Intraspecific variation in the diet of the Mexican garter snake *Thamnophis eques*

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Abstract

The Mexican Garter Snake (*Thamnophis eques*) is a terrestrial-aquatic generalist that feeds on both aquatic and terrestrial prey. We describe size-related variation and sexual variation in the diet of *T. eques* through analysis of 262 samples of identifiable stomach contents in snakes from 23 locations on the Mexican Plateau. The snake *T. eques* we studied consumed mostly fish, followed in lesser amounts by leeches, earthworms, frogs, and tadpoles. Correspondence analysis suggested that the frequency of consumption of various prey items differed between the

24   categories of age but not between sex of snakes, and the general pattern was a reduction of prey

25   item diversity with size of snake. Snake length was correlated positively with mass of ingested

26   prey. Large snakes consumed large prey and continued to consume smaller prey. In general, no

27   differences were found between the prey taxa of male and female snakes, although males ate two

28   times more tadpoles than females. Males and females did not differ in the mass of leeches,

29   earthworms, fishes, frogs and tadpoles that they ate, and males and females that ate each prey

30   taxon were similar in length. We discuss proximate and functional determinants of diet and

31   suggest that the observed intraspecific variation in *T. eques* could be explored by temporal

32   variation in prey availability, proportions of snake size classes and possible sexual dimorphism

33   in head traits and prey dimensions to assess the role of intersexual resource competition.

34   **Introduction**

35   The Mexican Garter Snake (*T. eques*) is a medium-sized garter snake classified as a generalist

36   predator because it feeds on both aquatic and terrestrial prey; mostly frogs, tadpoles, and fish,

37   supplemented by lizards and mice (Drummond & Macías Garcia, 1989; Manjarrez, 1998).

38   Drummond & Macías Garcia (1989) found that *T. eques* at Tecocomulco, Hidalgo, is locally

39   specialized in feeding on only two to three taxa. This snake forages in vegetative cover along the

40   shore and an attack may include a sudden lunge across the surface toward prey (Drummond &

41   Macías Garcia, 1989).

42   Although *T. eques* is widely distributed over the Mexican Plateau, in this area, the

43   disturbance and loss of habitat have caused the isolation and fragmentation of their populations

44   (Conant, 2003; Manjarrez, Contreras-Garduño, & Janczur, 2014), with low population densities

45   and constricted distribution (Rossman, Ford, & Seigel, 1996; Manjarrez, 1998). This scenario

46   raises the possibility of intraspecific dietary differences by spatial variation of the environment.

47   In general, garter snakes show important ecological intraspecific variation (Rossman, Ford, &

48   Seigel, 1996), and reports on diet for *T. eques* in Mexico showed sexual, ontogenetic (neonates-

49   adults) and seasonal (rainy-dry) divergence in prey size (Macías Garcia & Drummond, 1988;

50   Drummond & Macías Garcia, 1989; Manjarrez, Contreras-Garduño, & Janczur, 2014). For

51   example, at Lake Tecocomulco, Mexico, small snakes fed mainly on aquatic invertebrates

52   (leeches and earthworms), while large snakes fed on aquatic vertebrates (frogs, fish, and

53   salamander larvae). Fluctuations in prey availability was associated with seasonal variation in

54   prey (Macías Garcia & Drummond, 1988). At Toluca, Mexico, snakes *T. eques* were detected to

55   have eaten earthworms, tadpole, slugs and mice (Manjarrez, 1998; Manjarrez, Contreras-

56   Garduño, & Janczur, 2014).

57   In this study, we provide the first broad description of the diet of *T. eques* on the Mexican

58   Plateau. We looked for variation in consumption of prey type, sex and size-classes of snake from

59   three different drainages. To permit more extensive and novel comparisons, we pooled our

60   dietary records with those of Lozoya (1988) and Drummond & Macías Garcia (1989), as

61   described in Materials and Methods.

