Thermal ecology and baseline energetic requirements of a large-bodied ectotherm suggest resilience to climate change

Authors: Hayley L. Crowell*[ab], Katherine C. King[c], James M. Whelan[a], Mallory V. Harmel[d], Gennesee Garcia[a], Sebastian G. Gonzales[a], Paul H. Maier[a], Heather Neldner[a], Thomas Nhu[a], John T. Nolan[a], and Emily N. Taylor[a]

[a] Biological Sciences Department, California Polytechnic State University, San Luis Obispo, California, USA

[b] Ecology and Evolutionary Biology Department, University of Michigan, Ann Arbor, Michigan, USA

[c] Crocodile Lake Wildlife Refuge, U.S. Fish and Wildlife Service, Key Largo, FL, USA

[d] Jekyll Island Authority Conservation Department, Jekyll Island, Georgia, USA

*Corresponding Author: hlcrowel@umich.edu

Address: Biological Sciences Building, 1105 N. University Ave, Ann Arbor, MI 48109

Running Head: Comparative thermal ecology of rattlesnakes

DR HAYLEY LAYNE CROWELL (Orcid ID : 0000-0003-3531-6673)

Abstract

1. Most studies on how rising temperatures will impact terrestrial ectotherms have focused on single populations or multiple sympatric species. Addressing the thermal and energetic implications of climatic variation on multiple allopatric populations of a species will help us better elucidate how a species may be impacted by altered climates.

2. We used eight years of thermal and behavioral data collected from four populations of Pacific rattlesnakes (Crotalus oreganus) living in climatically distinct habitat types (inland and coastal) to determine the field-active and lab-preferred body temperatures, thermoregulatory metrics, and maintenance energetic requirements of snakes from each population.

3. Physical models showed that thermal quality was best at coastal sites, but inland snakes thermoregulated more accurately despite being in more thermally constrained environments. Projected increases of 1 and 2 ℃ in ambient temperature result in an increase in overall thermal quality at both coastal and inland sites.

4. Population differences in modeled standard metabolic rate estimates were driven by body size and not field-active body temperature, with inland snakes requiring 1.6x more food annually than coastal snakes.

5. All snakes thermoregulated with high accuracy, suggesting that small increases in ambient temperature are unlikely to impact the maintenance energetic requirements of individual snakes and that some species of large-bodied reptiles may be robust to modest thermal perturbations under conservative climate change predictions.

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Keywords

climate change, ectotherm, energetic requirements, metabolism, rattlesnake, thermal
ecology, thermal quality

Introduction:

The urgency of the biodiversity crisis is accelerating as scientists document how climate change, habitat loss, pollution, and other human-induced disturbances are causing extinction of many taxa, such as insects, amphibians (Deutsch et al., 2008), mammals (Thuiller et al., 2006; Davies et al., 2008), birds (Wormworth & Mallon, 2006; White & Bennett, 2015), and reptiles (Gibbons et al., 2000; Sinervo et al., 2010; Böhm et al., 2013; Urban, 2015). As ectotherms, reptiles depend heavily on their immediate surroundings to regulate body temperature, where even slight changes in environmental conditions can impact physiological functions (Huey, 1982; Walther et al., 2002; Besson & Cree, 2010). Researchers have begun to quantify the current and predicted effects of climate change on diverse reptile species using recently-available high-resolution climate change forecasts and technology for modeling thermal landscapes (Sinervo et al., 2010; Böhm et al., 2016; Brusch et al., 2016; Wright et al., 2016).

Small-bodied, heliothermic (sun-basking) lizards are the focus of many climate-based studies, largely because it is easy to obtain large sample sizes with limited effort and because these heat-loving species may be at high risk of further warming (Sinervo et al., 2010; Clusella-Trullas et al., 2011; Pelegrin & Bucher, 2012; Buckley et al., 2015). However, studies investigating thermal ecology in larger-bodied ectotherms have tended to only use single populations of a given species and/or focus on sympatric species (Moore, 1978; Beck, 1995; Blouin-Demers & Weatherhead, 2001; Blouin-Demers et al., 2002; Lelièvre et al., 2011; Bovo et al., 2012), potentially because these species tend to be less common, rendering the effort and expense involved in these studies prohibitive. This, in turn, limits the scope of these studies to certain localities and prevents inferences about the possibility that climate change and environmental variation will interact in their future impacts on a given species. Furthermore, many of these studies have focused on the direct impacts of altered temperatures on the body temperature (T_b) of the population while failing to address the implications of changing T_b on the population's energetic needs (Waldshmidt et al., 1986; Alford & Lutterschmidt, 2012). To the best of our knowledge, no study to date has compared the thermal ecology and energetic requirements of a large-bodied reptile across multiple populations that inhabit distinct thermal environments.

72   Here, we examine the thermal ecology of a large-bodied reptile, the Pacific

73   rattlesnake (Crotalus oreganus), across multiple populations while also extending our

74   inference to quantify the energetic implications of environmental variation. The

75   extraordinarily low metabolic rates and energetic allocation to specific physiological

76   functions are well-established in rattlesnakes (Beaupre & Duvall 1998a; Beaupre &

77   Duvall 1998b). We conducted intensive field studies collecting physiological and

78   temperature data from four field sites on the Central Coast of California over eight years

79   to quantify the thermal ecology (see Table S1 for explanations of terminology common in

80   thermal ecology studies) and energy requirements of snakes on a macroecological scale.

81   We then subjected these data to predicted increases in ambient temperature to examine

82   how snake annual maintenance energy requirements will be impacted in a warming

83   world. We hypothesized that precise thermoregulation and low metabolic rates allow

84   rattlesnakes to respond to variable thermal environments effectively, both now and in the

85   future due to climate change. At low temperatures the snakes expend very little energy,

86   but as temperatures rise, their precise thermoregulation allows them to remain at body

87   temperatures optimally suited for their physiological processes. Specifically, we

88   predicted that the thermal quality of habitats would differ, with hot and thermally variable

89   inland sites having poorer thermal quality than the cool and stable coastal sites.

90   Additionally, due to the climatic differences between these habitat types, we predicted

91   that coastal snakes would have lower field active T_b and therefore lower annual

92   maintenance energy expenditures than snakes at inland habitat, making them less

93   thermally constrained both currently and in the future.

94

95   **Materials and Methods:**

96   Study species

97   The Pacific Rattlesnake (Crotalus oreganus, Holbrook 1840) ranges in western

98   North America from southern British Columbia to Baja California, Mexico (Pook et al.,

99   2000; Sunagar et al., 2014). The taxonomy of this species is under debate, and our four

100   study sites fall into what is currently considered the integration zone of the northern (C.

101   o. oreganus) and southern (C. o. helleri) subspecies (Ashton & Queiroz, 2001). However,

102   recent evidence suggests that all these study populations genetically cluster (Holding et

103   al., 2021); for the purpose of this study we will refer to them as C. oreganus. They are

104   habitat and dietary generalists that prey primarily on small mammals and lizards

105   (Mackessy et al., 2003; Sunagar et al., 2014, Sparks et al., 2015). Body size varies widely

106   among localities, but typical snout-vent lengths (SVLs) of adult male C. oreganus in

107   California range from approximately 60 cm to 120 cm (Ashton, 2001; Aldridge, 2002).

108

109   Study sites

110   The four study sites used for this investigation were the Chimineas Ranch in the

111   Carrizo Plain Ecological Reserve (CR), Montaña de Oro State Park (MDO), the

112   University of California Sedgwick Reserve (SG), and Vandenberg Air Force Base

113   (VAFB; Figure 1a). MDO and VAFB are coastal sites characterized by rugged cliffs,

114   canyons, and coastal scrub plant communities that experience relatively stable and mild

115   seasonal temperatures (Figure 1b; Underwood et al., 2003; Capehart et al., 2016). CR and

