

A peer-reviewed version of this preprint was published in PeerJ on 13 March 2019.

[View the peer-reviewed version](https://peerj.com/articles/6601) (peerj.com/articles/6601), which is the preferred citable publication unless you specifically need to cite this preprint.

Valencia-Flores E, Venegas-Barrera CS, Fajardo V, Manjarrez J. 2019. Microgeographic variation in body condition of three Mexican garter snakes in central Mexico. PeerJ 7:e6601
<https://doi.org/10.7717/peerj.6601>

Microgeographic variation in body condition of three Mexican garter snakes in central Mexico

Erika Valencia-Flores¹, Crystian S Venegas-Barrera², Victor Fajardo³, Javier Manjarrez^{Corresp. 1}

¹ Facultad de Ciencias, Universidad Autónoma del Estado de México, Toluca, Estado de México, México

² División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Ciudad Victoria, Ciudad Victoria, Tamaulipas, México

³ Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

Corresponding Author: Javier Manjarrez

Email address: jsilva@uaemex.mx

Background. Geographic variation in body size and condition can reveal differential local adaptation to resource availability or climatic factors. Body size and condition are related to fitness in garter snakes (*Thamnophis*), thus good body condition may increase survival, fecundity in females, and mating success in males. Sympatric species are predicted to exhibit similar body condition when they experience similar environmental conditions. We focused on interspecific and geographical variation in body size and condition in three sympatric Mexican garter snakes from the highlands of Central Mexico. **Methods.** We assessed SVL, mass, and body condition (obtained from Major axis linear regression of ln-transformed body mass on ln-transformed SVL) in adults and juveniles of both sexes of *Thamnophis eques*, *T. melanogaster*, and *T. scalaris* sampled at different locations and over a 20-year period. **Results.** We provide a heterogeneous pattern of sexual and ontogenic reproductive status variations of body size and condition among local populations. Each garter snake species shows locations with good and poor body condition; juvenile snakes show similar body condition between populations, adults show varying body condition between populations, and adults also show sexual differences in body condition. We discuss variations in body condition as possibly related to the snakes' life cycle differences.

1

2 **MICROGEOGRAPHIC VARIATION IN BODY**
3 **CONDITION OF THREE MEXICAN GARTER SNAKES**
4 **IN CENTRAL MEXICO**

5

6 Erika Valencia-Flores¹, Crystian S. Venegas-Barrera², Victor Fajardo³ and Javier Manjarrez¹

7

8 ¹ Facultad de Ciencias, Universidad Autónoma del Estado de México, Toluca, Estado de México,
9 México

10 ² División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Ciudad Victoria,
11 Ciudad Victoria, Tamaulipas, México

12 ³ Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México,
13 Toluca, Estado de México, México

14

15 Corresponding Author:

16 Javier Manjarrez¹

17 Instituto Literario 100, Centro, Toluca, Estado de México, CP 50000, México

18 Email address: jsilva@uaemex.mx

19

20 **ABSTRACT**

21 **Background.** Geographic variation in body size and condition can reveal differential local
22 adaptation to resource availability or climatic factors. Body size and condition are related to
23 fitness in garter snakes (*Thamnophis*), thus good body condition may increase survival, fecundity
24 in females, and mating success in males. Sympatric species are predicted to exhibit similar body
25 condition when they experience similar environmental conditions. We focused on interspecific
26 and geographical variation in body size and condition in three sympatric Mexican garter snakes
27 from the highlands of Central Mexico.

28 **Methods.** We assessed SVL, mass, and body condition (obtained from Major axis linear
29 regression of ln-transformed body mass on ln-transformed SVL) in adults and juveniles of both
30 sexes of *Thamnophis eques*, *T. melanogaster*, and *T. scalaris* sampled at different locations and
31 over a 20-year period.

32 **Results.** We provide a heterogeneous pattern of sexual and ontogenic reproductive status
33 variations of body size and condition among local populations. Each garter snake species shows
34 locations with good and poor body condition; juvenile snakes show similar body condition
35 between populations, adults show varying body condition between populations, and adults also
36 show sexual differences in body condition. We discuss variations in body condition as possibly
37 related to the snakes' life cycle differences.

38

39 INTRODUCTION

40 Organisms usually respond to differences in environmental conditions by exhibiting local
41 adaptation in phenotypic traits. Geographic variation in phenotypic traits associated with body
42 size and condition can reveal differential adaptation of local populations to local biotic and
43 abiotic fluctuations as presence of related species, resource availability, or climatic factors

44 (Bronikowski & Arnold 1999, Bronikowski 2000, Miller et al. 2011). Also, geographic variation
45 in body size and body condition can reveal fundamental variation in selective pressures,
46 especially in reptiles such as snakes (Bronikowski & Arnold 1999, Miller et al. 2011). Thus,
47 analyses of geographic variation in body size and condition are important to explain locally
48 variable adaptations that produce morphological diversity in snake species.

49 Body condition is an expression of weight and length (size-adjusted body mass), and it is
50 correlated with body reserves (Hayes & Shonkwiler 2001), especially with energy stores in the
51 liver, muscle, and fat of snakes (Bonnet et al. 1998, Falk, Snow & Reed 2017). During periods of
52 low resource availability, starvation and low body reserves are a good predictor of mortality
53 (Shine et al. 2001, Kissner & Weatherhead 2005), decreased reproductive status (Naulleau &
54 Bonnet 1996, Lind & Beaupre 2015, Catherine, LeMaster & Lutterschmidt 2018), and low
55 growth rates in snakes (Bronikowski 2000).

56 Thus, there is a relation between body size and condition with fitness, but in different
57 ways for the two sexes, especially with reproductive status of snakes. For example, a good body
58 condition may be associated with enhanced survival of both sexes of garter snakes, greater
59 fecundity in female garter snakes, and increased mating success for males (Naulleau & Bonnet
60 1996); thereby, a reduction in body condition may reduce reproductive capacity (Lind & Beaupre
61 2015). Conversely, adult female snakes in poor condition that are carrying eggs experience
62 greater mortality (Madsen & Shine 1993, Brown & Weatherhead 1997, Shine et al. 2001).

63 Additionally, sympatric species are predicted to exhibit similar body condition when they
64 have similar ecology, because they share similar interspecific interactions and selective pressures
65 (i.e. Yom-Tov & Geffen 2006, Koyama et al. 2015, Sivan et al. 2015). For example, closely
66 related species of garter snakes with highly overlapping ranges in Mexico, *Thamnophis*

67 *melanogaster* and *T. eques*, show similar patterns of neonate body condition as a function of date
68 of birth (Manjarrez & San-Roman-Apolonio 2015).

69 To understand the complex evolution of body condition, we studied interspecific and
70 geographical variation in traits known to be associated with body condition in three sympatric
71 Mexican garter snakes (*Thamnophis* sp.) from the highlands of Central Mexico. We
72 hypothesized that traits associated with body condition of snakes would potentially reveal a
73 pattern of geographical variation among local populations that could be influenced by ontogenic
74 reproductive status (juvenile, adult) and sex of snakes. We discuss possible body condition
75 differences as they are related to life cycle differences.

