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Relative Tail Width as an Indicator of Body Condition in Central Texas *Eurycea* Salamanders

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Abstract.—**Measures of body condition are frequently used to assess the health and fitness of animals and are potentially useful for monitoring threatened and endangered species. I studied the suitability of relative tail width (tail width adjusted for body size) as a measure of body condition in populations of central Texas salamanders, the Georgetown Salamander (*Eurycea naufragia*) and the Salado Salamander (*E. chisholmensis*), by examining the association of relative tail width with ecologically important variables. Relative tail width was significantly wider in a population *E. naufragia* than in a population of *E. chisholmensis*. Within a population of *E. naufragia*, gravid salamanders had significantly smaller relative tail width than nongravid individuals. I observed no significant differences in relative tail width among salamanders from different sections of the spring run, but there was an association between relative tail width and number of salamanders observed within sections. Relative tail width varied seasonally, with lowest values occurring during winter months and increasing in summer and fall. These results are similar to those found in other species of central Texas *Eurycea* and suggest that relative tail width, as a measure of body condition, may be a useful tool in the conservation management of these threatened animals.**

Key Words.—body condition; *Eurycea naufragia*; *Eurycea chisholmensis*; salamanders; tail width

Introduction

Conservation management frequently requires assessment of the vulnerability of individuals and populations to environmental stress. One trait used to measure population health and vulnerability is body condition (e.g., Novinger and Rahel 2003; Cabezas et al. 2006; Hoare et al. 2006; Karraker and Welsh 2006; Prosser et al. 2016), usually defined as the relative energy reserves of an animal at a given point in time (Green 2001; Peig and Green 2009). Body condition, often assumed to be a proxy for overall health and fitness, has been assessed by measuring fat deposits (Weatherhead and Brown 1996), determining body composition (Peig and Green 2009), using observer scoring (Prosser et al. 2016), and, most commonly, by measuring associations between body mass and body size (Green 2001; Blackwell 2002; Peig and Green 2009; Băncilă et al. 2010). Among amphibians, variation in body condition has been associated with climate change (Reading 2007; Bucciarelli et al. 2020), population size (Unglaub et al. 2018), habitat availability and fragmentation (Janin et al. 2011), prey availability (Pope and Matthews 2002), fluctuating asymmetry (Davis and Maerz 2007), tail loss and regeneration (Pierce and Gonzales 2019), reproduction (Strickland et al. 2015; Nissen and Bendik 2020), competition (Liles et al. 2017), environmental temperature (Liles et al. 2017), and urbanization (Iglesias-Carrasco et al. 2017).

Because body condition is an indicator of fitness and response to stress, it potentially can be used as a tool in monitoring and management of threatened and endangered species. My study focused on endemic salamanders found on the Edwards Plateau, a large region of karst limestone in central Texas, USA. Fifteen described and several undescribed species of *Eurycea* occur in springs and caves of the Edwards Plateau (Chippindale et al. 2000; Bendik et al. 2013a; Devitt et al. 2019). All of the species are fully aquatic (paedomorphic) and dependent on groundwater, a diminishing resource that has been impacted by pumping, urbanization, and reduced recharge (Chippindale and Price 2005; Devitt et al. 2019). Most species of central Texas *Eurycea* have limited geographic ranges, and many are threatened or endangered (Devitt et al. 2019).

I studied body condition, as measured by relative tail width, in two species of *Eurycea* salamanders from the San Gabriel River drainage at the northern end of the Edwards Plateau, an area that is undergoing rapid urbanization. These salamanders were originally described as the Georgetown Salamander (*Eurycea naufragia*), but some northern populations in the drainage, including the Twin Springs population studied here, have now been reassigned to the Salado Salamander (*E. chisholmensis*) based on genomic analysis (Devitt et al. 2019). Both species were federally listed as Threatened in 2014 (U.S. Fish and Wildlife Service 2014).