116   SG are inland sites that experience higher and more variable daily and seasonal

117   temperatures (Figure 1b) and are dominated primarily by chaparral, oak savanna, and

118   grassland plain habitats (Chimineas Ranch Foundation, 2019; University of California

119   Reserve System: Natural Resources, 2019). CR and MDO are situated to the north and

120   are in San Luis Obispo County, CA, USA, whereas SG and VAFB are to the south in

121   Santa Barbara County, CA, USA, Table S2).

122

123   Preferred body temperature (T_{set})

124   We constructed a laboratory thermal gradient adapted from Bovo et al. (2012),

125   spanning a range of ecologically relevant temperatures from 8 - 46 °C to determine

126   preferred body temperature (T_{set}) (see Supplemental Methods; Thermal Gradient

127   Construction & Data Collection). Snakes (N = 45) of varying size (35 cm - 108.4 cm)

128   and sex (M = 41, F = 4) were collected from all four field sites opportunistically from

129   September 2017 - June 2018 (Table 1). Snakes were brought back to the California

130   Polytechnic State University (Cal Poly) campus, where basic morphometrics (mass(g)

131   and snout-vent length(mm)), sex, reproductive status, and presence of gut contents were

132   recorded. Snakes were excluded from the analysis if they were found to have visible

133   meals in their gut or detectable follicles/offspring, as these factors could dramatically

134 alter preferred temperatures. Snakes were placed in the thermal gradient for a two-hour

135 acclimation period (Bovo et al., 2012) followed by a 12-hour data recording period. We

136 defined T_{set} as the interquartile range of the data (Blouin-Demers and Weatherhead, 2001;

137 Fitzgerald et al., 2003). Data collected for this and the following components of this

138 project were analyzed in JMP v14.0 (JMP®, SAS Institute Inc., Cary, NC, USA. 1989-

139 2007) unless otherwise specified. Site, sex, mass, season (Charland, 1990), and

140 presence/absence of internal radio-transmitter (see below) were evaluated as predictor

141 variables in model examining the response variable median T_{set} (Blouin-Demers &

142 Weatherhead, 2001; Fitzgerald et al., 2003).

143

144 **Table 1.** Summary mass and sex data of Pacific rattlesnake (Crotalus oreganus; N = 45 snakes)

145 used for preferred body temperature (T_{set}) trials, range of temperatures reached during trials, and

146 median site T_{set}.

Site	n	No. of individuals with radio transmitters	Sex	Mass Range (g)	T_{set} Range (°C)	Mean Median T_{set}
CR (inland)	8	3	M=5 F=3	370-790	30.0-36.3	32.25
SG (inland)	11	5	M=10 F=1	347-912	11.7-36.6	26.47
MDO (coastal)	15	6	M=15	60-860	14.8-37.6	30.55
VAFB (coastal)	11	3	M=11	165-990	15.8-36.7	27.74

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148

149 Field Active Body Temperatures (T_b)

150 Between the years of 2010-2017, we collected 85 adult, male rattlesnakes across

151 each of the four study sites (CR = 23, SG = 28, MDO = 15, VAFB = 19; Table S3). We

152 studied snakes at one site in each of 2010-16 (CR: 2010, SG: 2015, MDO: 2014, VAFB:

153 2012, 2013), and we studied snakes simultaneously at all four sites in 2017. Subjects

154 were transported to the laboratory at California Polytechnic State University (San Luis

155   Obispo, California, USA) and surgically implanted with radio-transmitters (Holohil

156   models SB-2, 5.2g and SI-2, 11 g, 13.5 g; Holohil Systems Ltd., Carp, Ontario, CA) and

157   Thermochron iButtons (DS1922L-F5 and DS1921G-F5 models, accuracies = ± 0.5°C and

158   ± 1°C respectively, Maxim Integrated Products Inc., Rio Robles, San Jose, CA), which

159   were set to record field active body temperatures (T_b) every hour. Temperature loggers

160   and radiotransmitters were implanted intracoelemically as separate units following the

161   procedures of Claunch et al. (2017). Snakes were released within 1-2 days of surgery at

162   the location of capture. At the end of each active season, we recaptured snakes and

163   brought them back to the laboratory to remove iButtons and radio-transmitters. To

164   compare the mean field active T_b of snakes across the four sites, we used a mixed-effects

165   model with an AR(1) covariate structure to capture the serial correlation of the within-

166   snake observations of temperature (Millar & Anderson, 2004). Site, month, and time of

167   day acted as fixed effects, snake ID was a random effect, and a unique time of day/date

168   value for each data point was used for the repeated structure. Additional sin- and cos-

169   transformed time-of-day variables were also included as fixed effects to account for the

170   cyclic, continuous nature of time.

171

172   Snake Physical Model Temperatures (T_{mod}):

173   In 2017, we deployed physical models in exposed, shaded, and burrow

174   microhabitats throughout the four field sites to characterize the thermal landscapes

175   potentially available to each of the rattlesnake populations (Dzialowski 2005;

176   Lutterschmidt & Reinert, 2012). Physical model microhabitat sites were selected based

177   on previous observations of snake use within those areas, and also with an effort to

178   distribute them evenly throughout the area where snakes were being radiotracked.

179   Importantly, these models represent a range of low and high temperatures available to the

180   snakes, but do not encompass the relative amount of each microhabitat available to

181   snakes at each field site. Physical models consisted of water-filled copper pipes, painted

182   to approximate C. oreganus reflectance, with iButtons suspended inside in accordance

183   with Bakken (1992) and Lutterschmidt & Reinert (2012) (see Supplemental Methods;

184   Physical Model Construction & Validation). Model temperature (T_{mod}) was recorded

185   every hour for one year from June 2017 to June 2018. Five physical models were placed

186   at each of the four study sites in various microhabitats (Blouin-Demers & Weatherhead,

187   2002; Lutterschmidt et al., 2012): two inside typical snake refugia (e.g., ground squirrel

188   burrows, rock burrows), two in exposed habitat (open field, gravel road), and one in a

189   shaded habitat (under a shrub or bush). We validated models using similar-sized, adult

190   rattlesnake carcasses. Because our models contained water (therefore had thermal

191   inertia), they did not meet the definition of an operative temperature model (Bakken &

192   Gates, 1975). Rather, we built our models to have thermal properties similar to those of a

193   non-thermoregulating snake, meaning that the temperatures collected by our models

194   reflected the full range of maximum high and low temperatures an average, adult-size

195   animal could reach if it were to remain in the most thermally stable and most thermally

196   variable microhabitats within each of the four study sites. For a large-bodied, non-

197   heliothermic, non-active shuttling ectotherm, we believe that these models were most

198   accurate for quantifying the thermal conditions of each habitat as opposed to smaller, air-

199   filled models (Lutterschmidt & Reinert, 2012).

200   To compare T_{mod} values across different sites, we used a mixed model with an

201   AR(1) covariate structure similar to the model used for T_b (see above section on body

202   temperature). No T_{mod} values for exposed microhabitats for SG were included because

203   the T_{mod} exceeded iButton temperature limits, causing all exposed models at SG to fail.

204   Additionally, T_{mod} values for the shaded CR model are unavailable for months July-

205   December due to iButton failure. Site, month, and time of day were included as fixed

206   effects.

207

208   Thermal Ecology Variables Measured:

209   We used thermal indices developed by Hertz et al. (1993) to evaluate the extent to

210   which a given habitat temperature (T_{mod}) permits a T_b within T_{set} to be achieved (thermal

211   quality) and the extent to which an animal actually experiences T_b within its T_{set}

212   (thermoregulatory accuracy). We calculated thermal quality of the environment (d_e) as

213   the absolute value of the difference between T_{mod} and T_{set}, where high d_e values mean that

214   the thermal quality of the environment is low and d_e values approaching zero represent

215   more favorable thermal habitat. The metric of d_e by definition uses operative temperature

216   (T_e, a value obtained from physical models without thermal inertia instead of the metric

217   T_{mod}); however, we use T_{mod} here with the caveat that the models' inertia could impact

218   their cooling and heating rates. We calculated thermoregulatory accuracy (d_b) as the

219   absolute value of the difference between T_b and T_{set}, where high d_b values mean poor

220   thermoregulatory accuracy (i.e., the snake's actual body temperature is much higher or

221   lower than its T_{set}), and d_b values approaching zero represent accurate thermoregulation

222   (Hertz et al., 1993; Blouin-Demers et al., 2001). Calculation of thermal variables was

223   performed in R v. 3.4.4 (R Development Core Team, 2015) using the package "dplyr"

224   (François et al., 2018).