76 In this study we assessed snout-vent length (SVL), mass, and body condition in adults
77 and juveniles of both sexes from three sympatric garter snakes in the Central Mexican Highlands
78 (Fig. 1); Mexican Garter Snake (*Thamnophis eques*), Mexican Black-bellied Garter Snake (*T.*
79 *melanogaster*), and Longtail Alpine Garter Snake (*T. scalaris*). They are grouped within the
80 well-supported clade of garter snakes composed of species found mostly in Mexico (de Queiroz,
81 Lawson & Lemos-Espinal 2002, Guo et al. 2012, McVay & Carstens 2013). Garter snakes are
82 the most abundant snake genus in Central Mexico (Flores-Villela, Canseco-Marquez & Ochoa-
83 Ochoa 2010). In general, garter snakes are sexually dimorphic in body size (Shine 1993) with
84 females regularly larger than males (Shine 1994). Almost all studies comparing the body
85 condition of garter snake species were conducted separately for each sex; therefore, there is
86 scarce information of possible sexual differences in garter snake body condition, but see
87 (Rollings et al. 2017).

88 We chose the species *T. eques*, *T. melanogaster*, and *T. scalaris* that occur in Central
89 Mexico because there are no studies that describe the body condition or its possible interspecific

90 or spatial variations under natural conditions for these three species. Only one study of *T.*
91 *melanogaster* and *T. eques* detected body condition patterns in offspring born from females
92 caught in the wild (Manjarrez & San-Roman-Apolonio 2015). For both species, body condition
93 of neonates differed by being lower in the early season and higher in the late season. Snout-vent
94 length of neonates and mean mass of neonates per litter did not change throughout the birth
95 season (Manjarrez & San-Roman-Apolonio 2015).

96 *Thamnophis eques* is widely distributed from Central Mexico to southern New Mexico
97 and Arizona in the United States (Rossman, Ford & Seigel 1996). It is a generalist snake because
98 it preys on both terrestrial and aquatic prey such as frogs, fish and tadpoles, and occasionally,
99 mice and lizards (Drummond & Macías García 1989, Manjarrez 1998, Manjarrez, Pacheco-
100 Tinoco & Venegas-Barrera 2017). *Thamnophis melanogaster* is endemic to the Central Mexican
101 Plateau. It is a semiaquatic snake present at the edge of water bodies and preys mostly on
102 tadpoles, fish, and leeches (Rossman, Ford & Seigel 1996, Manjarrez, Macías García &
103 Drummond 2013). *Thamnophis scalaris* is also endemic to Central Mexico (Rossman, Ford &
104 Seigel 1996). It inhabits forests and grasslands, where it specializes on earthworms, although it
105 can eat vertebrates such as lizards and mice (Manjarrez, Venegas-Barrera & García-Guadarrama
106 2007).

107

108 **MATERIALS & METHODS**

109 In Central Mexico, we irregularly sampled garter snakes at eight different locations in the Rio
110 Lerma drainage (Fig. 1A) over a period of 20 years, however, we selected only those five
111 populations (Fig. 1B) with more than 24 records of snakes, which allowed us to make spatial and
112 sex comparisons. We selected the records of snakes collected over three different years for *T.*

113 *scalaris* (2003, 2005, and 2010) at three locations; seven years for *T. melanogaster* (2005–2011),
114 at two locations, and eleven years for *T. eques* (2000–2003, 2005–2011) at three locations (Table
115 1). Among the five sites, mean annual temperature ranged from 13.7°–18.1°C and mean annual
116 precipitation ranged from 116 mm–755.8 mm (Table 1). *Thamnophis eques* were captured
117 between March and November, *T. melanogaster* between January and December, and *T. scalaris*
118 between June and November.

119 We found snakes by searching under rocks and tree trunks, and some were found simply
120 basking on the ground. All snakes were captured by hand. Adult females were carefully
121 examined for the presence of embryos, and those identified as gravid were excluded from
122 analysis. Measurements of captured snakes included sex (visual inspection of tail-base breadth or
123 by everting the male hemipenes in small snakes), snout-vent length (SVL), and mass (measured
124 on an electronic scale [± 0.1 g]). Immediately after processing, snakes were released where they
125 had been captured.

126 **Analysis**

127 Individual body condition was calculated using residuals from the Major axis (MA) linear
128 regression of ln-transformed body mass on ln-transformed SVL. This residual index is
129 considered an excellent estimator of true snake body condition because it shows a strong
130 association with body fat mass but not SVL (Falk, Snow & Reed 2017). This regression was
131 significant for all species (*T. eques*, $r = 0.90$, $P < 0.0001$; *T. melanogaster*, $r = 0.93$, $P < 0.0001$;
132 *T. scalaris*, $r = 0.95$, $P < 0.0001$). Residuals were used to categorize body condition, with
133 positive residuals corresponding to individuals with good body condition and negative residuals
134 corresponding to individuals with poor body condition (Weatherhead & Brown 1996, Falk, Snow
135 & Reed 2017).

136 Because the optimal body condition should approximate the true body condition of the
137 snakes and should be unbiased with respect to body size, we evaluated this relationship with
138 Kendall rank correlation coefficient to test for a correlation between body condition and ln-
139 transformed SVL as a measure of size and estimated the percent variation in body condition.
140 Also, with the coefficient of determination (R^2), we estimated the percent variation in body
141 condition and mass that can be explained by SVL.

142 **Geographic comparison.**

143 The SVL and mass of snakes were transformed with natural logarithms prior to analyses. We
144 utilized one-way analyses of variance (ANOVA) to compare body condition, SVL, and mass as
145 dependent variables among populations of each species. In these analyses, we pooled male and
146 female snakes because a three-way ANOVA (locality, year, and sex) indicated that SVL and
147 mass within each species did not differ between sexes, but did differ among locality and between
148 years (Table 2). We used a Chi-square goodness-of-fit test to determine if sex ratio among
149 species was different than 1:1 (Table 3). Statistical significance was assessed at $\alpha = 0.05$. All
150 data are reported as means ± 1 *SD*.

151 **Sexual and size status comparison**

152 Each snake was assigned an ontogenic reproductive status (juvenile, adult) according to size at
153 capture (adult snakes > 39.0, 33.0, and 34 cm SVL for *T. eques*, *T. melanogaster* and *T. scalaris*,
154 respectively; Manjarrez 1998, Manjarrez, Venegas-Barrera & García-Guadarrama 2007). We
155 performed a discriminant function analysis (DFA) for testing intraspecific differences (between
156 location, sex, and size category) according to the mean of the exploratory variables (SVL, mass,
157 and body condition) and for generating linear combinations that classify snakes as a function of
158 their morphological traits associated with snake body condition. The grouping variables were

159 location, sex (male, female) and ontogenic reproductive status. DFA is an inferential, descriptive
160 multivariate procedure for testing differences between groups according to the mean of all
161 variables and for generating linear combinations that classify objects as a function of their
162 characteristics (Statistica, ver. 12; StatSoft 2012).

