The majority of the studies investigating changes in web structures due to abiotic and biotic factors have been conducted for only a small number of taxa, mainly in the family Araneidae (Sandoval 1994; Sherman 1994; Vollrath et al. 1997). The most commonly recorded web parameters for these studies were web area, thread length and mesh size. Sherman (1994) found that Larinioides cornutus (Clerck, 1757) increased their web area and total thread length when prey was limited but there was no significant change in mesh size. Sandoval (1994) found that Parawixia bistriata (Rengger, 1836) spun larger webs with increased mesh size when larger prey episodically became more abundant. Under controlled lab conditions, Vollrath et al. (1997) found that Araneus diadematus Clerck, 1757 alter their webs in response to changes in abiotic factors such as temperature and humidity. Decreases in temperature resulted in similar web areas, but larger mesh sizes; while a decrease in humidity resulted in smaller capture areas, shorter spiral lengths, and smaller mesh sizes (Vollrath et al. 1997). Substrate is another important factor because spiders would have to alter web dimensions in relation to available attachment points and when confined to smaller areas, orb-weaving spiders were found to spin significantly smaller webs (Vollrath et al.

1997). Our results of a tetragnathid species in response to urbanization showed significant changes in capture area and length of spirals, but not in mesh size. However, due to differences in how web structures were measured and with possibly inherent differences in behavior between taxa, it may be difficult in comparing results from the few studies conducted up to this point and different species of orb-weaving spiders may respond differently to similar stimuli.

Apart from obvious changes in web-spinning behavior due to differences in body size and ontogeny (Eberhard 1988), or changes in behavior induced by the presence of harmful toxins (Samu & Vollrath 1992; Hesselberg & Vollrath 2004), it may be difficult to determine the underlying reasons for changes in web structures. One overall trend however is that it appears that capture area and total spiral length are web structures commonly adapted to both abiotic and biotic factors, while other web parameters such as free zone area, and number of radii appear to be more static. In conclusion, we found that the web structures and body condition of riparian tetragnathids can be significantly altered by increasing levels of urbanization that impact stream ecosystems. Within a heavily urbanized watershed riparian, tetragnathids are most likely being affected, at least in some part, by some combination of several factors. Therefore, future studies focused on how particular mechanisms affect T. boydi web-spinning behaviors would be beneficial in determining how specific factors associated with urbanization are impacting these important riparian consumers within urban watersheds. As urban areas continue to dominate the landscape, it is becoming increasingly important to understand how the behavior and fitness of organisms is being affected by these anthropogenic activities.

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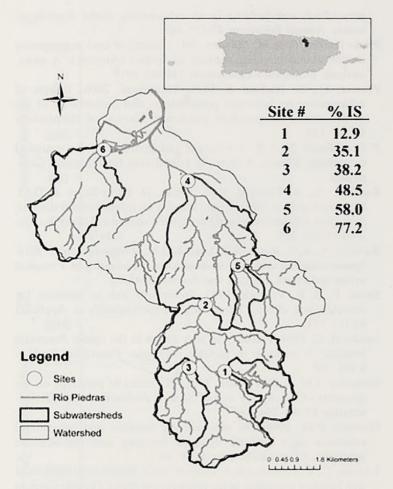
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Appendix 1.—Study sites within the Río Piedras watershed. Site 1 is the least urbanized with the lowest amount of surrounding percent impervious surface (%IS) and Site 6 the most urbanized with the greatest amount of surrounding percent impervious surface

Appendix 2.—Mean \pm standard deviation for the seven web parameters and spider body condition (ratio index = mass/cephalothorax width ²)
at each site (Site 1-6), along with the percent of impervious surface (%IS) for each site's sub-watershed and the number of webs and individual
spiders measured at each site (n), with the exception s of Site 1 (only 8 spiders measured) and Site 6 (10 spiders measured).

the descention of the set	Site 1 12.9 %IS (n = 10)	Site 2 35.1 %IS (n = 10)	Site 3 38.2 %IS (n = 9)	Site 4 48.5 %IS (n = 10)	Site 5 58.0 %IS (n = 10)	Site 6 77.2 %IS (n = 7)
Web parameters						
capture area (cm ²)	1783 ± 720	1019 ± 695	1532 ± 1144	452 ± 180	601 ± 339	434 ± 330
freezone area (cm ²)	33.0 ± 11.0	31.9 ± 13.1	49.4 ± 25.6	18.0 ± 4.0	20.0 ± 7.4	14.5 ± 9.9
mesh size (cm)	0.69 ± 0.18	0.70 ± 0.26	0.86 ± 0.17	0.42 ± 0.08	0.55 ± 0.15	0.55 ± 0.16
number of radii	11.5 ± 1.8	12.9 ± 1.9	11.8 ± 2.8	12.4 ± 1.4	12.8 ± 2.0	12.4 ± 1.5
number of spirals	27.4 ± 4.6	20.6 ± 7.5	21.4 ± 7.4	20.2 ± 5.8	19.8 ± 5.2	17.4 ± 2.9
radii length (cm)	283 ± 79	225 ± 99	253 ± 90	144 ± 45	181 ± 66	148 ± 67
spiral length (cm)	2700 ± 869	1690 ± 847	1960 ± 1075	941 ± 347	1108 ± 508	951 ± 576
Spider body condition						
ratio index	2226 ± 660	2337 ± 519	2530 ± 841	1967 ± 374	1724 ± 720	1337 ± 351



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