225   Individual T_{mod} values for physical models of the same site and type (for example,

226   all CR burrow models) were averaged to calculate a mean d_e value for each time of day

227   observation (h) for each month. A repeated measures ANOVA was conducted to compare

228   overall d_e values of each study site and then rerun with results blocked by physical model

229   (burrow, shaded, exposed) to examine differences in thermal quality among microhabitat

230   types. Because of the need to compare multiple levels/groups (both site and microhabitats

231   within site), the repeated measures analyses with the AR(1) covariate structure was not

232   used for d_e. Site, month, and time of day were included as fixed effects in this model as

233   well as their interactions in a full factorial to account for these variables. For d_b, because

234   we were only examining differences at the site level, we used the same statistical analysis

235   that we used for T_b and T_{mod} values (see above sections) with site, month, and time of day

236   included as fixed effects.

237

238   Energetics

239   We used snake field active body temperatures (T_b) and morphometric data to

240   calculate theoretical standard metabolic rates (SMR) based on the following equation

241   established by Beaupre and Duvall (1998b, see Supplemental Methods: Energetics):

242    $$SMR = log_{10}VO_2 = X_1 + log_{10}mass + X_2*temperature + X_3$$

243   We converted the inverse log of the SMR into annual maintenance energy requirement in

244   Joules (19.874 J ml^{-1} O$_2$) and then Calories (2.39006 x 10^{-4} kcal J^{-1}). We then calculated

245   the approximate annual prey requirements to meet maintenance costs of an average-sized

246   male rattlesnake for each of the four study sites using their most common prey item, the

247   California ground squirrel (Otospermophilus beecheyi; Rowe et al., 1990; Sparks et al.,

248   2015). Based on previously published food assimilation experiments in the genus

249   Crotalus (Secor & Nagy, 1994; Beaupre & Zaidan, 2012), we assumed an 80% energetic

250   assimilation efficiency and that an average, adult ground squirrel weighs approximately

251   500 g (Evans & Holderied, 1943) and contains roughly 690 kcal (Kaufman, 1975; Dorcas

252   et al., 2004). An ANCOVA was performed to compare the daily SMR (ml O_2 day^{-1}) of

253   the four populations of snakes (N = 85) during their active season (April-Oct). Snake

254   mass, site, and the site x mass interaction were included in the model. Because mass was

255   used to estimate SMR, it will inevitably be a significant predictor variable for SMR.

256   However, we included it in the models to account for variation in snake body size among

257   sites.

258

259   Climate Change Projections

260   We used the Cal-Adapt (2019) representative concentration pathway (RCP)

261   climate scenario 4.5 as a "best case" scenario (emissions peak around the year 2040 then

262   steadily decline) to estimate the changes in habitat thermal quality and therefore energetic

263   consequences of anthropogenic climate change on snakes. We used the "modeled

264   projected annual mean" tool to identify the years in which the annual average

265   temperatures increase one degree from the 2017 average for each of the four study sites

266   (CR/SG/VAFB = 2030, MDO = 2047). We repeated this procedure for a two-degree

267   increase as well. To make macroecological predictions, we then assumed that a 1 °C

268   increase in annual average temperature would be equivalent to the same increase in T_{mod}

269   of all microhabitats. We calculated the proportion of current mean hourly T_{mod} for each

270   site and microhabitat type that fell within T_{set} for 2017 as well as with 1 °C and 2 °C

271   increases in mean hourly temperatures. We then calculated the percent change in these

272   proportions between each of these three climate scenarios as well as the change in mean

273   d_e for each site and microhabitat type. Lastly, we calculated the mean increase in annual

274   energetic needs (kcal year^{-1}) assuming snake T_b increased along with T_{mod} by adding 1

275   °C and 2 °C to the mean hourly T_b of each snake and using the Beaupre and Duvall

276   (1998b) equation to recalculate mean SMR for each of the four sites. We used a repeated-

277   measures ANOVA to compare differences in current energetic needs and those projected

278   with 1 °C and 2 °C increases, with site included as a factor.

279

Results:

281 Preferred Body Temperature (T_{set})

282   The mean of the median T_{set} of all snakes (N = 45) was 29.22 ± 0.92 °C with a
283 50% interquartile range of 26.28 ± 1.01 °C - 32.34 ± 0.84 °C (Blouin-Demers &
284 Weatherhead, 2001). None of the factors tested (site, sex, mass, season, or presence of
285 internal radio transmitter) significantly affected median T_{set} ($F_{8.36} = 1.30$, p = 0.27; Figure
286 S1).

287

288 Field Active Body Temperature (T_b)

289   After accounting for monthly and diel variation in temperature, we found that T_b
290 differed significantly among sites, with snakes from both CR and SG having higher mean
291 body temperatures than snakes from both MDO and VAFB ($F_{3,83.2} = 26.16$, p < 0.0001).
292 Tukey-Kramer post-hoc tests showed no significant differences in T_b between the two
293 coastal populations or between the two inland populations (Figure 2).

294

295 Physical Model Temperatures (T_{mod})

296   After accounting for monthly and diel variation in temperature, we found that
297 mean monthly T_{mod} differed significantly among sites ($F_{11} = 366.18$, p < 0.0001; Figure
298 3) although overall annual T_{mod} did not ($F_3 = 0.41$, p = 0.75).

299

300 Thermal Quality (d_e)

301   A total of 3,048 mean hourly d_e values were obtained from physical models (n =
302 5/site) at all sites. There was a significant difference in d_e among study sites ($F_{1151,1896} =$
303 3.62, p < 0.0001); post-hoc analysis showed no significant difference in overall mean d_e
304 among the two coastal sites (MDO & VAFB) and SG but did show that mean CR d_e was
305 significantly higher (= poorer thermal quality) than all other sites. Almost all interaction
306 terms within the full factorial model were significant (Table S4). When the same test was
307 run with d_e values blocked by microhabitat type, there was still a significant difference in
308 d_e among sites within each microhabitat (Burrow, $F_{3,1114} = 137.29$, p < 0.0001; Shaded,
309 $F_{3,994} = 75.13$, p < 0.0001; Exposed, $F_{2,827} = 19.38$, p < 0.0001; Figure 4). Because of the

310   failure of SG exposed physical models, no exposed d_e values were included for the

311   overall calculations for SG, therefore resulting in a lower overall d_e value than the

312   expected actual value and reducing the SG model sample size to n = 3. Due to similar

313   ambient temperatures, we expect that SG exposed d_e values would have been similar to

314   CR exposed values.

315

316   Thermoregulatory Accuracy (d_b)

317   Thermoregulatory accuracy (d_b) differed among study sites, with inland snakes

318   (CR, SG) being more accurate thermoregulators than coastal snakes (MDO, VAFB;

319    $F_{3,84.4} = 37.17$, $p < 0.0001$; Figure 5). Snakes at both CR and SG spent an overall larger

320   portion of time in or near preferred body temperatures throughout the entirety of the

321   study (Figure 5). On average, 50.89% of hourly T_b observations fell within the T_{set} for

322   inland snakes versus only 21.63% for coastal snakes. Post-hoc tests revealed no

323   significant differences between the two inland sites or between the two coastal sites

324   (Figure 6).