62   Sexual differences in snake diets show that females sometimes ingest larger prey than

63   males (Shine, 1993; Seigel, 1996; Daltry, Wuster, & Thorpe, 1998) and this difference is usually

64   attributed to sexual dimorphism in body size when females are bigger that males. The maximum

65   size of prey that can be ingested is constrained by a snake's gape (e.g. King, 2002), and in most

66   species, larger snakes take larger prey and appear to drop small prey from their diet, although

67   data from very young snakes is usually limited (review in Arnold, 1993). Garter snakes are

68   sexually dimorphic in body size (Shine, 1993) and their diet can vary with age/size-classes

69   (Mushinsky, 1987; Macías Garcia & Drummond, 1988; amongst others). Female garter snakes

70 are usually larger than males (Shine, 1994) but sexual differences in garter snake diets have not

71 been well studied (Seigel, 1996). *Thamnophis eques* is sexually dimorphic with adult females

72 being 5.6% larger than males in snout-vent length (SVL; Manjarrez, 1998; Manjarrez, Contreras-

73 Garduño, & Janczur, 2014).

74 **Materials & Methods**

75 This study received the approval of the ethics committee of the Universidad Autónoma del

76 Estado de México (Number 4047/2016SF). All subjects were treated humanely on the basis of

77 guidelines outlined by the American Society of Ichthyologists and Herpetologists (ASIH, 2004).

78   We collected snakes along streams, rivers, canals, ponds, and lakes. We measured each

79 captured snake (SVL in cm), and also recorded sex in adults by visual inspection of the tail-base

80 breadth or by manually everting the hemipenis in small snakes (Conant & Collins, 1998).

81 Although the snakes were weighed, these data were not used in this study. Each snake was

82 forced to regurgitate stomach contents by abdominal palpation (Carpenter, 1952). After

83 processing, snakes were released at their capture sites. We measured the wet mass of each prey

84 item and then fixed them in 10% formalin and preserved them in 70% alcohol.

85   We sampled snake stomach contents at 23 sites on the Mexican Plateau (Lerma, Tula, and

86 Nazas, drainages, Table S1) sporadically during the active reproductive season (February to

87 November) over a period of 16 years (Table S2). The sites in the Lerma and Tula drainages were

88 sampled from 1980-1986 and 1991-1995. We obtained 194 regurgitations from 22 sites in

89 Jalisco, Michoacán, México, Hidalgo, and Queretaro. The records obtained for these two

90 drainages are partially reported by Lozoya (1988, 19% of the total regurgitations), a reference

91 inaccessible to most readers.

92   In the Nazas drainage we obtained 68 regurgitations. The Nazas population inhabits an

93   isolated 0.36 ha spring-fed cattle pond in the Chihuahuan Desert with a rainy season from June

94   through October. The records were obtained during 27 2-4 day visits, bimonthly during April to

95   November 1981, and monthly during February through December 1982, and February through

96   November 1983. Drummond & Macías Garcia (1989) previously reported 13% of the total these

97   records. Snakes were captured on the first two days of each visit and released on the second day

98   to prevent repeat sampling during the same visit. We counted and classified prey items as fish,

99   leeches, frogs, tadpoles, and earthworms.

100   **Analysis**

101   We classified snakes as neonates (< 20.5 cm SVL), juveniles (20.5–39.5 cm) or adults (> 39.5

102   cm; Manjarrez, 1998). Percentages of regurgitations containing each prey taxon were normalized

103   by arcsine transformation (Zar, 1984). We used MANCOVA (with snake length as a covariate)

104   to explore variation in the mass of prey consumed by prey taxa and by the sex of each snake. We

105   included complementary analyses of dietary variation in relation to snake size whenever these

106   could contribute to understanding variation in diet. For analyses of prey mass we excluded taxa

107   represented by fewer than five prey items. Prey mass and snake length were natural log

108   transformed prior to calculating correlations because of the lack of homoscedasticity and skewed

109   distributions.