163 The objective of DFA was to test differences between groups and identify which
164 variables discriminate between two or more groups. Comparisons between groups were
165 performed under the null hypothesis that morphological traits between categories of grouping
166 variables were similar, and the estimated value was contrasted with the theoretical value of the F-
167 distribution. We employed a probability of 0.05 to test the hypothesis, where P values lower than
168 0.05 were associated with groups of snakes showing different morphological traits, whereas
169 values greater than or equal to 0.05 were associated with groups with similar morphological
170 traits. The canonical average of the observations from each category (centroid) for the significant
171 roots (canonical scores) was plotted, which reflects morphological variations between categories
172 of grouping variables. The position of the centroids was interpreted using the variables that
173 contributed most to discriminating between groups.

174 We chose those variables that exhibited a coefficient of the factor structure higher than
175 0.5 or lower than -0.5. The coefficients represent the correlation between the original variables
176 and the roots. We applied one-way ANOVAs or Student-t with Statistica software (ver. 8.0
177 StatSoft, Tulsa, Oklahoma, USA) when only one morphological variable exhibited a coefficient
178 of the factor structure higher than 0.5 or lower than -0.5.

179 This study received the approval of the ethics committee of the Universidad Autónoma
180 del Estado de México (Number 4047/2016SF). All subjects were treated humanely on the
181 basis of guidelines outlined by the American Society of Ichthyologists and Herpetologists

182 (ASIH, 2004).

183

184 RESULTS

185 The biggest species of garter snake was *T. eques* with a mean body size of SVL 43.43 ± 17.57
186 cm (range 12.51–81.30), mass of 55.62 ± 60.56 g (range 1.40–335.86, $n = 253$). *Thamnophis*
187 *melanogaster* was slightly larger than *T. scalaris* (*T. melanogaster*: SVL 29.17 ± 41 cm [range
188 14.40–66.0], mass 19.10 ± 23.3 g [range 1.62–196.0], $n = 686$; *T. scalaris*: SVL 28.70 ± 9.21 cm
189 [range 12.10–53.0], mass 16.44 ± 12.59 g [range 1.30–60.70], $n = 80$).

190 The number of males and females collected was independent of locations sampled for *T.*
191 *melanogaster* ($\chi^2 = 0.001$, $df = 1$, $P = 0.97$), and *T. scalaris* ($\chi^2 = 3.69$, $df = 2$, $P = 0.15$), but
192 dependent on location for *T. eques* ($\chi^2 = 10.4$, $df = 2$, $P = 0.006$). Considering all individuals
193 collected, the sex ratio was biased toward females. For *T. eques* and *T. scalaris*, the sex ratio was
194 skewed toward females in two of three locations analyzed (Table 3), whereas the sex ratio for *T.*
195 *melanogaster* was biased toward females in Cuitzeo but not in Lerma (Table 3). For *T. scalaris*
196 the female bias was very distinct, especially Zempoala where no males were found (Table 3).

197 Both body condition (residuals from MA linear regression of ln-transformed body mass
198 on ln-transformed SVL) and body mass were related to ln-SVL in each garter snake (Table 4).
199 The R^2 values suggest that more than 80% of the variation in body mass is explained by SVL,
200 and less than 12% of the variation in body condition is explained by SVL (Table 4).

201 Geographic comparison

202 *Thamnophis eques*. For the three locations that we analyzed for *T. eques* (Lerma, Cerrillo and
203 Cuitzeo), we observed a difference in mean body condition. *Thamnophis eques* from Lerma
204 showed a mean poor body condition that was the lowest of the three populations ($F_{2,250} = 10.7$, P

205 < 0.0001; Fig. 2), although snakes in this location were significantly larger than in the other two
206 (ln-SVL $F_{2,250} = 6.7$, $P = 0.001$). Conversely, *T. eques* from Cuitzeo showed the best body
207 condition, but the shortest length (Fig. 2). Mean body mass was not different between locations
208 of *T. eques* (ln-mass $F_{2,250} = 2.2$, $P = 0.11$).

209 *Thamnophis melanogaster*. For *T. melanogaster*, the statistical test did not detect a significant
210 difference in mean body condition between the two locations, Lerma and Cuitzeo ($F_{1,684} = 3.1$, P
211 $= 0.07$). However, the Lerma snakes were significantly larger (ln-SVL $F_{1,684} = 42.3$, $P < 0.0001$),
212 and heavier than those collected in Cuitzeo (ln-mass $F_{1,684} = 56.4$, $P < 0.0001$; Fig. 2).

213 *Thamnophis scalaris*. In this species the mean SVL and mass showed no differences among the
214 three locations analyzed (Lerma, S. Morelos and Zempoala, ln-SVL $F_{2,77} = 1.55$, $P = 0.21$; ln-
215 mass $F_{2,77} = 0.58$, $P = 0.56$), however, mean body condition was good in the individuals from S.
216 Morelos and poor for those from Zempoala ($F_{2,77} = 20.9$, $P < 0.0001$; Fig. 2).

217 **Sexual and size status comparison**

218 The results of DFA showed that each garter snake had a unique pattern of intraspecific
219 differences.

220 *Thamnophis eques*. Juvenile females of Cuitzeo had a better body condition than juvenile
221 females of Lerma ($t_{36} = 2.17$, $P = 0.03$), but body size (SVL and mass) were similar between
222 Juvenile females of both locations ($F_{2,35} = 2.9$, $P = 0.06$). Juvenile males *T. eques* have similar
223 body size and body condition between Lerma and Cuitzeo.

224 Adult males *T. eques* of Cuitzeo had a higher mass (140 ± 130.1 g) than adult males of
225 Lerma (57.2 ± 32.7 g) and Cerrillo (49.7 ± 18.7 g, ANOVA $F_{2,37} = 8.2$, $P < 0.0001$). Adult
226 female *T. eques* of Lerma presented greater body size (SVL 59.0 ± 9.3 cm; mass 104.0 ± 68.1 g)

227 than adult females of Cerrillo (SVL 49.7 ± 8.5 cm; mass 71.0 ± 41.3 g; DFA $F_{4,158} = 3.51$, $P =$
228 0.008, Fig. 3).

229 *Thamnophis melanogaster*. Juvenile male *T. melanogaster* showed that body size traits and the
230 body condition were similar between Lerma and Cuitzeo ($F_{1,165} = 1.3$, $P = 0.25$). In the case of
231 juvenile female *T. melanogaster*, SVL was greater in Lerma (26.2 ± 4.8 cm) than Cuitzeo ($23.3 \pm$
232 4.7 cm), and body condition was similar between both locations ($F_{1,260} = 5.06$, $P = 0.02$).