325

326   Energetics

327   The daily SMR of snakes across all four study sites differed significantly, with inland

328   snakes (CR, SG) having higher overall SMR than coastal (MDO, VAFB) snakes ($F_{7,77} =$

329   136.68, $p < 0.0001$, Figure S2). We suspect that the effect of site on SMR was not due to

330   T_b differences among sites, but to body size differences (inland snakes are larger, Table

331   S5). When correcting for mass (by dividing out mass from overall SMR), we found no

332   significant site differences among SMRs ($F_{3,81} = 1.50$, $p = 0.22$). However, given that the

333   goal of this study is to examine overall differences in SMR and energetic requirements,

334   we will only focus on whole-animal values as non-mass corrected data will be most

335   informative for our initial questions (Lighton & Halsey, 2011). When mean SMR values

336   were converted to annual energetic needs, we found that individual snakes from all four

337   populations needed to eat less than the equivalent of one adult ground squirrel year^{-1} to

338   satisfy maintenance energetic requirements. An average-sized, adult male inland snake

339   would need to consume a mean of 0.80 ground squirrels per year whereas a coastal snake

340   would need an average of 0.51 (Figure 7).

341

342   Climate Change Projections

343   During the physical model deployment period (June 2017-June 2018), the overall

344   proportion of hourly T_{mod} that fell below C. oreganus T_{set} was considerably higher

345   (0.856) than the proportion that fell within (0.056) or above (0.085) this range. While

346   these proportions varied among and within sites as well as microhabitat types (Table S6),

347   this general trend held true across all categories. With a 1 °C increase in T_{mod}, the overall

348   mean proportion of hourly temperatures that fell within T_{set} increased to 0.064, with still

349   the majority of hourly readings falling below T_{set} (0.084) and 0.091 falling above. A 2 °C

350   increase shows the same pattern, with a higher proportion of T_{mod} falling within the T_{set}

351   range (0.075) than the previous two climate conditions, the proportion below T_{set}

352   decreasing (0.825), and the proportion above T_{set} increasing (0.096, Figure S3).

353   Additionally, thermal quality (d_e) of each microhabitat type and the overall thermal

354   quality of each site are projected to improve with increases in ambient temperature

355   (Figure 8). With the greatest increase of 2 °C, CR, SG, MDO and VAFB will respectively

356   experience an overall 10, 12, 13 and 11% increase in d_e. These data suggest that rising

357   temperatures associated with anthropogenic climate change could actually benefit C.

358   oreganus as the thermal quality of their habitats increases.

359

360   Maintenance energy requirements (kcal year^{-1}) were overall significantly higher

361   when incorporating annual increases of 1 °C and 2 °C in mean snake T_b ($F_{5,6}$ = 106.97, p

362   < 0.00001). However, post-hoc tests revealed that whole model significance is driven by

363   the differences between current and 1 °C requirements, and requirements incorporating

364   the 2 °C increase. There are no significant increases from current energetic requirements

365   to energetic requirements with an increase in snake T_b of 1 °C (8.4% increase in annual

366   maintenance energetic requirements). With a 2 °C increase from current snake T_b, mean

367   energetic requirements increased by another 17.6%, which was a significant increase

368   from the current and 1 °C requirements. However, when converted to mean number of

369   squirrels per year, all populations still required roughly 1 or less large meal (Table 2).

370

Table 2. Mean number of ground squirrels required by an average-sized, adult male Pacific rattlesnake (Crotalus oreganus) from four populations on the Central Coast of California required to meet annual maintenance energy requirements at current body temperature (T_b) conditions and with theoretical 1 °C and 2 °C increases in T_b.

Site	Current no. required squirrels	No. required squirrels with 1 °C increase	No. required squirrels with 2 °C increase
CR (Inland)	0.84±0.04	0.90±0.04	1.06±0.04
SG (Inland)	0.77±0.04	0.86±0.05	1.01±0.05
MDO (Coastal)	0.49±0.04	0.53±0.05	0.62±0.04
VAFB (Coastal)	0.52±0.02	0.57±0.02	0.68±0.02

Discussion:

In this study, we demonstrate that populations of C. oreganus experience dramatically different climatic conditions but utilize thermal variation in microhabitats to thermoregulate such that differences in mean active T_b are minor, and that these differences may not have major impacts on the snakes' overall energetic needs. As a moderately precise thermoregulator (Blouin-Demers & Weatherhead, 2001), C. oreganus will likely be able to mitigate the effects of inhabiting low-quality thermal habitat by altering thermoregulatory behaviors, and furthermore, climate change is predicted to increase the thermal quality of C. oreganus habitat. Thus, this rattlesnake species and others may be fairly resilient to and even positively impacted by changing climates.

The T_{set} range (26.28 ± 1.01 °C - 32.34 ± 0.84 °C) was similar in all four populations of C. oreganus and is notably wide in range, potentially because, as habitat generalists and ambush foragers, rattlesnakes are adapted to experiencing a wide range of environmental temperatures (Alford & Lutterschmidt, 2012). Other vipers also exhibit high variation in T_{set} within and among species (Table S7). This large range of temperature preferences exemplifies the tendency of larger-bodied ambush-predator

392 ectotherms to exhibit more eurythermic behaviors that are reflective of the environments
393 in which they are found (Brattstrom, 1965; Moore, 1969; Bovo et al., 2012). Snakes from
394 coastal sites had significantly lower T_b than snakes from inland sites, which we expected
395 based on different ambient temperatures. However, variation in T_b may also be related to
396 environmental trade-offs impacting the thermoregulatory behavior, such as site
397 differences in predation risk, as well as body size and thermal inertia (Putman & Clark,
398 2017), which in the case of our study is inextricably linked to source population. That is,
399 inland snakes could experience higher T_b in part because their larger body sizes impart
400 slower cooling rates (Stevenson 1985). Regardless of site, both inland and coastal snakes
401 typically exhibited field active T_b below their T_{set}. Snakes often select ambush sites in
402 shaded areas along small mammal trails in grass or near ground squirrel burrows
403 (Theodoratus & Chiszar, 2000; Putman et al., 2016) and remain for an extended period of
404 time until a prey item passes within striking distance. Lower T_b resulting from ambush
405 site selection may represent a thermoregulatory trade-off as snakes passively
406 thermoconform to low quality ambush sites (Alford & Lutterschmidt, 2012).

407   Our prediction that coastal sites would have overall better thermal quality than
408 inland sites was supported (if we assume that SG would have a similar thermal quality to
409 CR with the inclusion of values from exposed physical models), although there was no
410 significant difference in annual overall mean T_{mod}. The lack of overall difference in mean
411 T_{mod} is likely due to the greater variance of temperatures in inland sites throughout the
412 year relative to coastal sites, creating similar means. However, the variation in
413 temperature among and within these sites is best demonstrated by the significant variation
414 in thermal quality of each microhabitat. Overall, burrows have the best thermal quality
415 due to their more stable temperatures, and it is likely that they offer cooler temperatures
416 and further thermal stability deeper in the burrow systems which were unattainable to us
417 for measurement. Snakes are able to utilize this microhabitat as dependable thermal
418 refugia when surface temperatures are either too hot or cold. To our surprise, shaded
419 physical models often experienced temperatures close to those of exposed models at their
420 respective sites. It is possible that even though these models were shaded, the extreme
421 heat of the inland sites and frequently windy and wet conditions of the coastal sites still
422 drove the temperatures of these models far above and below T_{set} due to conduction and

423   convection respectively. Because thermal quality is more variable at inland sites, this may

424   mean that snakes need to spend more energy shuttling between thermally favorable

425   habitat during their active summer season as well as experience restricted daily and

426   annual activity patterns. Meanwhile, the lack of large temperature fluctuations at coastal

427   sites means snakes can remain active for a larger portion of daylight hours and for most,

428   if not all of the year. Although physical models allowed us to produce a coarse estimate

429   of the thermal landscape, we are unable to account for the entire thermal configuration of

430   each site (Sinclair et al., 2016), and a more detailed analysis within each of these sites

431   would help elucidate some of the drivers of differences in field active T_b observed in

432   these populations of C. oreganus. Additionally, our study utilized environmental and

433   body temperature data that only partially overlapped in study periods; future studies

434   interested in making comparisons of fine-scale relationships between microhabitat

435   temperatures and snake body temperatures should collect data across the exact same time

436   period, even if long-term climates at study sites are relatively stable.

