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24 **Abstract**

25

26 Ectotherms in cold environments often spend long winters underground. In 1941
27 Raymond Cowles proposed a novel ecological trade-off involving depth at which
28 ectotherms overwintered. On warm days, only shallow reptiles could detect warming
29 soils and become active; but on cold days, they risked freezing. Cowles discovered that
30 most reptiles at a desert site overwintered at shallow depths. To extend his study we
31 compiled hourly soil temperatures (5 depths, 90 sites, continental USA) and
32 physiological data, and then simulated consequences of overwintering at fixed depths. In
33 warm localities shallow ectotherms have low energy costs and largest reserves in spring;
34 but in cold localities, shallow ectotherms risk freezing. Ectotherms shifting to the coldest
35 depth potentially reduce energy expenses, but paradoxically sometimes have higher
36 expenses than those at fixed depths. Biophysical simulations for one desert site predict
37 that shallow ectotherms should have elevated opportunities for mid-winter activity but
38 may need to move deep to digest captured food. Our simulations generate testable eco-
39 physiological predictions but rely on physiological responses to acute cold rather to
40 natural cooling profiles. Furthermore, testing ecological predictions requires natural-
41 history data that do not exist. Thus, our simulation approach uncovers “unknown
42 unknowns” and suggests research agendas for studying ectotherms overwintering
43 underground.

44

45 **INTRODUCTION**

46

47 *“...as we know, there are known knowns; there are things we know*
48 *we know. We also know there are known unknowns; that is to say we*
49 *know there are some things we do not know. But there are also*
50 *unknown unknowns—the ones we don't know we don't know.”*

51

52 *Donald Rumsfeld (2002),*
53 *United States Secretary of Defense*
54 *News briefing 12 February 2002*

55

56 Ectotherms living in cold climates may spend months hibernating underground or in
57 other retreats. Some remain underground, but others emerge on warm days. Conditions
58 underground will affect risk of freezing or cold injury as well as energy reserves in
59 spring. Despite a legacy of studies of the physiology of cold tolerance and of hibernation
60 (Gregory 1982; Storey 1990; Addo-Bediako *et al.* 2000; Costanzo *et al.* 2008; Denlinger
61 & Lee 2010; Zani *et al.* 2012; Williams *et al.* 2014; Sinclair 2015), an understanding of
62 the dynamics of overwinter physiology, behaviour, and ecology has striking gaps. As we
63 will argue here, many ‘unknown unknowns’ exist.

64 Almost eight decades ago, Raymond B. Cowles (1941) explored the overwinter
65 biology of squamate reptiles in a California desert. Cowles observed that some squamates
66 spent the entire winter buried in the sand but that some others emerge and are active on
67 warm days. He knew that soil temperatures changed with depth and were both coldest

68 and warmest near the ground surface (Figs. 1, S1, S2-animation), and that the diurnal heat
69 pulse on sunny days penetrated slowly downward (Smith 1929). Cowles proposed a
70 novel trade-off: only reptiles in shallow retreats could potentially become active on warm
71 days in winter or spring because the diurnal heat pulse descending into the soil would
72 reach them during daylight hours (Smith 1929) and be a reliable cue that surface
73 temperatures were warm enough for activity. Nevertheless, Cowles also noted that
74 shallow reptiles risked freezing and predation by endotherms (see p. 129 in Cowles
75 1941). Thus, Cowles (1941) is a pioneering example of “trade-off” and optimality
76 thinking in ecology.

77 Testing such ideas involves determining where organisms are overwintering
78 underground. In 1944 that was – and still is – a logistic challenge (but see Karlstrom
79 1956; Kenagy & Smith 1971; van Gelder *et al.* 1986; Grenot & Heulin 1988;
80 Lutterschmidt *et al.* 2006; Maritz & Alexander 2009; Harris *et al.* 2015; Berman *et al.*
81 2016; DeNardo *et al.* 2018). Cowles was opportunistic: he followed a large tractor and
82 ‘scraper’ that was progressively scraping off the tops of hummock dunes, thus converting
83 native desert to farmland (“brushing”, photo in Fig. S3). Cowles caught any reptiles in
84 the “dirt spill,” took their body temperatures (T_b), and estimated hibernation depths from
85 soil-temperature measurements (T_{soil}). His efforts were “exceedingly gratifying”: after
86 only “four and a half” days, he caught 96 individuals of 14 species and estimated depth
87 for 49. Most were shallow: 76% were between 2 and 30 cm deep (Fig. S4).