I used relative tail width to assess body condition in a population of *E. naufragia* and a population of *E. chisholmensis*. I focused on relative tail width because salamanders often deposit lipids in the tail, which can then be mobilized for energy-intensive functions (Fitzpatrick 1973; Bernardo and Agosta 2005), and because relative tail width has been used to assess body condition in other studies (Bendik and Gluesenkamp 2012; Gutierrez et al. 2018; Pierce and Gonzalez 2019; Nissen and Bendik 2020). Here, I examined the use of relative tail width as a measure of body condition by analyzing how relative tail width of *Eurycea* salamanders varied among: (1) taxa; (2) reproductive states; (3) seasons; and (4) locations within a surface spring.

Materials and Methods

Field surveys.—I examined body condition of salamanders at two sites, Swinbank Spring (*E. naufragia*) and Twin Springs (*E. chisholmensis*), located in Williamson County, Texas, USA. Both sites consist of permanent springs emanating from the Edwards Aquifer and associated shallow spring runs approximately 1 m wide. Most salamanders at each site occur within 25–30 m of the primary spring outlet and are active year-round (Pierce et al. 2010). Information on habitat characteristics, reproduction, and population sizes at these sites is available elsewhere (Pierce et al. 2010, 2014).

Researchers surveyed salamanders at both sites monthly between October 2012 and July 2015 (34 surveys at each site). During each survey, my student assistants and I examined the entire wet surface of the spring run along a transect, beginning at the spring origin and extending downstream 24 m at Swinbank Spring and 36 m at Twin Springs. I divided each transect into approximately 5 m sections. We overturned all potential cover objects (rocks, leaf litter, plants, sticks, etc.) along the transect, captured salamanders with small aquarium nets, and placed them in mesh boxes within the spring run until they were photographed. We recorded the 5-m section within which a salamander was captured. To determine if salamanders were gravid, we placed each in a water-filled petri dish and held the dish up to sunlight or used a small flashlight to pass light through the abdominal wall to illuminate eggs, if present. We then used a Canon EOS Digital Rebel XTi camera and Canon EF-S 60 mm macro lens (Canon U.S.A., Inc., Melville, New York, USA) held directly above the salamander to photograph each salamander against a 0.634 × 0.634 cm grid. After we photographed all salamanders, we returned each salamander to the 5-m section of the spring from which it was originally captured.

Identification and measurement of salamanders.—I used unique patterns of melanophores on the head and Wild ID (vers. 1.0, Hanover, New Hampshire, USA; Bolger et al. 2012), a pattern-recognition software, to identify each salamander from digital photographs. Previous studies (Bendik et al. 2013b) demonstrated that this method can reliably identify individual *Eurycea* salamanders. I used digital photographs and ImageJ software (vers. 1.48, U. S. National Institutes of Health, Bethesda, Maryland, USA; https://imagej.nih.gov/ij/) to measure head-trunk length (HTL) and tail width (TW). HTL is the distance from the tip of the snout to the middle of a line drawn through the anterior-most insertion of the hind limbs (Fig. 1). I used head-trunk length as a measure of overall body size instead of the more traditional snout-vent length because I was unable to determine the location of vent from the dorsal views of the salamanders available from the photographs. Following Bendik and Gluesenkamp (2012), I operationally defined TW as the width of the body at the posterior-most insertion of the hind limbs (Fig. 1).

Data analysis.—To assess temporal and spatial variation in salamander tail width, I analyzed measurements on all salamander observations, including observations from recaptured salamanders. There were no hatchlings and few juveniles present in the samples (only 9% of all observations were salamanders with HTL < 23 mm); therefore, I combined adults and juveniles for the analyses. Tail width was strongly correlated with HTL ($r = 0.81$, $t = 51.99$, df = 1 and 1,470, $P < 0.001$, Fig. 2). To standardize tail width for body size, I performed a Linear Regression of tail width on HTL and used the residuals as measures of relative tail width (RTW; tail width corrected for body length). A positive residual indicates that tail width is greater than expected for a salamander of that size, whereas a negative value means the tail width is smaller than expected for a salamander of that size.