110   To avoid making Type I or II errors when many Chi square (X^2) tests are performed, we

111   used a Multiple Correspondence Analysis (MCA) to associate prey items consumed more

112   frequently (earthworm, tadpole, fish, frog and leech, considered as not continuous data) by the

113   combination of two categorical variables, the sex of snake (male-female) and snake size

114   (neonates, juveniles, and adults). The analysis is a modification of X^2 used to analyze

115 contingency tables and creates a Cartesian diagram based on the association between more than

116 three categorical variables (Legendre & Legendre, 2003). The diagram display simultaneously

117 the relative position (canonical position) of studied variables categories (Gotelli, 2001). The

118 nearest canonical positions of different variables represent a high association, while distant

119 categories show lower association. The degrees of freedom (*df*) and probability (*P*) for MCA

120 have no statistical relevance, because both were used only in two-way table, therefore, we do not

121 report these values. However, similar to X^2 test, the MCA estimate the differences between

122 observed and expected values, which allow estimate contribution of each variable to X^2 test

123 value. The analyses generates a coordinate system of reference that account variations of all

124 variables (dimensions), where we reported the first two dimensions, which represents the higher

125 variations. Finally, the center of graph (coordinate 0, 0) was the average of all variables,

126 therefore categories nearest to center of graph show a lower association to rest of categories.

127   Additionally, we performed a Cluster analysis to identify groups of categories of age and

128 sex of snakes with similar consumption of prey, we used the Morisita index of similarity between

129 frequencies of prey consumed, and the Ward's Method of amalgamation (Rencher, 2002).

130 Ward's Method attempts to minimize the Sum of Squares (SS) of any two hypothetical clusters

131 that can be formed at each step (Legendre & Legendre, 2003), and it is considered as very

132 efficient method, due reduce cluster long chains produced by other joining amalgamation

133 methods as nearest neighbor or UPGMA. The number of groups retained were determined by

134 graph of amalgamation schedule. The graph shows a line graph of the linkage distances at

135 successive clustering steps, the optimal cut-off to deciding how many clusters to retain is when

136 the linkage distance line forms a plateau. Multiple Correspondence and Cluster Analyses are

137 complementary, the MCA identify associations among categories of different categorical

138   variables, but cannot determine differences; while cluster create groups of different elements, but

139   we cannot know why they are different.

140   **Results**

141   **Prey items**

142   We obtained identifiable stomach contents from 262 (38%) of 690 *T. eques* collected. Thirteen

143   regurgitations (5.7% of total) included more than one prey species, and hence contributed more

144   than one data point for some snakes. In order of percentage of prey items identified we found

145   42.4% (n =111 regurgitations) consumed fish, 23.7% ($n = 62$) leeches, 10.6% ($n = 28$)

146   earthworms, 10.2% ($n = 27$) frogs, 9.8% ($n = 25$) tadpoles, and 2.3% ($n = 6$) axolotls

147   (*Ambystoma sp*.). Lizard, slug, and mouse were recovered from only 1 stomach each.

148   Our Multiple Correspondence Analysis ($X^2 = 2{,}410.1$) shows that prey type variable

149   contributes with 71.6% of X^2 test value ($X^2 = 1{,}725.9$), while snake size with 19.5% ($X^2 = 468.6$)

150   and sex with 8.9% ($X^2 = 215.6$). Canonical position of adult snakes was closer to tadpoles, frogs,

151   and axolotls, therefore adults tend to feed more frequently these prey types than juveniles and

152   neonates snakes (Fig. 1). Juveniles consumed more earthworms, while neonates principally

153   leeches (Fig. 1). Canonical position of male and female snakes showed a lower variation on the

154   prey type consumed (Fig. 1). Cluster analysis indicated that the three size classes differ in prey

155   type consumed (Fig. 2), where adult snakes were closer to juveniles, and both distant to neonate.

156   Prey types consumed by female and male snakes were similar, due linkage distance nodes of

157   males and females of the same size classes was lower than cutoff value. This grouping of snakes

158   suggests a scheme of ontogenetic change in the taxon of prey, with lower relevance of the

159   grouping by sex (Fig. 2).