88 Cowles’s insights inspired us to ask three basic questions about the eco-
89 physiological consequences of overwintering at various depths. Cowles addressed the
90 first two. (1) How deep must a reptile go to avoid dangerously or lethally cold

91 temperatures? (2) Which depth best enables reptiles to detect a thermal cue of a warm
92 day in winter, thus maximizing opportunities for above-ground activity? (3) What depths
93 minimize cumulative energetic expenses over the winter? Cowles did not ask this last
94 question, but low expenses may promote overwinter survival and maximize energy
95 reserves at spring emergence (Hahn & Tinkle 1965; Wilson & Cooke 2001; Zani 2008;
96 Williams *et al.* 2014; Sinclair 2015).

97 Ideally, such questions should be answered with field data of known hibernating
98 depths, T_b , and physiological profiles, all from geographically diverse sites. However,
99 data for squamate reptiles are incomplete and scattered (e.g., Parker & Brown 1974;
100 Ruby 1977; Congdon *et al.* 1979; Bauwens 1981; Grenot & Heulin 1988; Sexton *et al.*
101 1997; Wilson & Cooke 2001; Bishop & Echternacht 2004; Zani 2008; Zani *et al.* 2012;
102 Harris *et al.* 2015; Berman *et al.* 2016; Cecchetto *et al.* 2019), except for the few species
103 hibernating communally in rocky dens at mid- to high-latitude (Gregory 1982; Norberg &
104 Cobb 2017). Geographic surveys of overwinter biology are rare (Tsuji 1988; Sexton *et al.*
105 1997; Wilson & Cooke 2001). Moreover, physiological data for squamates are typically
106 acquired from acute-exposure assays, which may have limited relevance to ectotherms
107 experiencing slow cooking in nature (Fig. 1B) (Halpern & Lowe 1968; Patterson &
108 Davies 1984; Tsuji 1988; Storey 2006; Huang & Tu 2008; Berman *et al.* 2016).

109 Given limitations of empirical data, we used concepts in Cowles (1941) as a
110 ‘narrative’ (Otto & Rosales 2020) to guide simulations that explore consequences of
111 overwintering at different depths (Fig. 2). We downloaded hourly soil temperature data
112 for five depths from 90 sites for 2017-2018 (Fig. S5) in the continental United States
113 **(Material and Methods)**. Next, we incorporated physiological data (e.g., cold

114 tolerance) and predicted how overwintering depth affects cold injury and energy
115 expenditures. Also, we used a biophysical model (Kearney & Porter 2017, 2019) to
116 simulate how retreat depth at a desert site affects opportunities for activity on warm days.

117 While attempting to parameterize variables (e.g., lethal temperature) in these
118 simulations, we discovered that specific behavioural, ecological, and physiological data
119 required for our simulations – as well as field data required to test their predictions –
120 often do not exist or are unsuitable. These issues likely reflect the logistic difficulty of
121 determining the depth, temporal T_b profiles, and behaviour of overwintering ectotherms
122 along environmental gradients (Sinclair 2001a), the logistic challenge of quantifying time
123 series of physiological responses to chronic rather than acute temperature exposures
124 (Sinclair 2001a; Huang & Tu 2008), and the lack of information on cues used for mid-
125 winter emergence (Heath 1962; Lutterschmidt *et al.* 2006). Our simulations thus rely on
126 uncertain parameter values but nonetheless provide “approximate answers” (Tukey 1962)
127 to ecologically relevant questions. Most importantly, they help uncover what needs to be
128 measured (‘unknown unknowns’).

129 The three questions we address are diverse (cold risk, energy reserves, activity).
130 Therefore, we present separate methods, results, and discussion sections for each question
131 and for the initial section of T_{soil} patterns. When appropriate, we include an “unknown
132 unknowns” section. In **CONCLUDING REMARKS** we synthesize key issues and
133 propose a research agenda.

134 We made several global assumptions. We assumed that ectotherms are buried in
135 soil (thus not in burrows, under rocks, or in rock crevices) and that T_b equals adjacent

136 T_{soil} . [Note: the median absolute deviation of T_b from T_{soil} was only 0.2 °C for 11 lizards
137 (*Phrynocephalus vlangalii*) dug up in voluntarily selected, underground retreats in Aksai,
138 China (L. Ma, unpublished observations).] We assumed that soil moisture (or ice),
139 desiccation, or gas tensions did not influence results (but see, Costanzo 1989; Burke *et al.*
140 2002; Berman *et al.* 2016; Rossi *et al.* 2020).