Figure 1. A Georgetown Salamander (*Eurycea naufragia*) illustrating measurements used to determine relative tail width. Head-trunk length was measured as the distance from the tip of the snout to the anterior insertion of the hind limbs. Tail width was defined as the width of the tail immediately posterior to the hind limbs. (Photographed by Benjamin Pierce).

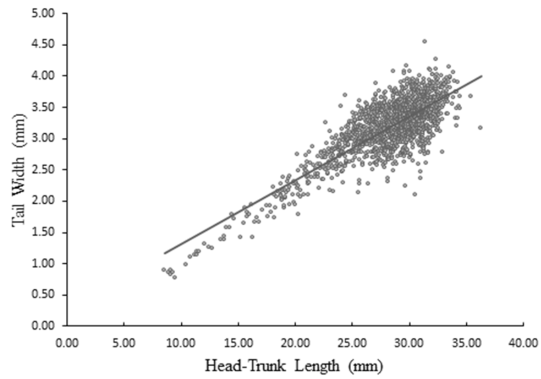

FIGURE 2. Regression of head-trunk length and tail width in Georgetown Salamanders (*Eurycea naufragia*) from Swinbank Spring and Salado Salamanders (*Eurycea chisholmensis*) from Twin Springs, Williamson County, Texas, USA.

FIGURE 3. Relative tail width among Georgetown Salamanders (*Eurycea naufragia*) from Swinbank Spring and Salado Salamanders (*Eurycea chisholmensis*) from Twin Springs, Williamson County, Texas, USA.

To assess differences between *E. naufragia* at Swinbank Spring and *E. chisholmensis* at Twin Springs, I used residuals from a regression of all salamander observations from both sites. Because *E. naufragia* and *E. chisholmensis* differed in relative tail width and because the numbers of *E. chisholmensis* that were gravid, present in some months, and among sections at Twin Springs were relatively small, I analyzed the effects of season, location, and reproductive state on *Eurycea naufragia* salamanders from Swinbank Spring only. For these analyses, I calculated residuals using a regression of *E. naufragia* from Swinbank Spring only.

The residuals of the regression of tail width on head-trunk length were normally distributed (one sample Kolmogorov-Smirnov test = 0.014, $P = 0.200$), but for each of the independent variables tested (population, whether gravid, section, and month), the group variances of the residuals were not homogeneous (assessed with Levene's statistic), so I used nonparametric Mann Whitney U tests and Kruskal-Wallace tests to examine differences in relative tail width among salamanders from different populations, seasons, sections, and reproductive states. I used an alpha level of 0.05 for all statistical tests. I used IBM SPSS Statistics (Version 24 Armonk, New York, USA) to perform all statistical tests.

RESULTS

During the monthly surveys between October 2012 and July 2015, I recorded 1,110 salamander observations (from 703 unique individuals) at Swinbank Spring and 362 salamander observations (from 210 unique individuals) at Twin Springs. Salamanders from Swinbank Spring (*E. naufragia*) had significantly greater relative tail widths (mean = 0.066 ± 0.008 standard error) than *E. chisholmensis* from Twin Springs (-0.203 ± 0.013; $U = 91{,}167$, $P < 0.001$; Fig. 3). The relative tail width of gravid *E. naufragia* at Swinbank Spring was smaller (-0.063 ± 0.024) than that of nongravid individuals (0.006 ± 0.009; $U = 39{,}804.5$, $P = 0.011$). There was no significant difference in mean RTW among salamanders from different sections of the spring run at Swinbank Spring ($H = 8.64$, df = 4, $P = 0.071$); however, there was a strong negative correlation between average RTW and the total number of salamanders captured within a section over the 34-month period: salamanders from sections with more salamander observations had lower relative tail width ($r = -0.89$, $t = -3.43$, df = 1, 3, $P = 0.041$; Fig. 4).