233 Adult *T. melanogaster* of both sexes presented a similar pattern. A better body condition
234 in Lerma than Cuitzeo (males: 0.09 ± 0.29 vs. -0.04 ± 0.24 ; females 0.18 ± 0.34 vs. $-0.07 \pm$
235 0.33), and similar body size (SVL and mass) between Lerma and Cuitzeo (males: $F_{1,86} = 4.9$, $P =$
236 0.02; females: $F_{2,98} = 8.07$, $P = 0.0006$).

237 *Thamnophis scalaris*. Only number of female *T. scalaris* (juvenile and adult) was enough to
238 make comparisons between locations. Juvenile female *T. scalaris* of Zempoala were significantly
239 longer, lighter, and had poor body condition than other locations. Lerma snakes showed lower
240 SVL, mass, and average body condition, while snakes from Cerrillo and S. Morelos presented a
241 better body condition, average SVL, and higher mass ($F_{1,165} = 1.3$, $P = 0.25$, Fig. 4A).

242 Adult female *T. scalaris* of Lerma and Zempoala had poorer body condition than those of
243 Cerrillo and S. Morelos ($F_{6,62} = 8.4$, $P < 0.0001$, Fig. 4C).

244

245 DISCUSSION

246 In this study, we provide a heterogeneous pattern of sexual and ontogenic reproductive status
247 variations in body size and condition among populations of three sympatric garter snakes
248 collected in the Central Mexico Highlands over several years. We found: (1) each garter snake
249 species shows good and poor body condition in a variety of locations, (2) juvenile garter snakes

250 show similar body condition between populations, (3) adults show different body conditions
251 between populations, and (4) adults also show sexual differences in body condition. Thus,
252 geographical differences in body condition were present in juvenile female *T. eques*, both sexes
253 of adult *T. melanogaster*, and juvenile and adult females of *T. scalaris*.

254 Several problems may confound these inter- and intraspecific patterns of differences in
255 body condition because each responds to complex interactions between sexual and ontogenic
256 reproductive status with local environmental variables and local resource availability (Congdon
257 1989, Shine et al. 2001). Thus, the differences in body condition between sites may reflect result
258 from differences in local prey availability, dietary quality, or predation efficiency (Britt, Hicks &
259 Bennett 2006), or a complex spatio-temporal interaction that is reflected in micro-geographic
260 diet variation, a pattern common in garter snakes (Seigel 1996).

261 Sympatric and closely related species are expected to exhibit a similar body condition
262 due to the ecological similarities that impose common selective pressures (i.e. Manjarrez & San-
263 Roman-Apolonio 2015). However, we cannot assume that the garter snakes we studied make
264 similar use of local energy supplies, which may vary according to intra-interspecific competition
265 and available resources (Congdon 1989), especially on prey availability.

266 We hypothesized that body condition of garter snakes would reveal a pattern of
267 geographical variation influenced by ontogenic reproductive status (juvenile, adult) and sex.
268 Growth and body condition in snakes may reflect intraspecific competition intensity that would
269 correspond to availability and allocation of energy (Bronikowski 2000, Bronikowski & Arnold
270 1999, Blouin-Demers, Prior & Weatherhead 2002). This is especially applicable for female
271 garter snakes because they are generally heavier bodied and have greater reproductive energy
272 demands than males (Naulleau & Bonnet 1996, Shine et al. 2001, Blouin-Demers &

273 Weatherhead 2007). In general, our results of both sexes showed similar variation in body
274 condition among locations, therefore we lack evidence to support a difference in body condition
275 based on sex.

276 Ontogenic differences in body condition can result from differential resource use. For
277 example, studies on *T. melanogaster*, *T. eques*, and *T. scalaris* have reported intraspecific
278 differences in the diet of snakes, such as the changing of aquatic invertebrate to terrestrial
279 vertebrate prey between small and large snakes (Macias-Garcia & Drummond 1988, Manjarrez,
280 Venegas-Barrera & García-Guadarrama 2007, Manjarrez, Macías García & Drummond 2013,
281 Manjarrez, Pacheco-Tinoco & Venegas-Barrera 2017). This suggests different trade-off
282 strategies between growth rate and body mass for resource allocation among sites, according to
283 sex and ontogenic reproductive status (Naulleau & Bonnet 1996, Lind & Beaupre 2015). This
284 trade-off has been sparsely studied in neonate snakes (i.e *Nerodia sipedon* and *Elaphe obsoleta*;
285 Weatherhead et al. 1999, Blouin-Demers & Weatherhead 2007).

286 Another reason for geographic variation in the body condition of juvenile and adult
287 snakes includes geographic variation in the percentage of juveniles and adults in the population.
288 For *T. melanogaster*, 94% of juveniles and 76% of adults were collected from Cuitzeo; while for
289 *T. eques* 70% of juveniles and 77% of adults were collected Lerma. In *T. scalaris* locations, this
290 age bias was less evident, with collection percentages of juveniles ranging from 17% to 31% by
291 location, and 15% to 27% for adults.

292 According to our results, the models propose different paths for population fitness of each
293 garter snake species assuming the current body condition. Thus, for *T. eques*, in Lerma with poor
294 body condition, we predict a lower fitness, especially for juvenile females. Conversely, *T. eques*
295 of Cuitzeo, with good body condition, show an improved fitness. For *T. melanogaster*, an

296 increase in survival and reproduction is predicted in Lerma but not in Cuitzeo, especially for
297 adults of both sex. Finally, for *T. scalaris*, an increase in fitness is expected for S. Morelos and
298 Cerrillo, but a decreased fitness in Zempoala and Lerma. In this way, the future scenario
299 responds according to the local geographic variation of each population, however, this prediction
300 is difficult to rely upon because environmental fluctuations can be unpredictable, and changes in
301 the climate, vegetation, topography, and land use variables will reduce the future potential
302 distribution of these three garter snakes, as has been predicted in Gonzalez et al. (2018).

303 Another important pattern in this study is the interspecific difference of body condition
304 within the same location. For example, in Cuitzeo, the body condition of *T. eques* is good, and in
305 Lerma it is poor, while in *T. melanogaster* the body condition is inverse; poor in Cuitzeo and
306 good in Lerma. This difference could be explained by interspecific differences in resource use
307 and its differential microdistribution. In this sense, *T. eques* is a generalist in its diet, ingesting
308 aquatic and terrestrial prey, while *T. melanogaster* is a specialist ingesting only aquatic prey. The
309 majority of specialist-generalist trade-offs are related with wide ecological traits that result in
310 distinct performance between specialists and generalists (Drummond 1983, Futuyma & Moreno
311 1988). If these species exploit different foraging environments, it is likely that they are exposed
312 to different environmental conditions. For example, Cuitzeo is a permanent lake that offers a
313 constant aquatic foraging environment for the aquatic specialist *T. melanogaster*, while Lerma is
314 a wetland environment, more suitable for the aquatic-terrestrial *T. eques*, a differential pattern
315 that is reflected in the interspecific differential body condition within both locations.