141 **Patterns of soil temperatures**

142 **Methods (soil temperatures)**

143 Using the R package ‘*soilDB*’ (Skovlin & Beaudette 2019), we downloaded hourly soil
144 temperatures at five depths (-5, -10, -20, -50, and -100 cm) from 660 sites from the
145 continental USA from the Climate Analysis Network (SCAN,
146 <http://www.wcc.nrcs.usda.gov/scan/>) and from the NRCS National Water and Climate
147 Center (SNOTEL, <https://www.wcc.nrcs.usda.gov/snow/>) for 2017-18. Site metadata
148 (longitude, latitude, and elevation, etc.) were also downloaded (table S1). Site
149 descriptions and images are available at the above URLs. Note that measured T_{soil} are site
150 specific, as T_{soil} will vary even on a local scale (shading, aspect, soil).

151 Downloaded T_{soil} data were messy (Kearney 2020). We numerically scanned T_{soil}
152 and examined plots of T_{soil} versus date for obvious errors. Questionable sites were
153 omitted (blind as to location), as were sites missing data for more than 5% of hours at any
154 depth from October-March. This left 90 sites (table S1, black dots in Fig. S5). The
155 minimum completeness (by depth and month) was 97.3%, and 71.9% of samples were
156 100% complete. To characterize T_{soil} at each site, depth, and month, we computed the
157 median T_{soil} and the 2.5% and 97.5% quantiles for T_{soil} . We chose quantiles as estimates
158 of minimum and maximum T_{soil} to exclude potential measurement errors and extremes.

159 SCAN/SNOTEL sites were established to support natural resource assessments.
160 To determine whether sites were relevant to squamate reptiles, we downloaded and
161 cleaned squamate locality records (<https://www.gbif.org>). T_{soil} sites are close to known
162 squamate localities (see legend Fig. S5), but some regions with high species density of
163 squamates (e.g., southeast US) are under sampled.

164 We used a linear model (gls) to analyse how minimum T_{soil} varied with latitude,
165 elevation, and depth. We accounted for spatial autocorrelation of residuals by including a
166 spatial error structure (Zuur *et al.* 2009). After examining residuals, we substituted
167 log(depth) for depth and checked for normality, homogeneity, and spatial autocorrelation.
168 Because the 3-way interaction was significant (*p*-value and AIC scores), model
169 simplification was unnecessary. The model was fit (gls) by the R package ‘nlme’
170 (Pinheiro *et al.* 2020). All statistics were generated via R version 3.6.2 (2019-12-12).

171 **Results (soil temperatures)**

172 Monthly variation in minimum and in maximum T_{soil} (September – April) versus
173 depth are depicted for a random sample of 25 sites (Fig. 3A,B): each line connects
174 minimum or maximum T_{soil} with depth for one site. In most months and sites, the coldest
175 and warmest T_{soil} are both at shallow depths. In mid-winter, gradients of minimum T_{soil}
176 with depth are relatively steep; and warmest T_{soil} are deep.

177 The coldest T_{soil} were usually at -5 cm in all months (between 52.0 and 93.9% of
178 sites), except June and July, when coldest T_{soil} were at -100 cm (Table S2A). The
179 warmest T_{soil} were also at -5 cm in all months (between 49.1 and 90.9% of sites) except
180 November through January, when the warmest T_{soil} switched to -100 cm (Table S2B).
181 Not surprisingly, T_{soil} was least variable (inter-quartile range, IQR) at -100 cm in all

182 months (between 34.9 and 93.1% of sites, Table S2C); and T_{soil} was most variable at -5
183 cm in all months (between 59.8 and 93.9% of sites, Table S2D).

184 Temporal constancy of T_{soil} increased with depth. The average absolute difference
185 in median T_{soil} (at each site, depth) for January 2017 versus January 2018 was inversely
186 correlated with depth (-5 cm = 1.17°C, -10 cm = 1.21°C, -20 cm = 1.13°, -50 cm =
187 0.89°C, -100 cm = 0.60°C; $r = -0.993$, $P = 0.0007$, Pearson correlation, 2-tailed). Thus,
188 between-year, within-year (Fig. 1) and within-month (Table S2D) variation in T_{soil} was
189 greatest at shallow depths.