437   We were somewhat surprised to find that snakes in habitats with poorer thermal

438   quality (inland sites) thermoregulated more accurately than snakes with access to higher

439   quality habitats (coastal populations). While this phenomenon has been documented in

440   multiple small lizard species (Gunderson & Leal, 2012; Sagonas et al., 2013), the number

441   of studies reporting this in large-bodied reptiles is limited (Blouin-Demers &

442   Weatherhead, 2001, Row & Blouin-Demers, 2006; Besson & Cree, 2010), particularly in

443   ambush predators (Bovo et al., 2012). It is possible that the higher thermal quality of

444   coastal sites may result in longer periods of time where ambient temperatures are closer

445   to the T_{set} of C. oreganus, putting less physiological pressure on snakes to actively

446   thermoregulate. Additionally, snakes at the poorer quality inland sites may be

447   thermoregulating more accurately to increase physiological performance for other

448   behaviors (e.g. mate searching, ambush, etc.) during the more limited activity hours, as

449   Besson & Cree (2010) reported in tuatara. The results may also reflect the thermal

450   heterogeneity of the habitats: the thermal quality of microhabitats in inland sites was

451   more variable than those in coastal sites (Figure 4), potentially affording inland snakes a

452   wider range of choices when thermoregulating and allowing them to find ambush sites or

453   refugia closer to their T_{set}.

454   Our estimates of the SMR of snakes from all four populations revealed that snakes from inland sites require on average 1.6x as much food as coastal snakes for maintenance metabolism. The equations to estimate SMR (Beaupre & Duvall 1998a; Beaupre & Duvall 1998b) use mass and T_b; while both of these were higher at inland sites, the larger body size of inland males was the major contributor to their higher SMR and therefore energy requirements. Our data show that high variation in ambient temperatures among sites translate into only minor inter-population differences in T_b due to effective thermoregulation, and that these differences do not have a great impact on maintenance energy requirements. The driving factor for differences in overall metabolic rates, and therefore energetic needs, is the actual mass of the animal (Dorcas et al., 2004). Why are males larger at inland sites? There are many possibilities, including a warmer active season promoting a longer growing season (Mousseau, 1997), higher rainfall and water availability at our inland sites offsetting the negative effects of living in semi-arid habitats (Amarello et al., 2010), more competition for resources at coastal sites due to higher rattlesnake population densities and/or lower prey densities (Beaupre, 1995; Madsen & Shine, 1993), or population differences with inland snakes genetically predisposed to grow larger and/or surviving longer (Forsman, 1993). Regardless of the cause, the implications of body size and temperature variation among adult male rattlesnakes at each of these sites results in minor differences in energetic needs to fuel maintenance metabolism, with snakes from each site needing less than one adult ground squirrel per year. Importantly, our metabolic calculations are only estimates of maintenance metabolism and do not encompass energetically costly activities including digestion and movement through the environment for mate-seeking, predator avoidance, and ambush site selection (Beaupre, 1996; 2008). Furthermore, energetic needs of female rattlesnakes to produce a litter of offspring would be much higher (Beaupre and Duvall, 1998b; Beaupre, 2002). Population and sex differences in overall energy requirements could only be ascertained by collecting field metabolic data (e.g., Beaupre, 2008).

       Climate change is generally predicted to have a negative effect on most ectotherm species, especially those at lower latitudes (Sinervo et al., 2010). However, it appears that small increases in ambient temperature may prove beneficial to rattlesnakes in central California because the overall thermal quality of all microhabitats is projected to increase

485   at all field sites. With a larger proportion of daily T_{mod} falling within the T_{set} range,

486   snakes will be less thermally constrained, choose among a wider range of ambush sites,

487   and be active for a longer time during the day. Specifically, snakes will be able to emerge

488   from overwintering earlier in the year and in turn, wait until later months before going

489   back into hiding. This may translate into additional opportunities to find resources such

490   as mates and food, as well as longer annual active seasons, and could feasibly result in

491   higher reproductive output in females and therefore increased population densities of

492   rattlesnakes (references showing more food and longer active season contributes to more

493   reproduction and pop density). It is possible that summer temperatures may exceed T_{set}

494   for longer periods of time during daylight hours (particularly at the inland sites), resulting

495   in altered behavior, such as a shift to more crepuscular/nocturnal foraging. This could

496   lead to utilization of alternate prey sources which in turn could start a cascade of

497   ecological effects at the community level. If the metabolic rates of these snakes rise with

498   increasing temperatures, they would need to obtain additional energetic resources.

499   However, as our calculations and other studies have shown, the metabolic needs of these

500   snakes are incredibly low (Beaupre, 1995; Beck, 1995; Beaupre & Duvall, 1998b), with

501   current baseline maintenance energetic demands being met with less than a single large

502   meal per year. Even if mean active T_b increased 1 °C or 2 °C along with ambient

503   temperature, the annual caloric requirements for maintenance would still be met with a

504   single large prey item. That said, evidence from this study suggests that C. oreganus is an

505   accurate enough thermoregulator that overall small changes in ambient temperature will

506   likely not dramatically shift the snakes' T_b. These theoretical calculations are limited to

507   the scope of energetic needs of the snakes from which they were calculated (i.e., resting,

508   fasted snakes unable to thermoregulate in a metabolic chamber), so these results must

509   also be considered in an ecological context. To fully understand the implications of

510   climate change for rattlesnakes, we would need to take into account possible impacts on

511   prey populations as well as changes in snake behavior as a response to changing

512   temperatures, which may increase energetic needs beyond the scope of our models.

513   Overall, rattlesnakes are ideal model organisms for examining the physiological

514   effects of climate on large-bodied ectotherms. Their life history traits, simple behaviors,

515   and metabolism are well-studied, providing a strong foundation for examining their

516 thermal ecology and implications of climate change on their energetic requirements.
517 Large-scale comparative studies among multiple populations of a given species can
518 greatly enhance our understanding of the effects of anthropogenic climate change on
519 biodiversity. While small increases in ambient temperature may prove thermally
520 beneficial to rattlesnakes on the Central Coast of California, alterations in climates may
521 affect rattlesnake environments in negative ways and have cascading effects within their
522 biotic communities. It is apparent that in rattlesnakes, a wide-ranging T_{set}, plasticity in
523 thermoregulatory behavior, and low energetic demands may help mitigate the changes in
524 environmental temperatures these animals will experience, even across extremely
525 variable habitat types.

544 **Authors' Contributions:**

545 HLC and ENT conceived the ideas and designed methodology; HLC, KCK, JMW and

546 MVH analyzed the data; all authors contributed to data collection, manuscript

547 preparation, and gave final approval for publication.

548

549 **Conflict of Interest:**

550 None declared.

551

552 **Data Accessibility:**

553 All data associated with this project can be found on Dryad

554 (doi:10.5061/dryad.mpg4f4qzv).

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557 **Literature Cited:**

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832   **Figure Legends**

833

834   **Figure 1a.** Map of locations on the Central Coast of California of the four Pacific rattlesnake

835   (Crotalus oreganus) populations (two coastal, two inland) used in this study. **1b**. Average

836   monthly temperatures (°C) for the same corresponding sites from 2010-2017 (National Ocean and

837   Atmospheric Administration & Western Regional Climate Center, 2017). **1c.** An adult male

838   Crotalus oreganus (larger snake) resting with a smaller female mate.

839

840   **Figure 2.** Mean hourly field active body temperatures (T_b) for four populations of Pacific

841   rattlesnake (Crotalus oreganus; N = 85; CR = 23, SG = 28, MDO = 15, VAFB = 19) on the

842   Central Coast of California during the active season (April-Oct). Black shapes denote inland sites,

843   white shapes denote coastal sites; circles denote northern sites, triangles denote southern sites.