Relative tail width in *E. naufragia* at Swinbank Spring varied seasonally ($H = 26.76$, df = 11, $P = 0.005$); it was highest in October, declined during winter months and increased in spring and summer (Fig. 5). For nongravid *E. naufragia* only, there were also significant differences in relative tail width among months ($H = 23.50$, df = 11, $P = 0.015$) and the seasonal trend was similar. After excluding small salamanders (HTL < 23 mm), there was also still a significant difference in relative tail width

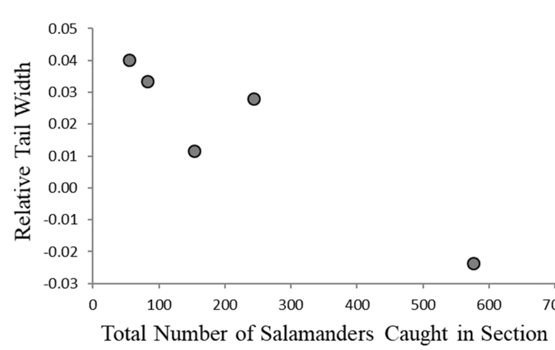

FIGURE 4. Correlation of average relative tail width with the total number of Georgetown Salamanders (*Eurycea naufragia*) caught per 5-m section at Swinbank Spring on the Edwards Plateau of central Texas, USA, during a 34-month study period.

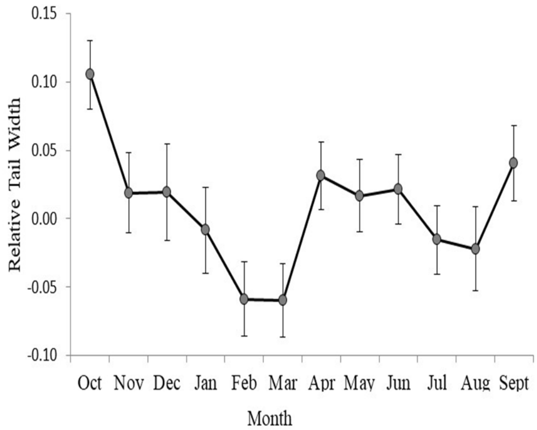

Figure 5. Relative tail width of Georgetown Salamanders (*Eurycea naufragia*) varied seasonally at Swinbank Spring on the Edwards Plateau of central Texas, USA, from October 2012 to July 2015.

among months ($H = 24.97$, df = 11, $P = 0.009$) and the seasonal trend (Fig 5) was the same.

Discussion

Body condition, defined as the relative energy reserves of an animal (Green 2001; Peig and Green 2009), is a useful tool in conservation management to assess the health and fitness of organisms (e.g., Novinger and Rahel 2003; Hoare et al. 2006; Prossar et al. 2016). In this study, I evaluated relative tail width as a potential measure of body condition in two threatened species of central Texas salamanders by examining the association of relative tail width with several important ecological parameters, including site, reproduction, season, and salamander abundance. Although my data indicate that relative tail width of *Eurycea naufragia* at Swinbank Spring is greater than that of *E. chisholmensis* at Twin Springs, I cannot determine if the difference is associated with genetic characteristics of the taxa or with differences in site ecology, such as food availability. Chippindale et al. (2000) observed morphological differences between *E. naufragia* and *E. chisholmensis* but did not examine relative tail width. Studies of relative tail width at additional populations of both species would be helpful in determining whether differences occur in relative tail width of the two species.