190 In the spatial model, minimum T_{soil} declined with latitude and elevation, but
191 increased with depth. Several interactions were significant (Table 1). The negative effect
192 of latitude weakened with elevation, and the negative effect of elevation weakened with
193 depth, with significant and negative 3-way interaction between depth, latitude, and
194 elevation). The implications of these patterns will be discussed in the topic-specific
195 sections (below).

196 **Question I. Which depth minimizes risk of cold injury or death?**

197 Cowles (1941) expected that the risk of cold injury or death would be highest at shallow
198 depths. At many sites (Fig. 3A), T_{soil} just below the ground surface indeed dropped below
199 freezing in winter. However, predicting whether shallow squamates are at risk from cold
200 requires knowing which T_b are damaging or lethal. Specifying such temperatures proved
201 problematic because traditional assays are of questionable relevance to risk in winter
202 (below).

203 **Methods (cold risk)**

204 In traditional cold tolerance assays (e.g., CT_{min}), squamates are cooled quickly until their

205 righting response is lost (Andersen *et al.* 2015; Bennett *et al.* 2018). Another assay
206 measures survival over hours or days, but typically after sudden drop to one or more
207 fixed T_b (e.g., Heatwole *et al.* 1969; Gregory 1982; Burke *et al.* 2002; Storey 2006; but
208 see Huang & Tu 2008; Olson *et al.* 2013; Berman *et al.* 2016).

209 In nature, T_b of squamates underground will not drop suddenly in autumn but
210 rather drop slowly over months, especially near the surface (Figs. 1B, S6). Traditional
211 assays (above) fail to approximate natural cooling patterns (Sinclair 2001a) and thus
212 their relevance to overwinter cold risk is at best correlational (Andersen *et al.* 2015).
213 Accordingly, we used three acute measures in an attempt to bracket cold risk.

214 **Critical Thermal Minimum:** CT_{min} is the most common index of cold tolerance
215 (Cowles & Bogert 1944). We compiled CT_{min} data for 40 species of North American
216 lizards (Grigg & Buckley 2013; Muñoz *et al.* 2014; Muñoz *et al.* 2016) and used the
217 median as a baseline CT_{min} (11.2 °C, range 2.2 to 15.5 °C). However, CT_{min} often varies
218 geographically (van Berkum 1988; Araújo *et al.* 2013; Sunday *et al.* 2014):
219 consequently, we computed a geographically adjusted CT_{min} by regressing CT_{min} on
220 absolute latitude and elevation (see **Supplemental Methods: Details**). The best-fitting
221 model (by AIC) was $CT_{min} \sim 17.69 (\pm 1.333) - 0.22 (\pm 0.049) \times \text{abs}(\text{latitude}) - 0.63 (\pm$
222 $0.224) \times \log(\text{elevation, m})$, with variance structure not added to elevation. We calculated
223 the percentage of time each month (and over winter) when temperatures at each depth
224 dropped below base or adjusted CT_{min} .

225 **Freezing point:** we calculated the percentage of time in each month in which
226 T_{soil} at each depth was below -0.63 °C, which is the median temperature at which a
227 sample of 23 species of North American lizards froze (Lowe *et al.* 1971). These

228 temperatures have a narrow range (-0.7 to -0.3 °C) and are uncorrelated with latitude ($r =$
229 -0.057, $P = 0.796$). Exposure to freezing temperatures for a few hours or days is likely
230 lethal (see table 1 in Storey 2006), but several *Zootoca vivipara* from France survived
231 temperatures of -0.8 to -3.5 °C for three weeks (Costanzo *et al.* 1995), and one *Z.*
232 *vivipara* from Siberia survived -10 °C for 34 days (Berman *et al.* 2016). Because
233 repeated cold exposures can be damaging (Sinclair 2001a; Marshall & Sinclair 2012,
234 2018), we calculated the number of times T_{soil} dropped below -0.63 °C as well as the
235 single longest run (hours).

236 **Supercooling point:** we calculated the percentage of time when T_{soil} at each
237 depth were below -6.0 °C, which is the median supercooling temperature for 23 species
238 of lizards (Lowe *et al.* 1971). Exposure to T_{soil} at or below this level should be lethal (see
239 table 1 in Storey 2006). Supercooling temperatures have a narrow range (-7.2 to -3.9°C)
240 and are uncorrelated with latitude ($r = 0.344$, $P = 0.1078$), as is the case 12 populations of
241 lab-reared *Uta stansburiana* (Michels-Boyce & Zani 2015).