844   Data were collected for varying lengths of time from 2010-17. Different letters represent a

845   significant difference between means, and bars represent 95% confidence intervals.

846

847   **Figure 3:** Mean monthly Pacific rattlesnake (Crotalus oreganus) physical model temperatures

848   (T_{mod}) for each of the four study sites (inland: CR, SG; coastal: MDO, VAFB) on the Central

849   Coast of California (measured June 2017-June 2018). Mean monthly T_{mod} differed significantly

850   among sites but overall annual T_{mod} did not. SG does not include exposed physical model values,

851   as all exposed SG models failed, as well as the CR shaded model from months Jul-Dec. Grey bar

852   represents the preferred body temperature range (T_{set}) of C. oreganus. Error bars represent $1 \pm$

853   standard deviation from the mean.

854

855   **Figure 4:** Mean thermal quality (d_e) values for each microhabitat at each of four study sites

856   (inland: CR, SG; coastal: MDO, VAFB) from June 2017 to June 2018. Thermal quality values

857   closer to zero are indicative of better thermal quality. Different letters indicate significant

858   differences among physical models in the same microhabitat type (capital letters = burrows,

859   italicized = shaded, lowercase = exposed). SG does not include exposed physical model values, as

860   all exposed SG models failed. Values are shown with 95% confidence intervals.

861

862   **Figure 5:** Mean thermoregulatory accuracy (d_b) for each of four populations of Pacific

863   rattlesnake (Crotalus oreganus; N = 85; CR = 23, SG = 28, MDO = 15, VAFB = 19) on the

864   Central Coast of California. Black shapes denote inland sites, white shapes denote coastal sites;

865   circles denote northern sites, triangles denote southern sites. Values closer to zero reflect higher

866   thermoregulatory accuracy. Mean daily d_b differed significantly among sites, with inland snakes

867   (CR, SG) thermoregulating more accurately than coastal snakes (MDO, VAFB). Different letters

868   represent significant differences between means, and bars represent 95% confidence intervals.

869

870   **Figure 6.** Mean hourly physical model temperatures (T_{mod}, in burrows, exposed, or shaded) and

871   Pacific rattlesnake (Crotalus oreganus) body temperatures (T_b) over the duration of the active

872   season (Apr-Oct) for each of the four study sites (inland: CR, SG; coastal: MDO, VAFB) on the

873   Central Coast of California. CR shaded means do not include data from Jul-Dec. Grey bars

874   represent the preferred body temperature range (T_{set}) of C. oreganus.

875

876   **Figure 7.** Number of California ground squirrels (Otospermophilus beecheyi) an average-

877   sized adult male Pacific rattlesnake (Crotalus oreganus) from each of four populations on the

878   Central Coast of California (CR, SG, MDO, VAFB) would need to consume to meet annual

879   maintenance energetic requirements.

880

881   **Figure 8.** Overall thermal quality (d_e) for four study sites (CR, SG, MDO, VAFB) on the Central

882   Coast of California at current conditions and with a 1 °C and 2 °C increase in ambient

883   temperatures. Values approaching zero represent higher thermal quality. All sites will experience

884   an increase in d_e as ambient temperatures increase, creating potentially more favorable thermal