I also observed that gravid *E. naufragia* at Swinbank Spring had smaller relative tail width compared to nongravid salamanders. Similarly, Nissen and Bendik (2020) found that gravid Barton Springs Salamanders (*E. sosorum*) and Jollyville Plateau Salamanders (*E. tonkawae*) had lower relative tail width than nongravid salamanders during winter months, when most gravid salamanders occur. Reproduction also has been associated with lower body condition in other salamanders (Strickland et al. 2015). Reduced tail width during periods of reproduction is likely due to mobilization of energy reserves in the tail for oocyte production (Fitzpatrick 1973; Bernardo and Agosta 2005), which results in a lower relative tail width during oogenesis.

Seasonal variation in relative tail width occurred among *E. naufragia* at Swinbank Spring with relative tail width highest in September and October, dropping during the winter months, and then increased in spring and summer. This is similar to the seasonal pattern that Nissen and Bendik (2020) observed for *E. sosorum* and *E. tonkawae*. The seasonal decline in relative tail width occurs when most gravid salamanders are present, but is independent of reproduction state, as nongravid salamanders also have lower relative tail width during winter months. This seasonal trend in relative tail width is also not simply the result of the presence of more juvenile salamanders, which may have less fat reserves and smaller relative tail widths, because excluding salamanders < 23 mm HTL from the analysis does not alter the overall pattern. Seasonal variation in relative tail width might be associated with changes in food abundance, but I have no quantitative data on this variable. Seasonal variation in availability of food has been correlated with variation in body condition of other salamanders (Huntsman et al. 2011; Fenolio et al. 2014). Bendik and Gluesenkamp (2012) also found that relative tail width of salamanders decreased during drought conditions, when salamanders were unable to occupy surface habitat.

The entire spring run at Swinbank Spring is only 24 m long, but most salamanders at this site are relatively sedentary and do not move great distances. For example, only 24% of recaptured salamanders at Swinbank Spring moved beyond their original 5 m section of original capture over a 32-mo period (Gutierrez et al. 2018). In spite of this low vagility, relative tail width of salamanders occupying different sections of the spring run do not differ. Although the differences among sections are not significant, there is a significant relationship between the number of salamanders observed in a section over the 34-mo period of the study and relative tail width, with higher salamander abundance associated with lower relative tail width. Total number of salamanders observed is not a direct estimate of salamander density, but the observed relationship is consistent with the prediction that relative tail width is smaller in areas of the spring run where density of salamanders is higher. Such an association could result from competition for food, physical interactions among salamanders, or other factors correlated with salamander abundance.

A correlation exists between relative tail width and regeneration of injured tails in *E. naufragia* at Swinbank Spring (Pierce and Gonzalez 2019) but not between relative tail width and movement (Gutierrez et al. 2018). Nissen and Bendik (2020) found a small positive effect of streamflow on relative tail width of *E. tonkawae* and a negative effect on relative tail width of *E. sosorum*. I did not have measures of streamflow in this study and so was not able to test this relationship.

Rosa et al. (2021) cautioned that relative tail width in salamanders may not be a reliable indicator of body condition measured for all salamander taxa, as they found a relationship between relative tail width and scaled mass index (a widely used measure of body condition) in only five of the six species they examined. They did not examine the relationship between tail width and ecological variables, however. My data support previous observations (Nissen and Bendik 2020) that relative tail width in *Eurycea* salamanders from central Texas is related to important ecological parameters, including reproduction, season, and abundance. These observations suggest that relative tail width may be useful for assessing the health and nutritional status of salamanders and may be a useful tool for conservation management of these threatened species. For example, a consistent decline in relative tail width within a population over time might be an indicator that the environment is being degraded. Similarly, when resources for conservation are limited, relative tail width might be useful for deciding which populations are healthy and which need conservation efforts. Measuring relative tail width from digital photographs does not require invasive procedures or sacrificing the animals and can be easily carried out in the field as a part of long-term monitoring studies. Long-term monitoring projects for these salamanders already use digital photographs for salamander identification (Pierce et al. 2014; Bendik 2017; Gutierrez et al. 2018; Bendik et al. 2021) and can be easily adapted for measuring relative tail width as an indicator of body condition, providing useful information about salamander body condition.

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