242 **Results (cold risk)**

243 Although CT_{min} is the most commonly measured cold-tolerance index of reptiles, T_{soil} in
244 January dropped below the median lizard CT_{min} (11.2°C) at least once at -5, -20, and even
245 -30 cm at all sites and even at -50 and -100 cm at most sites (Table S4A). The pattern is
246 similar though less extreme for CT_{min} adjusted for latitude and elevation (Table S4B). At
247 -5 cm, 64.3% of sites experienced below-freezing temperatures (Table S4C), and 17.4%
248 experienced below-supercooling temperatures (Table S4D).

249 Two-thirds of sites at -5 cm had below-freezing runs of T_{soil} at least once over
250 winter (Table 2). The median and maximum number of cold runs occurred at -5 cm, but

251 declined with depth, as did the median length of the longest freezing event (Table 2). The
252 single longest below-freezing run (-5 cm) was 131 days (Crow Creek, WY, 2539 m).

253 Geographic patterns in the proportion of hours with below-freezing temperatures
254 by depth in January (coldest month) are plotted in Fig. 4. Many southern sites never
255 experienced below-freezing temperatures at any depth (red dots, Fig. 4). At northern and
256 montane sites, freezing was common at shallow depths. Freezing was uncommon at -50
257 cm, and did not occur at -100 cm.

258

259 **Discussion (cold risk)**

260 Cowles (1941) predicted that reptiles overwintering at shallow depths potentially
261 face cold injury or death. Not surprisingly, T_{soil} – especially at shallow depths – are often
262 low (Figs. 1, 2, & 3, Table S4), especially at high latitudes and elevations (Table 1, S4).
263 But are those T_{soil} low enough to cause physiological stress or death?

264 CT_{min} is the most common cold-tolerance assay. However, almost all sites and
265 depths experienced T_{soil} below the median CT_{min} of lizards during winter (Table S4A),
266 and most still did so even with site-adjusted CT_{min} (Table S4B). Squamates live near most
267 sites (Fig. S5) and thus may regularly experience below CT_{min} temperatures in winter.
268 CT_{min} thus appears to be of limited direct relevance to cold survival or to geographic
269 range limits (Lowe *et al.* 1971; Huang & Tu 2008). If squamates tolerate near freezing
270 temperatures, they must over winter at or below -20 cm at most sites (Fig. 3, Table S4C).
271 If they survive to near super-cooling temperatures (Tables S4C,D), they can survive at
272 most sites – except perhaps at very shallow depths in the coldest sites (Tables S4D).

273 ‘*Unknowns*’ for cold tolerance. — The question “how cold is ‘too cold’ for

274 reptiles?” cannot presently be answered, because no ecologically relevant protocol and
275 assay exists. CT_{\min} is the most common assay (Lutterschmidt & Hutchison 1997; Bennett
276 *et al.* 2018), but the median cooling rate for 51 CT_{\min} studies with squamates is -1.0
277 °C/min (Bennett *et al.* 2018) – roughly four orders of magnitude faster than natural
278 cooling rates (Fig. 1B, S6; Sinclair 2001b). At -50 cm at Ford Dry Lake (Fig. 1B), for
279 example, T_{soil} drops only about 11.8 °C from October through December. At -20 or -10
280 cm, T_{soil} also drops slowly but fluctuates daily (Fig. 1B). Thus, rapid and monotonic
281 patterns of CT_{\min} assays bear no resemblance to natural cooling patterns and thus yield
282 point estimates of uncertain relevance to overwinter survival or cold injury.

283 A less common protocol involves scoring survival (or length of survival) after
284 sudden transfer to one or more low and fixed T_b (e.g., Storey 2006; but see Huang & Tu
285 2008; Olson *et al.* 2013). Such “drop and hold” assays (“response surfaces”) are
286 experimentally tractable and are probably more relevant to natural cold tolerance than is
287 CT_{\min} (e.g., Huang & Tu 2008; Olson *et al.* 2013), but still do not match the chronic and
288 fluctuating T_b profiles experienced by organisms in nature (Fig. 1B, S2 & S6, Sinclair
289 2001a; Marshall & Sinclair 2012, 2015). Do such temporal discrepancies render
290 traditional assays unreliable for predicting overwinter survival? That is unknown. We
291 suspect that drop and hold assays underestimate natural cold tolerance, as natural and
292 slow cooling offer ample time for acclimatization. This issue may never be resolved for
293 squamates, as survival experiments raise ethical issues.