885   environments for Pacific rattlesnakes (Crotalus oreganus) in both coastal and inland locations.
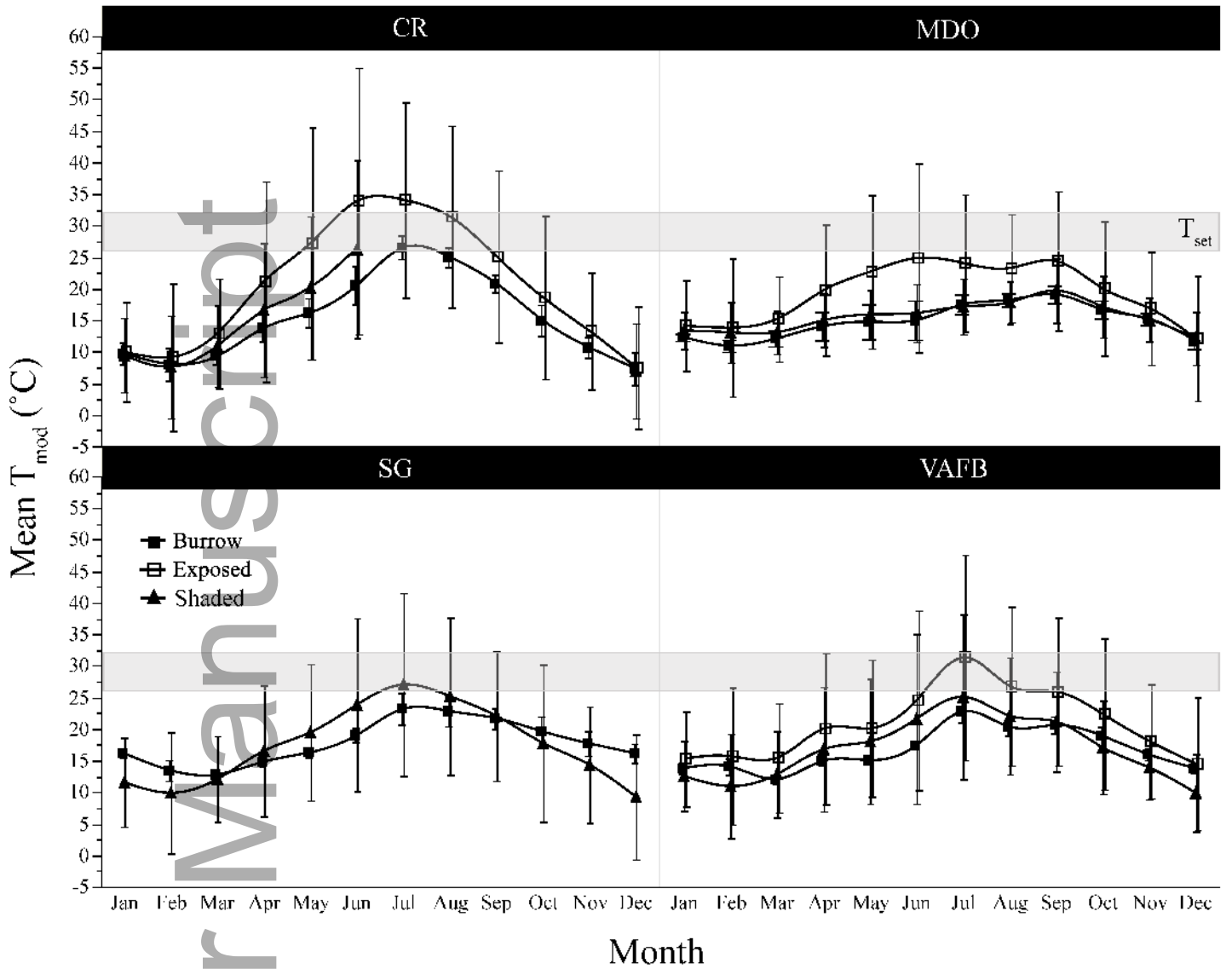
886   Error bars represent ±1 SEM.
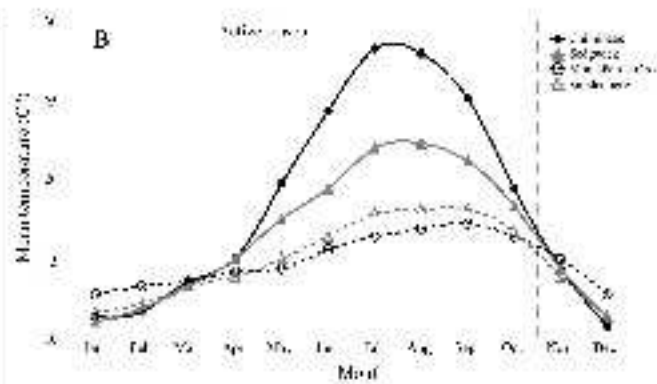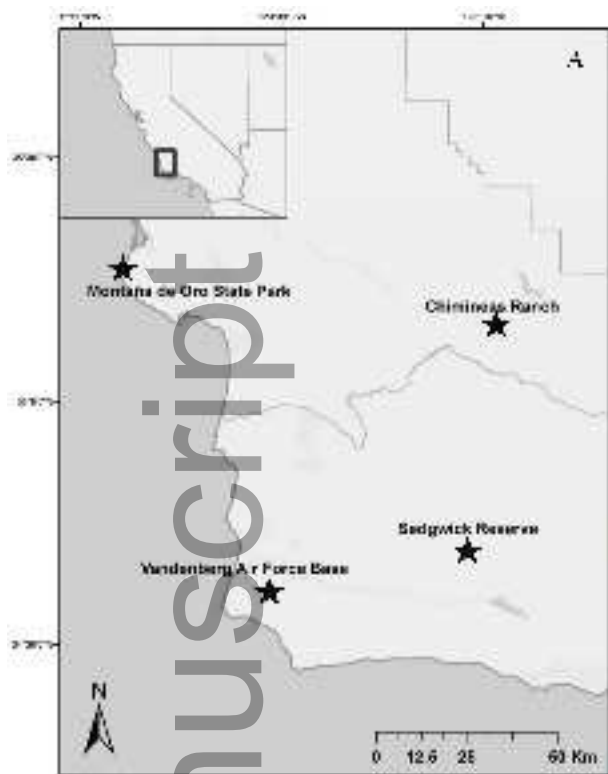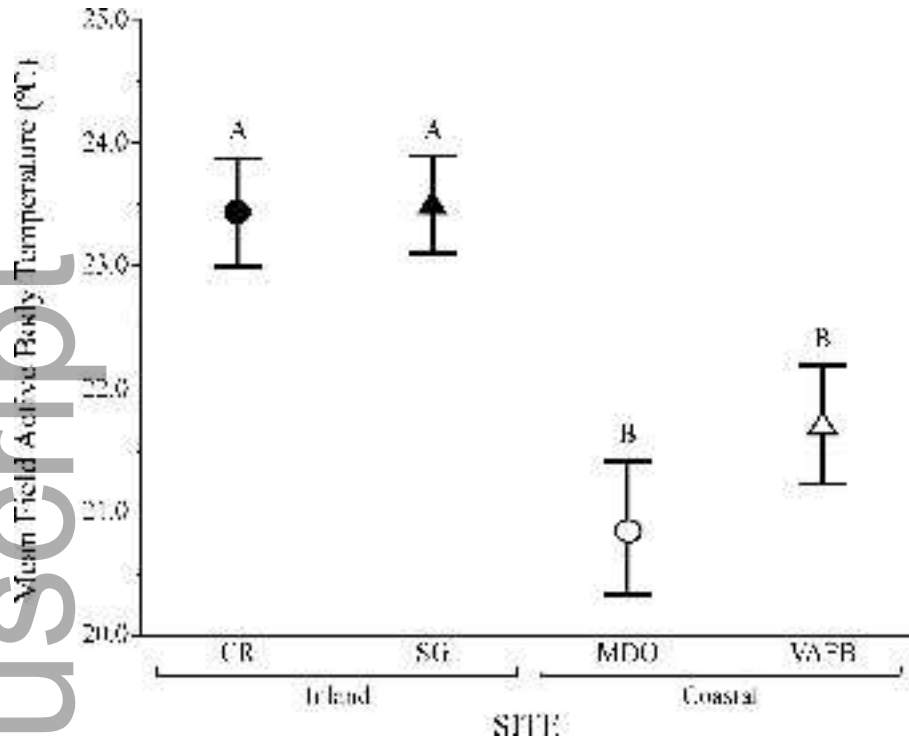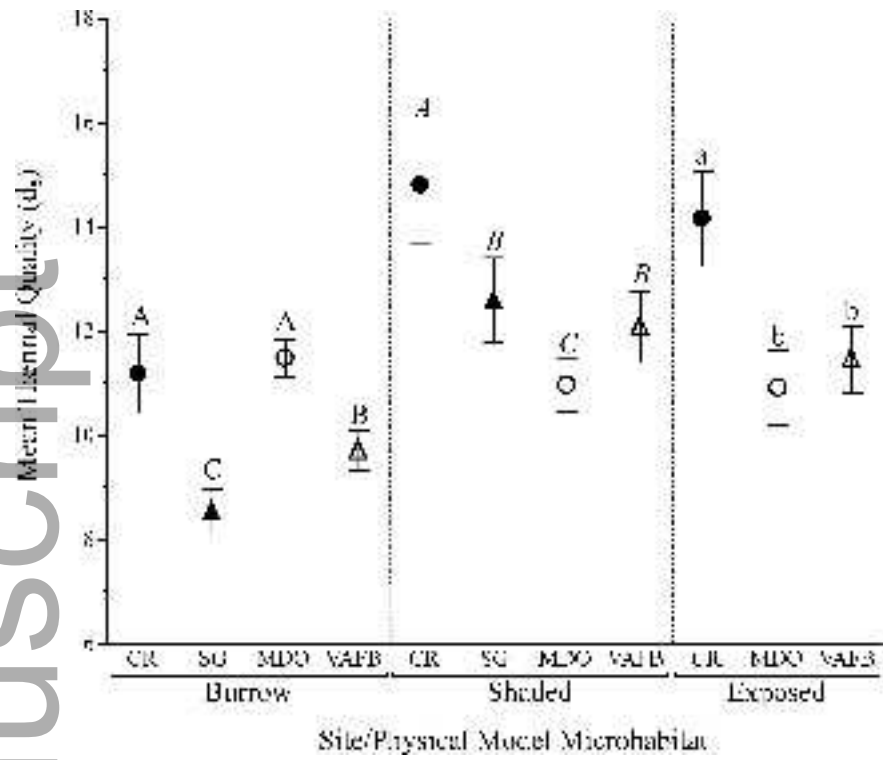