316

317 **CONCLUSIONS**

318 In conclusion, our analyses suggest that traits associated with body condition of sympatric
319 Mexican garter snakes *T. eques*, *T. melanogaster*, and *T. scalaris* in the Central Mexico
320 Highlands, reveal a pattern of microgeographical variation among local populations that differ
321 little by ontogenic reproductive status, and therefore, sex has little or no influence on body
322 condition in these garter snakes.

323

324 **ACKNOWLEDGEMENTS**

325 For their assistance in the field and laboratory work we thank all of the students of the
326 Evolutionary Biology Laboratory. Ruthe J. Smith provided comments and corrections regarding
327 the manuscript. EVF is grateful to the graduate program “Maestria en Ciencias Agropecuarias y
328 Recursos Naturales” of “Universidad Autonoma del Estado de Mexico” and to the “Consejo
329 Nacional de Ciencia y Tecnología”.

330

331 **REFERENCES**

- 332 Blouin-Demers G, Prior KA, Weatherhead PJ. 2002. Comparative demography of black rat
333 snakes (*Elaphe obsoleta*) in Ontario and Maryland. *Journal of Zoology*, London 256:1–
334 10. DOI: 10.1017/S0952836902000018.
- 335 Blouin-Demers G, Weatherhead PJ. 2007. Allocation of offspring size and sex by female black
336 ratsnakes. *Oikos* 116:1759–1767. DOI: 10.1111/j.0030-1299.2007.15993.x
- 337 Bonnet X, Shine R, Naulleau G, Vallas-Vacher M. 1998. Sexual dimorphism in snakes: different
338 reproductive roles favour different body plans. *Proceedings of the Royal Society B*
339 265:179–183.

- 340 Britt E, Hicks J, Bennett AF. 2006. The energetic consequences of dietary specialization in
341 populations of the garter snake, *Thamnophis elegans*. *The Journal of the Experimental*
342 *Biology* 209:3164–3169 DOI 10.1242/jeb.02366.
- 343 Bronikowski AM. 2000. Experimental evidence for the adaptive evolution of growth rate in the
344 garter snake *Thamnophis elegans*. *Evolution* 54:1760–1767.
- 345 Bronikowski AM, Arnold SJ. 1999. The evolutionary ecology of life history variation in the
346 garter snake *Thamnophis elegans*. *Ecology* 80:2314–2325.
- 347 Brown GP, Weatherhead PJ. 1997. Effects of reproduction on survival and growth of female
348 northern water snakes, *Nerodia sipedon*. *Canadian Journal of Zoology* 75:424–432.
- 349 Catherine AD, LeMaster MP, Lutterschmidt DI. 2018. Physiological correlates of reproductive
350 decisions: Relationships among body condition, reproductive status, and the
351 hypothalamus-pituitary-adrenal axis in a reptile. *Hormones and Behavior* 100: 1–11.
352 DOI: 10.1016/j.yhbeh.2018.02.004
- 353 Congdon JD. 1989. Proximate and evolutionary constraints on energy relations of reptiles.
354 *Zoology Physiological* 62:356–373.
- 355 de Queiroz A, Lawson R, Lemos-Espinal JA. 2002. Phylogenetic relationships of North
356 American Garter snakes (*Thamnophis*) based on four mitochondrial genes: How much
357 DNA sequence is enough?. *Molecular Phylogenetics and Evolution* 22:315–329. DOI:
358 10.1006/mpev.2001.1074
- 359 Drummond H. 1983. Aquatic foraging in garter snakes: a comparison specialist and generalist.
360 *Behaviour* 86:1–30.
- 361 Drummond H, Macías García C. 1989. Limitations of a generalist: a field comparison of
362 foraging snakes. *Behaviour* 108:23–43. DOI 10.1163/156853989X00033.

- 363 Falk BG, Snow RW, Reed RN. 2017. A validation of 11 body-condition indices in a giant snake
364 species that exhibits positive allometry. *PLoS ONE* 12: e0180791. DOI:
365 10.1371/journal.pone.0180791.
- 366 Flores-Villela O, Canseco-Márquez L, Ochoa-Ochoa L. 2010. Geographic distribution and
367 conservation of the herpetofauna of the highlands of Central Mexico. In: Wilson LD,
368 Townsend JH, Johnson JD, eds. Conservation of mesoamerican amphibians and reptiles.
369 Utah: Eagle Mountain Publishing Co., 303–321.
- 370 Futuyma DJ, Moreno G. 1988. The evolution of ecological specialization. *Annual Review of*
371 *Ecology, Evolution, and Systematics* 19, 207–233.
- 372 González-Fernández A, Manjarrez J, García-Vázquez U, D'Addario M, Sunny A. 2018. Present
373 and future ecological niche modeling of garter snake species from the Trans-Mexican
374 Volcanic Belt. *PeerJ* 6:e4618. DOI: 10.7717/peerj.4618
- 375 Guo P, Liu Q, Xu Y, Jiang KM, Ding L, Pyron RA, Burbrink FT. 2012. Out of Asia: natricinae
376 snakes support the Cenozoic Beringian Dispersal Hypothesis. *Molecular Phylogenetics*
377 *and Evolution* 63:825-833. DOI: 10.1016/j.ympev.2012.02.021
- 378 Hayes J, Shonkwiler J. 2001. Morphometric indicators of body condition: Worthwhile or wishful
379 thinking? In: Speakman JR, ed. Body composition analysis of animals: A handbook of
380 non-destructive methods. Cambridge: Cambridge University Press, 8–38.
- 381 Kissner KJ, Weatherhead PJ. 2005. Phenotypic effects on survival of neonatal northern
382 watersnakes *Nerodia sipedon*. *Journal of Animal Ecology* 74:259–265. DOI:
383 10.1111/j.1365-2656.2005.00919.x
- 384 Koyama T, Ito H, Kakishima S, Yoshimura J, Cooley JR, Simon C, Sota T. 2015. Geographic
385 body size variation in the periodical cicadas *Magicicada*: implications for life cycle