160   **Variation with snake length**

161 Snakes of different sizes ate a changing diversity of prey types. The general pattern was a

162 reduction of number of prey item with increasing snake body size. (Fig. 3). Snakes < 65 cm SVL

163 ate all prey types of all sizes, including invertebrates (leeches and earthworms) and vertebrates

164 (tadpoles, fish, and frogs). Vertebrate prey were taken by only the largest snakes (> 60 cm SVL).

165 At 15 cm SVL, snakes eat leeches as one of two major prey items, but at 55 cm SVL

166 consumption of leeches decreases drastically and disappears completely in larger snakes > 65cm

167 SVL (Fig. 3). The consumption of fish and tadpoles increases when snake body size increases.

168 However, the longer snakes > 75 cm SVL consume only fish and tadpoles while excluding all

169 other prey, possibly because longer snakes were a very small part of the entire sample ($n = 7$

170 stomach contents).

171 Snake length was correlated positively with mass of ingested prey ($r = 0.42$, $F_{1, 326} =$

172 71.52, $P < 0.001$; Fig. 4). Large snakes consume large prey and continue to consume smaller

173 prey. The same relationship was presented for leeches ($r = 0.42$, $F_{1,136} = 29.85$, $P < 0.00$; Fig. 5)

174 and fish ($r = 0.43$, $F_{1,88} = 20.30$, $P < 0.001$; Fig. 5), but not correlated with earthworm ($r = 0.14$,

175 $F_{1,32} = 18.32$, $P = 0.806$; Fig. 5) and tadpole mass ($r = 0.2$, $F_{1,25} = 2.36$, $P = 0.136$; Fig. 5).

176 **Variation with snake sex**

177 Mean body lengths of captured snakes did not differ between sexes of neonates (Student $t_{20} =$

178 0.08, $P = 0.94$), juveniles (Student $t_{94} = 0.95$, $P = 0.34$), or adults (Student $t_{131} = 0.13$, $P = 0.90$);

179 thus male and female snakes were similar in size.

180 No differences were found between the diets of male and female snakes. Pooling all sizes

181 of snake, males ($n = 124$) and females ($n = 121$) ate similar proportions of the five main prey

182 taxa ($X^2 = 3.82$, $P = 0.43$), both sexes eating mainly fishes, frogs, leeches, and earthworms, and

183 in similar proportions. Males ate two times more tadpoles (0.13) than females (0.06).

184   Males and females did not differ in the mass of leeches, earthworms, fishes, frogs and

185   tadpoles that they ate (MANCOVA $F_{1, 193} = 0.79$, $P = 0.37$), and males and females that ate each

186   prey taxon were similar in length ($F_{1, 235} = 0.91$, $P = 0.34$).

187   **Discussion**

188   In this study, we provide a broad description of the diet of *T. eques* on the Mexican Plateau. The

189   results indicate 69% of total regurgitations contain two major prey: leeches and fish, while the

190   other three main prey are ingested in similar percentages (earthworms, 10.6%; frogs, 10.2%;

191   tadpoles, 9.8%). The diet of *T. eques* included amphibious prey (frogs), terrestrial prey

192   (earthworms) and aquatic prey (leeches, fish and tadpoles), and occasionally other prey such as

193   axolotls, slugs, lizards, and mice. The main prey include three vertebrates (65%) and two

194   invertebrates (35%).