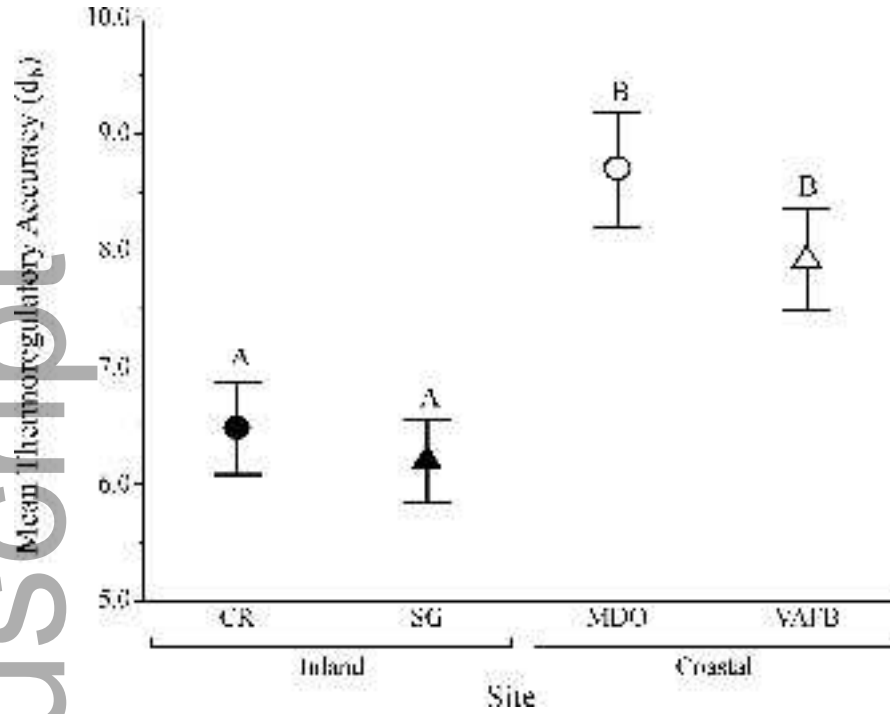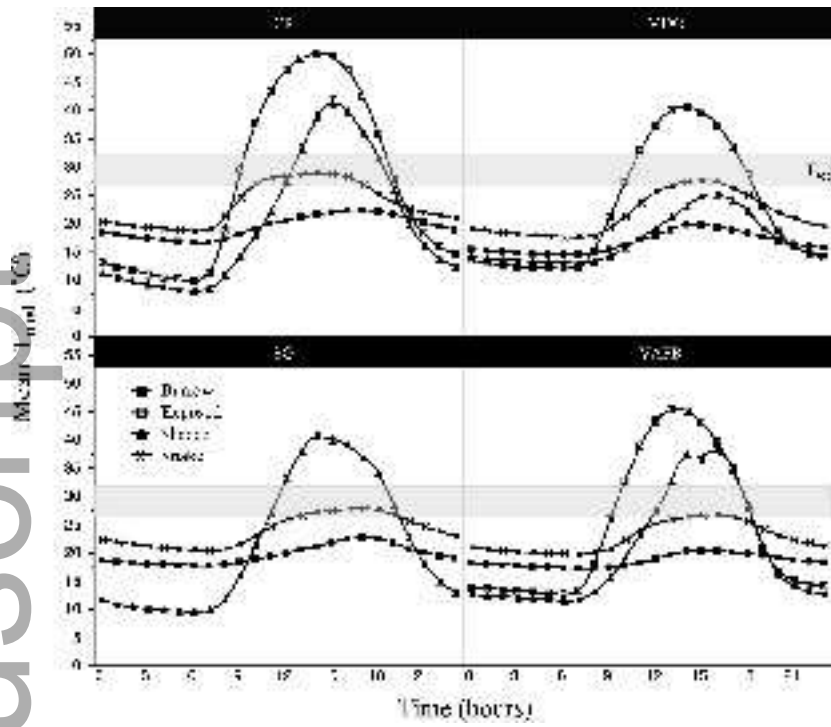
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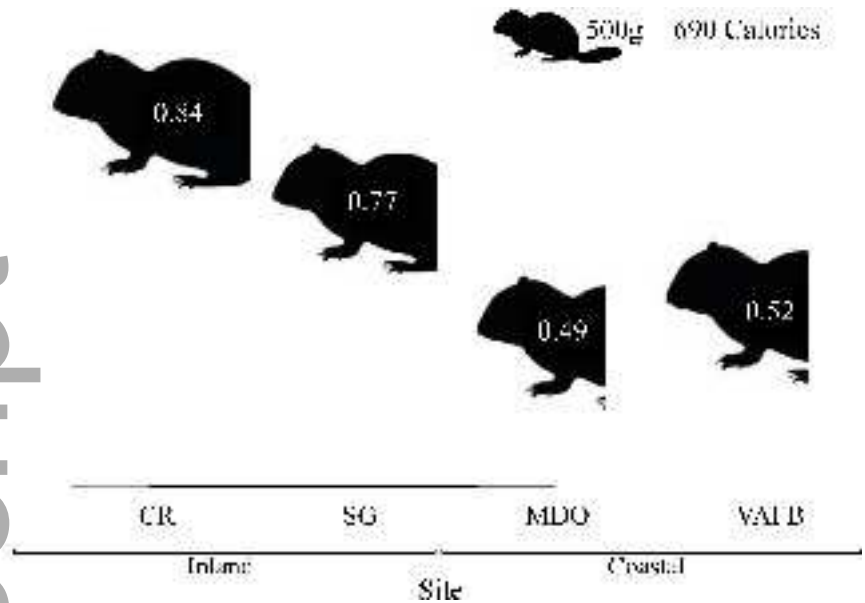
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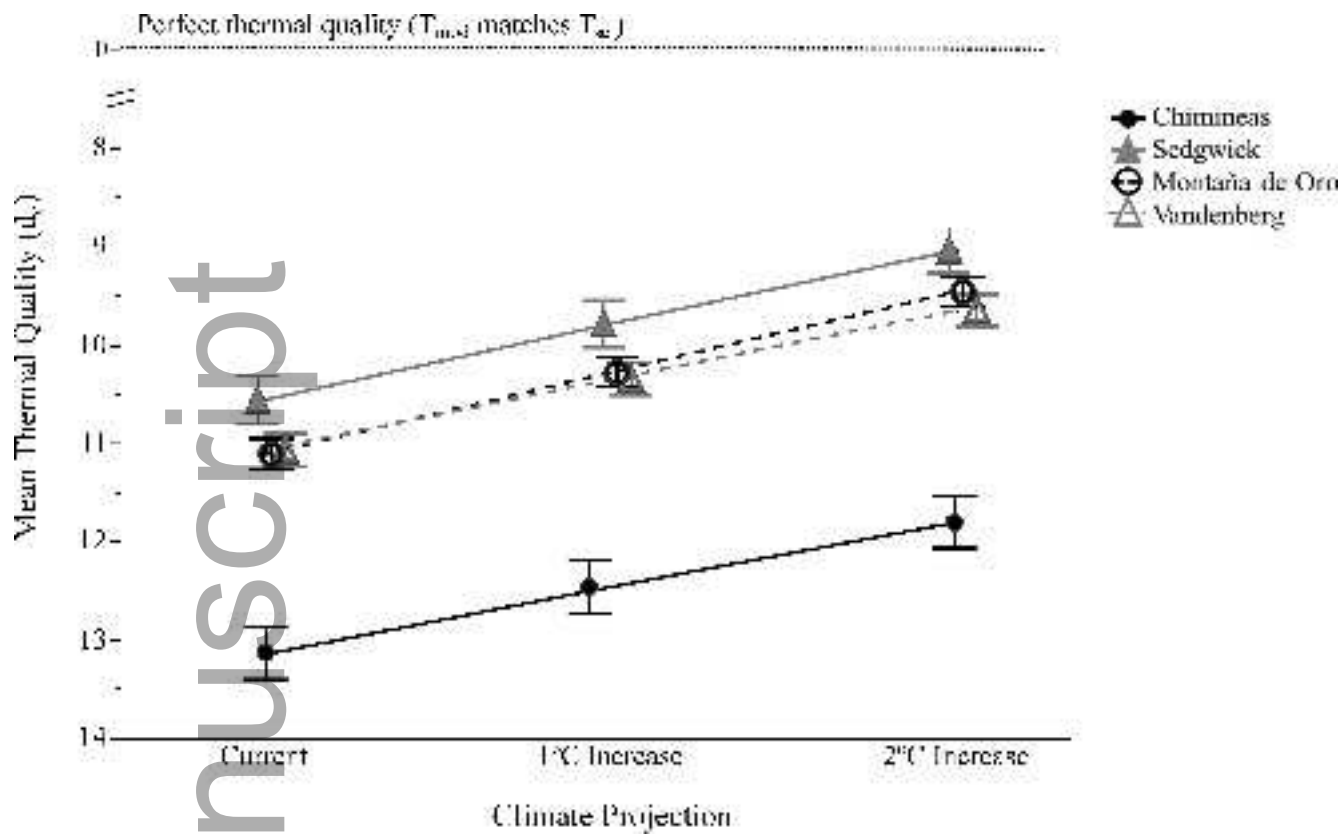
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