- 386 divergence and local Adaptation. *Journal of Evolutionary Biology* 28: 1270–1277. DOI:
387 10.1111/jeb.12653
- 388 Lind CM, Beaupre SJ. 2015. Male Snakes Allocate Time and Energy according to Individual
389 Energetic Status: Body Condition, Steroid Hormones, and Reproductive Behavior in
390 Timber Rattlesnakes, *Crotalus horridus*. *Physiological and Biochemical Zoology* 88:
391 624–633. DOI: 10.1086/683058
- 392 Macias-Garcia C, Drummond H. 1988. Seasonal and ontogenetic variation in the diet of the
393 Mexican garter snake, *Thamnophis eques* in Lake Tecocomulco, Hidalgo. *Journal of*
394 *Herpetology* 2:129–134.
- 395 Madsen T, Shine R. 1993. Costs of reproduction in a population of European adders. *Oecologia*
396 94, 488–495.
- 397 Manjarrez J. 1998. Ecology of the Mexican Garter snake (*Thamnophis eques*) in Toluca, Mexico.
398 *Journal of Herpetology* 32:464–468.
- 399 Manjarrez J, Venegas-Barrera CS, García-Guadarrama T. 2007. Ecology of the Mexican alpine
400 blotched garter snake (*Thamnophis scalaris*). *Southwestern Naturalist* 52:258–262. DOI:
401 10.1894/0038-4909(2007)52[258:EOTMAB]2.0.CO;2
- 402 Manjarrez J, Macías García C, Drummond H. 2013. Variation in the diet of the Mexican black-
403 bellied garter snake *Thamnophis melanogaster*: importance of prey availability and snake
404 body size. *Journal of Herpetology* 47: 413–420. DOI: 10.2307/1948469
- 405 Manjarrez J, San-Roman-Apolonio E. 2015. Timing of Birth and Body Condition in Neonates of
406 Two Gartersnake Species from Central Mexico. *Herpetologica* 71: 2015, 12–18. DOI:
407 10.1655/HERPETOLOGICA-D-13-00098.

- 408 Manjarrez J, Pacheco-Tinoco M, Venegas-Barrera CS. 2017. Intraspecific variation in the diet of
409 the Mexican garter snake *Thamnophis eques*. *PeerJ* 5:e4036; DOI: 10.7717/peerj.4036.
- 410 McVay JD, Carstens B. 2013. Testing monophyly without well-supported gene trees: Evidence
411 from multi-locus nuclear data conflicts with existing taxonomy in the snake tribe
412 *Thamnophiini*. *Molecular Phylogenetics and Evolution* 68:425–431. DOI:
413 10.1016/j.ympev.2013.04.028
- 414 Miller DA, Clark WR, Arnold SJ, Bronikowski AM. 2011. Stochastic population dynamics in
415 populations of western terrestrial garter snakes with divergent life histories. *Ecology*
416 92(8): 1658–1671. DOI: 10.1890/10-1438.1.
- 417 Naulleau G, Bonnet X. 1996. Body condition threshold for breeding in a viviparous snake.
418 *Oecologia* 107:301–306.
- 419 Rollings N, Uhrig EJ, Krohmer RW, Wayne HL, Mason RT, Olsson M, Whittington CM, Friesen
420 CR. 2017. Age-related sex differences in body condition and telomere dynamics of red-
421 sided garter snakes. *Proceedings of the Royal Society B* 284: 20162146. DOI:
422 10.1098/rspb.2016.2146.
- 423 Rossman DE, Ford NB, Seigel RA. 1996. The Garter snakes: Evolution and ecology. Norman:
424 University of Oklahoma Press.
- 425 Seigel RA. 1996. Ecology and conservation of garter snakes: Masters of plasticity. In: Rossman
426 DA, Ford NB, Seigel RA, eds. The Garter snakes. Evolution and ecology. Norman:
427 University of Oklahoma Press, 55–89.
- 428 Shine R. 1993. Sexual dimorphism in snakes. In: Seigel RA, Collins JT, eds. Snakes: ecology
429 and behavior. New York: McGraw-Hill, 49–86.
- 430 Shine R. 1994. Sexual dimorphism in snakes revised. *Copeia* 1994:326–346.

- 431 Shine R, Lemaster MP, Moore IT, Olsson MM, Mason RT. 2001. Bumpus in the snake den:
432 effects of sex, size, and body condition on mortality of red-sided Garter snakes. *Evolution*
433 55: 598–604. DOI: 10.1554/0014-3820(2001)055[0598:BITSDE]2.0.CO;2.
- 434 Sivan J, Kam M, Hadad S, Degen AA, Rosenstrauch A. 2015. Body size and seasonal body
435 condition in two small coexisting desert snake species, the Saharan sand viper (*Cerastes*
436 *vipera*) and the crowned leafnose (*Lytorhynchus diadema*). *Journal of Arid Environments*
437 114: 8–13. DOI: 10.1016/j.jaridenv.2014.10.013.
- 438 Weatherhead PJ, Brown GP. 1996. Measurement versus estimation of condition in snakes.
439 *Canadian Journal of Zoology* 74:1617–162.
- 440 Weatherhead PJ, Brown GP, Prosser MR, Kissner KJ. 1999. Factors affecting neonate size
441 variation in northern water snakes, *Nerodia sipedon*. *Journal of Herpetology* 33:577–589.
- 442 Yom-Tov Y, Geffen E. 2006. Geographic variation in body size: the effects of ambient
443 temperature and precipitation. *Oecologia* 148: 213–218. DOI: 10.1007/s00442-006-0364-
444 9.

Table 1 (on next page)

Capture locations of *T. eques*, *T. melanogaster* and *T. scalaris* in Central Mexico.

1 TABLE 1. Capture locations of *T. eques*, *T. melanogaster* and *T. scalaris* in Central Mexico.

Locality	Garter snake present	Coordinates N, W (Datum WGS84)	Elevation (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)
Lerma, Estado de México	<i>T. eques</i> , <i>T. melanogaster</i> , <i>T. scalaris</i>	19°14'28.73", 99°29'41.14"	2573	15.8	158.7
Cerrillo, Estado de México	<i>T. eques</i>	19°24'20.86", 99°41'41.05"	2550	13.7	116
S. Morelos, Estado de México	<i>T. scalaris</i>	19°18'49.58", 99°41'29.07"	2750	13.8	746.9
Cuitzeo, Michoacan	<i>T. eques</i> , <i>T. melanogaster</i> ,	19°55'32.83", 101°08'26.78"	1837	18.1	755.8
Zempoala, Morelos	<i>T. scalaris</i>	19°02'53.40", 99°18'44.54"	2800	14.2	514

2

3

Table 2 (on next page)

ANOVA of ln-SVL and ln-mass as dependent variables among locations, years and sex for each garter snake species.

1 TABLE 2. ANOVA of ln-SVL and ln-mass as dependent variables among locations, years and
 2 sex for each garter snake species.

	Location	Year	Sex
<i>T. melanogaster</i>			
SVL	21.58***	6.56***	0.18
mass	29.21***	5.50***	0.00
<i>T. eques</i>			
SVL	12.08***	22.75***	0.99
mass	7.47**	20.82***	1.66
<i>T. scalaris</i>			
SVL	7.12**	0.34	0.67
mass	2.42	3.73*	1.10

3 * $P < 0.05$

4 ** $P < 0.001$

5 *** $P < 0.0001$

Table 3 (on next page)

Sex ratio (male:female) of *T. eques*, *T. melanogaster*, and *T. scalaris* for each population collected from Central Mexican Highlands ($df = 1$ for all tests).