195   The studies that have analyzed the diet of *T. eques* in Mexico included four local

196   descriptions and in each study *T. eques* is locally specialized in feeding on only 2-3 prey taxa

197   (Table 1) (Drummond & Macías Garcia, 1989). This suggests a pattern of spatial variation in the

198   diet of *T. eques*, presumably by the local availability of prey, for example the temporal variation

199   of prey, which has not yet been explored for *T. eques* (Gregory & Nelson, 1991; Tuttle &

200   Gregory, 2009).

201   The diet of *T. eques* can also exhibit ontogenetic variations associated with individual

202   size, changing from terrestrial to aquatic prey as snake size increases (Macías Garcia &

203   Drummond, 1988; Drummond & Macías Garcia, 1989). Ontogenetic change by prey taxa in

204   gartersnakes, could be attributed to proximate mechanisms such as morphological constraints

205   that determine the size of ingested prey (Shine, 1991; Arnold, 1993), the availability of potential

206   prey (Krebs, 2009), energy or nutritional needs (Britt, Hicks, & Bennett, 2006), habituation and

207 learning (Halloy & Burghardt, 1990; Ford & Burghardt, 1993) or genetically programmed

208 preferences (Arnold, 1977; Arnold, 1981; Britt, Hicks, & Bennett, 2006). In Tecocomulco,

209 Hidalgo, the differential distribution of large and small snakes was interpreted as a possible

210 cause of differences in diet of *T. eques* with differences in the pattern of foraging, so that the

211 snake can be an effective predator in the air-water interface; preying on aquatic prey when they

212 are particularly vulnerable and terrestrial prey being added to the diet only opportunistically

213 (Drummond & Macías García, 1989).

214   In our study, the ontogenetic variation in diet of *T. eques* was also found in the

215 relationship between snake size and prey mass. The ingested prey size gradually increases with

216 snake size and large snakes continued eating small prey (Arnold, 1993). This relationship could

217 be interpreted as an ontogenetic telescope (Arnold, 1993), as previously reported for *T. eques* in

218 a Zacatecas population with *Rana berlandieri* (Drummond & Macías García, 1989).

219   The absence of ontogenetic variation in the regurgitated samples of tadpoles and

220 earthworms in *T. eques*, is common because in a previous study the annelids were ingested by *T.*

221 *eques* regardless of snake body size (Macías Garcia & Drummond (1988). The proximate

222 explanation for this phenomenon is the high availability of these prey during the annual active

223 period of the snake or by a stable ontogenetic preference for invertebrates (Ford & Burghardt,

224 1993). The proximate and functional diet determinants of intraspecific variation in *T. eques*

225 could be explored by local and temporal variation in prey availability and proportions of snake

226 size classes collected. Intraspecific differences in *Thamnophis* diet have been described for

227 different populations, seasons, years, size classes, and sexes (Rossman, Ford, & Siegel, 1996).

228 For example, in some species of *Thamnophis* have been described geographic dietary differences

229 (e.g., *T. elegans*, *T. radix*, and *T. sirtalis*; Kephart, 1982; Kephart & Arnold, 1982; Tuttle &

230 Gregory, 2009) explained by spatial or temporal variation in prey availability (Kephart and

231 Arnold, 1982; Seigel, 1996).

232 The intersexual variation in food habits has been associated with sexual differences in

233 body size (Shine et al., 1998). *Thamnophis eques* has been reported as sexually size dimorphic,

234 with males smaller than females in SVL (Manjarrez, 1998); however in this study, the average

235 size of male and female *T. eques* were similar and there were no sexual differences in diet,

236 except that males ate two times more tadpoles than females. This sexual difference in diet can

237 probably be attributed to real diet preference because females and males that ate each prey taxon

238 were similar in length. Generally, large samples revealed no differences between male and

239 female snakes in variety of prey taxa, proportions of different prey taxa and taxon specific prey

240 weight (Shine, 1993), and the males and females that ate each taxon were similar in size. Overall,

241 male and female *T. eques* differ in size (Manjarrez, Contreras-Garduño, & Janczur, 2014),

242 microhabitat use (Venegas-Barrera, 2001), seasonal foraging pattern (Drummond & Macías

243 Garcia, 1989), and diet (Macías Garcia & Drummond, 1988; Manjarrez, Contreras-Garduño, &

244 Janczur, 2014). As was found, males and females of this species do not differ in the body size of

245 prey and type of prey consumed. The possible small differences in diet and microhabitat can be

246 expose by larger sample sizes. Prey size and energetic demands may determine developmental

247 transitions to different prey sizes or taxa, whereas sex, in this snake lacking sexual size

248 dimorphism, has little or no influence on diet. However, sexual dimorphism in head dimensions,

249 and eaten prey shape have seldom been searched and it will be essential to measure prey and

250 head traits for *T. eques* to evaluate the function of resource competition between sexes.

251 **Conclusions**

252 In this study, we provide the first broad description of the diet of the snake *T. eques* on the

253 Mexican Plateau. The two major prey were leeches and fish. The diet of *T. eques* included

254 amphibious, terrestrial and aquatic prey with ontogenetic variations associated with individual

255 size, changing from terrestrial to aquatic prey as snake size increases. The ontogenetic variation

256 in diet of *T. eques* was also found in the relationship between snake size and prey mass. The

257 average size of male and female *T. eques* were similar and there were no sexual differences in

258 diet. The proximate and functional diet determinants of intraspecific variation in *T. eques* could

259 be explored by local and temporal variation in prey availability and proportions of snake size

260 classes collected.

261 **Acknowledgements**

262 For their assistance in the field and laboratory work we thank Hugh Drummond, Constantino

263 Macías García and all of the students of the Evolutionary Biology Laboratory. We also thank

264 Ruthe J. Smith for her comments and corrections of the typescript. All subjects were treated

265 humanely on the basis of guidelines outlined by the Society for the Study of Amphibians &

266 Reptiles. C. Zepeda, M. Manjarrez-Zepeda, and J. Manjarrez-Zepeda provided moral support to

267 JM.

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347 **Table 1.** Percentage of prey reported in the diet of *Thamnophis eques* in Mexico.

	Tecocomulco[1]	Cerrillo[2]	Toluca Valley[3]	Fresnillo[4]	Present study
	126 snakes	18 regurgitations	148 snakes	64 snakes	262 regurgitations
Invertebrate prey					
Earthworm	41	22	20.2	2.9	10.6 (*Eisenia foetida* and *Eisenia* sp.)
Leech	39	---	8.7	---	23.7 (*Erpobdella punctata* and *Mooreobdella* sp.)
Slug	1.0	5.5	---	---	0.4
Vertebrate prey					
Fish	11	---	29.0	---	42.4 (*Girardinichthys multiradiatus, Carassius auratus*)
Frog	5	28	10.1	69.0	10.2 (*Rana berlandieri* and *Rana sp.*)
Tadpole	1.5	33	22.2	23.4	9.8 (*R. berlandieri, Rana sp*)
Other (axolotl, lizard or mouse)	4.5	11.0	9.4	4.7	3.2

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349 [1]Macías García & Drummond, 1988; [2]Manjarrez, 1998; [3]Manjarrez, Contreras-Garduño &