- 1 Table 3. Sex ratio (male:female) of *T. eques*, *T. melanogaster*, and *T. scalaris* for each
 2 population collected from Central Mexican Highlands ($df=1$ for all tests).

	<i>T. eques</i>		<i>T. melanogaster</i>		<i>T. scalaris</i>	
	Sex Ratio	χ^2 test (<i>P</i>)	Sex Ratio	χ^2 test (<i>P</i>)	Sex Ratio	χ^2 test (<i>P</i>)
Lerma	1:1	0.45 (0.49)	1:1	2.0 (0.15)	1:1.5	12.46 (0.0004)
Cuitzeo	1:2	4.33 (0.03)	1:1.4	14.9 (0.0001)		
Cerrillo	1:3	6.76 (0.009)				
S. Morelos					1:1.7	9.94 (0.001)
Zempoala					0:23	23.0 (<0.0001)

- 3
4

Table 4(on next page)

Kendall rank correlation and R^2 coefficients of ln-mass and body condition on ln-SVL of *T. eques*, *T. melanogaster* and *T. scalaris*.

- 1 Table 4. Kendall rank correlation and R^2 coefficients of ln-mass and body condition on ln-SVL
 2 of *T. eques*, *T. melanogaster* and *T. scalaris*.

	<i>T. eques</i> <i>n</i> = 253	R^2	<i>T. melanogaster</i> <i>n</i> = 686	R^2	<i>T. scalaris</i> <i>n</i> = 80	R^2
Ln-mass	0.77*	0.84	0.81*	0.88	0.80*	0.92
Body condition	-0.25*	0.12	-0.19*	0.08	-0.21*	0.05

3 * $P < .0001$

4

Figure 1

(A) Geographic distribution of *T. eques*, *T. melanogaster* and *T. scalaris* in Central Mexico (digitalized from Rossman et al. 1996) and (B) capture locations.

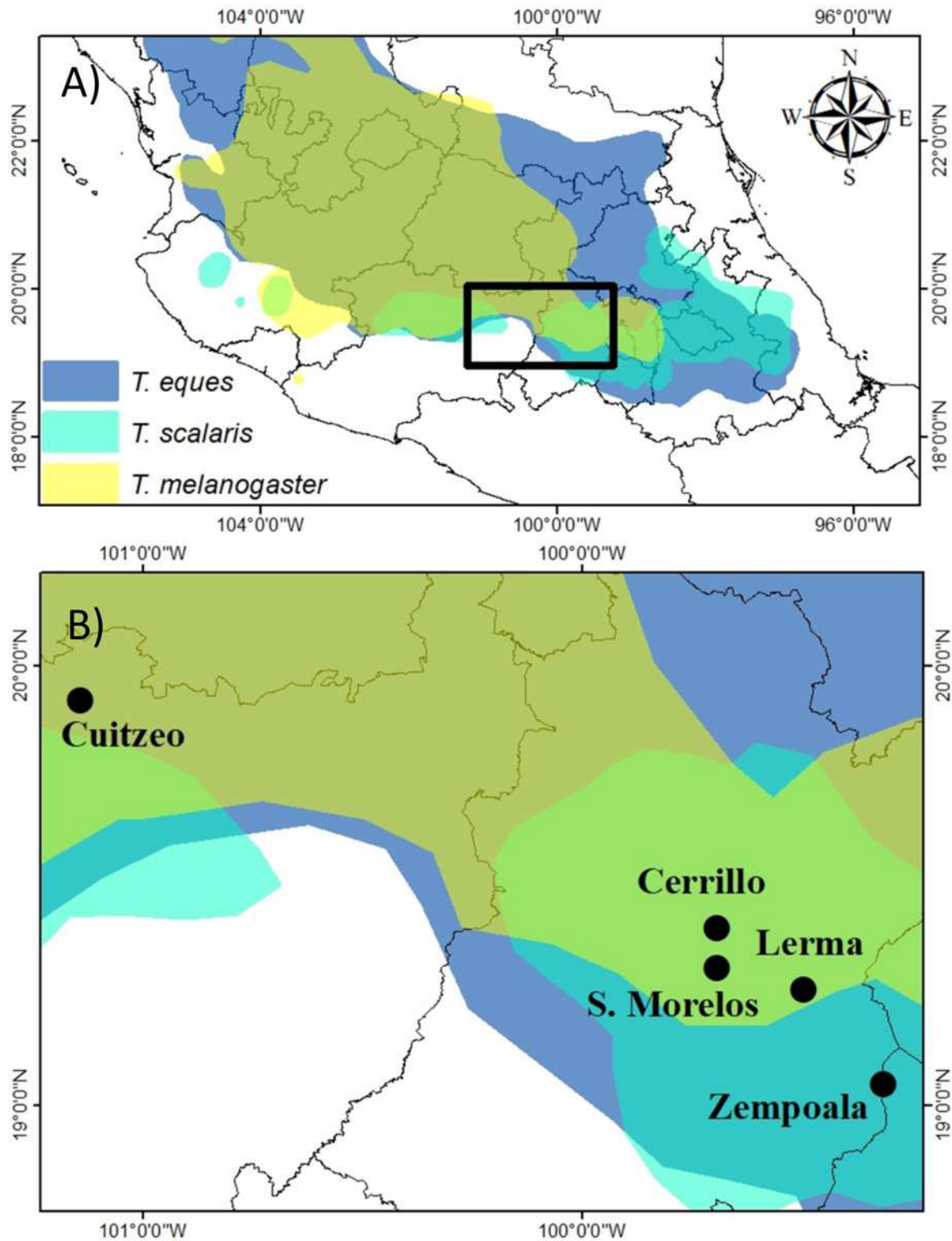


Figure 2

Body condition, SVL and mass (mean \pm 1 SE) of wild-caught snakes *T. eques*, *T. melanogaster*, and *T. scalaris* collected from locations in the Central Mexican Highlands over a period of 20 years.