350 Janczur; [4]Drummond & Macías García, 1989.

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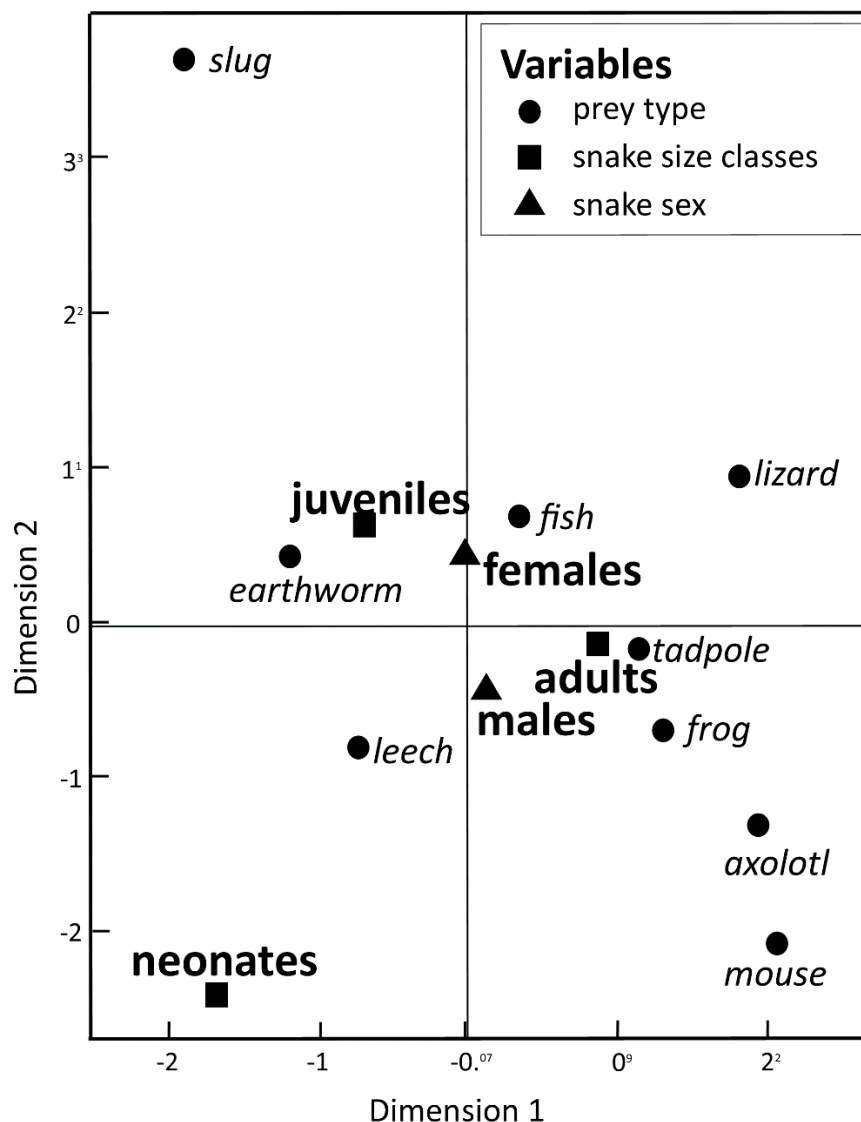
355 Fig. 1. Dimension variables obtained from a Multiple Correspondence Analysis to associate prey

356 items consumed by snake *T. eques* in the combination of snake sex (male-female) and snake size

357 classes (neonates, juveniles, and adults).

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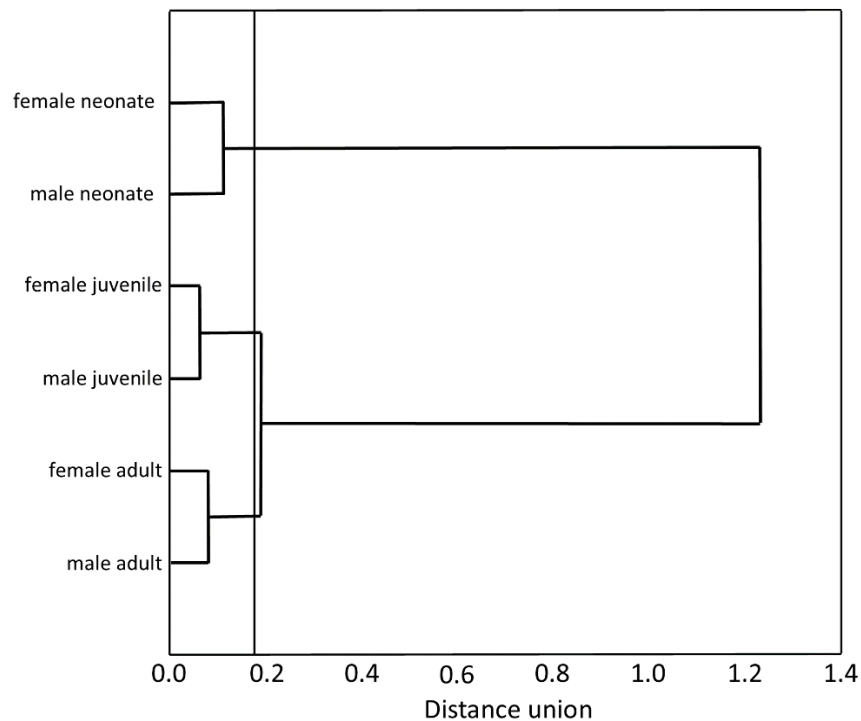
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361   Fig. 2. Hierarchical tree produced agglomeration of size classes (neonates, juveniles and adults)

362   and sex (female and male) of *T. eques* snakes in function of prey type consumed. The

363   amalgamation schedule (Ward's Method) defined in 0.8 the cutoff value for the tree diagram.

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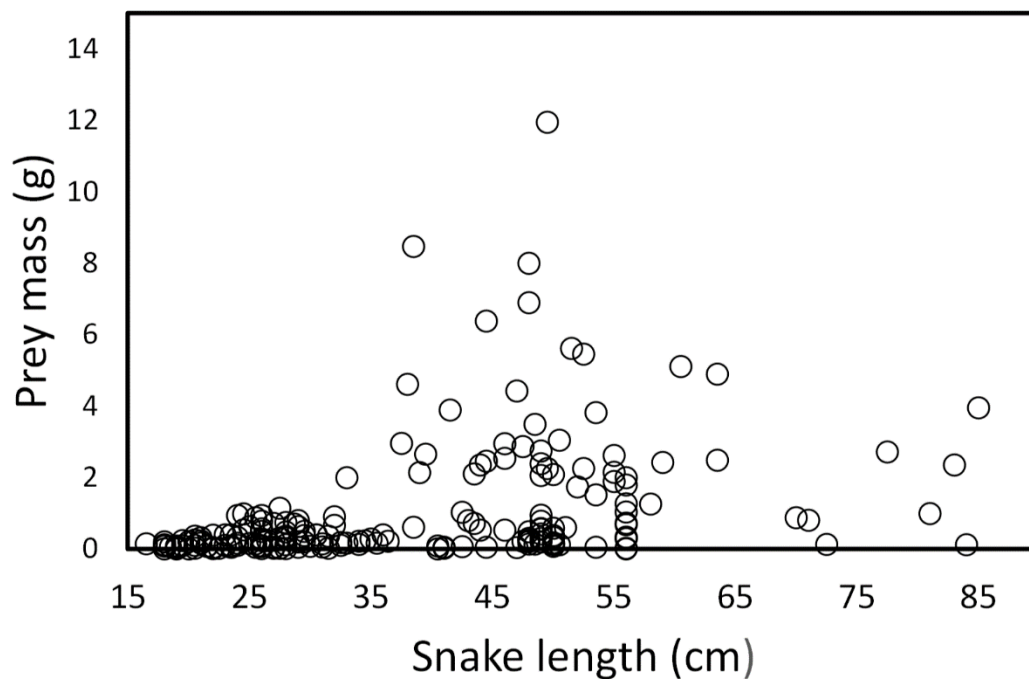
377   Fig. 3. Relationship between prey type and snake size (SVL, cm) of *T. eques* in México. Each

378   circle represents an individual snake with a type of prey item (262 regurgitations).

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380   Fig. 4. Prey mass as a function of snake length (SVL, cm) of (*T. eques*) in México. ($r = 0.42$, F

381    $_{1, 326} = 71.52$, $P < 0.001$). Each dot represents an individual prey item. The many circles on the

382   zero line of prey mass are because the low weights of leeches and earthworms.

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406   Fig. 5. Relation between prey mass and snake length of *T. eques*. (A) Leeches ($r = 0.42$, $P <$

407   0.00), (B) fish ($r = 0.43$, $P < 0.001$), (C) earthworm ($r = 0.14$, $P = 0.806$), (D) tadpole ($r = 0.2$, P

408    $= 0.136$), (E) frogs ($r = 0.597$, $P < 0.001$).