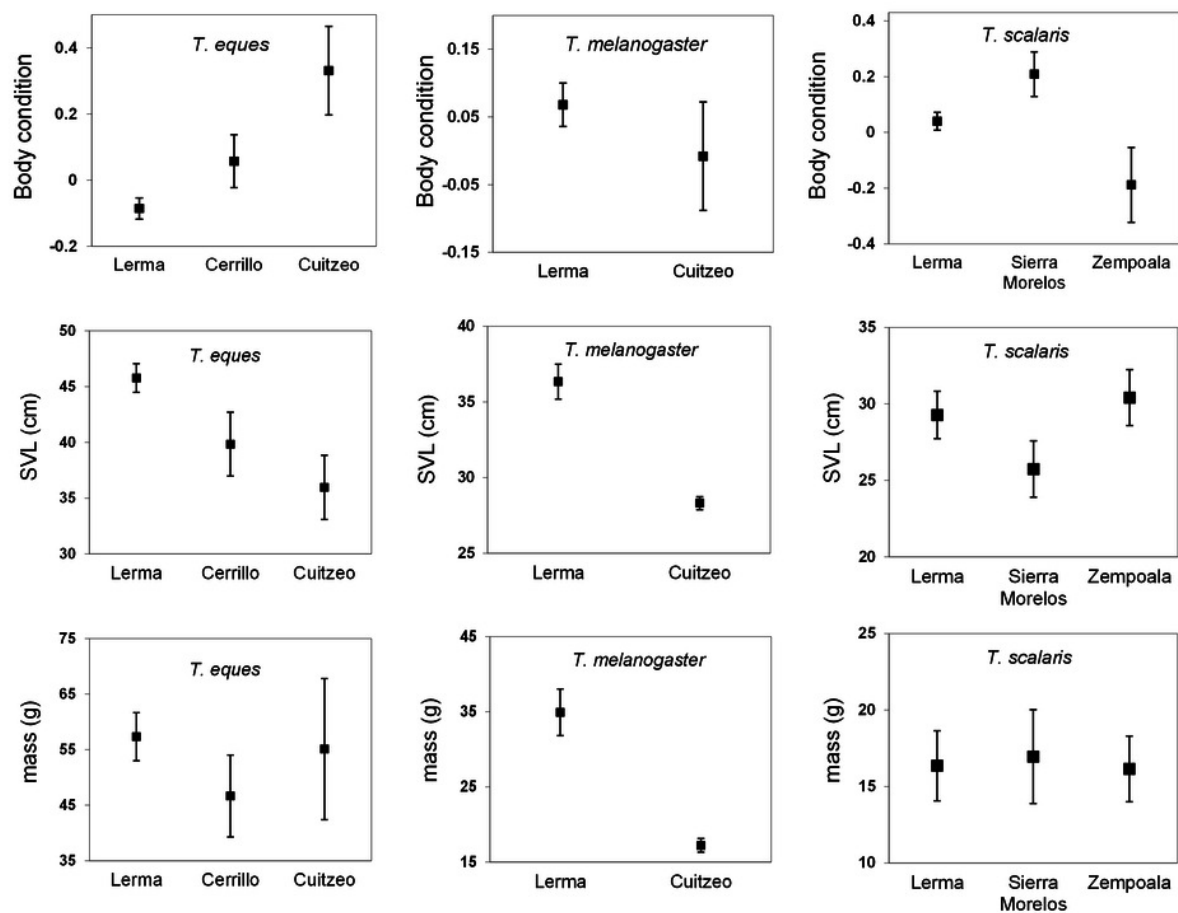


Figure 3

Average canonical position (centroid) for Lerma, Cerrillo and Cuitzeo, obtained from a discriminant function analysis of body condition traits in adult female garter snakes *T. eques* and factor structure.

Isoclines represent variation on SVL of snakes in Lerma, Cerrillo and Cuitzeo.

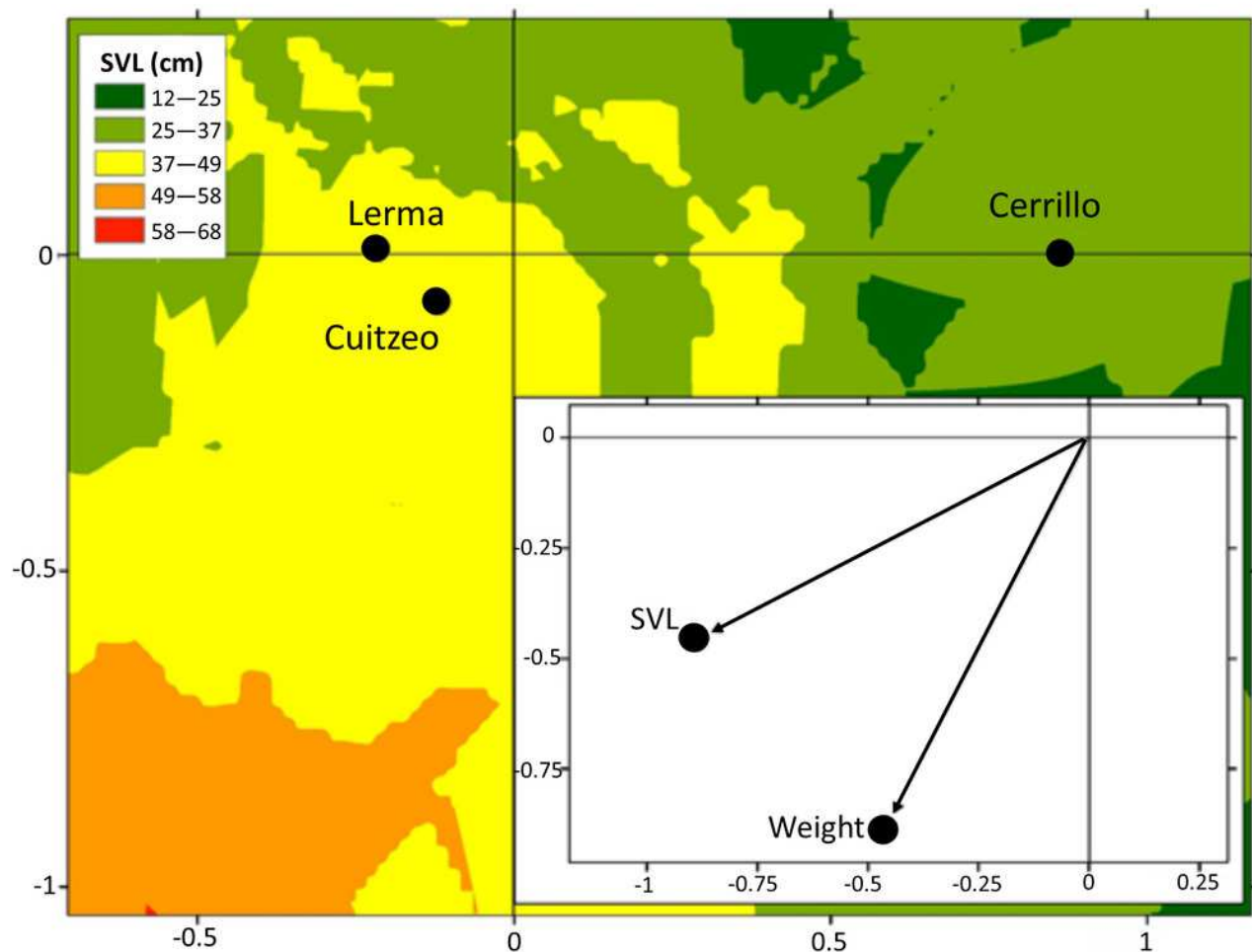


Figure 4

Canonical position of the centroids of juvenile (A) and adult (C) females of garter snakes *T. scalaris* captured from Lerma, Cerrillo, S. Morelos and Zempoala obtained from a discriminant function analysis and the variables that presented the greatest

Isoclines represent variation of body conditions of snakes in Lerma, Cerrillo, S. Morelos and Zempoala.