294 What new protocols might be suitable? We do not see a simple solution. A full
295 factorial design that incorporates fluctuating and shifting temperatures (Fig. 1B, S6),
296 varying exposure duration, and repeated cold-exposure bouts – and does so for multiple

297 populations and species – is logistically challenging (Sinclair 2001a; Huang & Tu 2008;
298 Marshall & Sinclair 2015, 2018) and potentially feasible only with insects or micro-
299 organisms. Moreover, any derived statistical model will be descriptive and not predictive
300 for other sites and perhaps other depths. A more pragmatic starting point would be to try
301 to determine whether cold tolerance estimates from ‘drop and hold’ or even ‘slow drop’
302 experiments (Huang & Tu 2008) are acceptably close to those derived from natural
303 patterns of cooling thermal profiles (Figs. 1B & S6) (Marshall & Sinclair 2015, 2018).

304 Perhaps asking what ‘temperature’ is damaging or lethal is an ‘inappropriate
305 question’ (sensu Hertz *et al.* 1993) because no single temperature likely exists. Lethal and
306 damaging temperatures might shift over winter and depend on each individual’s
307 idiosyncratic exposure, age (Kingsolver *et al.* 2011), and physiological state. But how
308 much individual (Bennett 1987) and season variation (Layne *et al.* 1985; Hu & Appel
309 2004; Pingor *et al.* 2016) is rarely known, even at a single site.

310 Rather than ask what temperature is lethal, one can turn the question around and
311 ask what depths are damaging and do those depths vary geographically and
312 interspecifically? Here two approaches seem feasible. First, subject sets of organisms in
313 the laboratory to empirical T_{soil} profiles measured over winter at different sites and depths
314 (e.g., Fig. 1B, S6) and later score survival or fecundity. Alternatively, bury organisms at
315 various depths and sites, monitor their thermal profiles, and score their survival in spring
316 (Tucker & Packard 1998; Kevin Roberts, personal communication); and then determine
317 whether traditional acute measurements of cold tolerance predict overwinter survival.
318 Either approach would illustrate how deep an organism needs to go to survive at a given
319 site, but any conclusions would be descriptive and applicable only locally. Again, such

320 survival experiments are feasible only with invertebrates or microorganisms.

321 It has been eight decades since Cowles asked how deep an ectotherm must
322 overwinter to reduce the risk of cold injury or death. In our view, that question is still
323 unanswered primarily because traditional cooling protocols yield unvalidated predictions
324 of cold tolerance in nature. Ecologically relevant protocols are essentially ‘unknown
325 unknowns,’ and will be logistically challenging to derive and implement.

326

327 **Question II. What depth maximizes the opportunity for activity** 328 **on warm days in winter?**

329

330 Cowles (1941) knew that some reptiles in California deserts would become active on
331 warm winter days. But how might buried reptiles detect whether a given day was in fact
332 sufficiently warm for activity? Cowles noted that the heat pulse moving slowly down into
333 the soil (Smith 1929) on sunny days could serve as a reliable cue of warm conditions on
334 the surface, but only if reptiles were shallow. To explore his prediction, we used the
335 microclimate model (Kearney & Porter 2017) and the steady state heat budget model in
336 NicheMapR (Kearney & Porter 2019) and predicted T_{soil} , potential activity times, and
337 metabolic rates for lizards (20-g) that had retreated underground at fixed depths
338 (below) at a California desert site (Ford Dry Lake, 33.7°N, -115.1°W). [Complete R code
339 in Supplement.]

340 **Methods (activity)**

341 We estimated hourly T_{soil} at multiple depths (2.5, 5, 10, 15, 20, 30, 50, 100 cm)
342 plus above-ground operative temperatures (T_e) for winter 2017-2018, but for 0% and 90%

343 shade. [Input parameter values are in Table S8.] We then used the ‘ectotherm’ function of
344 NicheMapR (Kearney & Porter 2019) to compute potential T_b of squamates active on the
345 surface and to determine potential activity time, contingent on minimum threshold
346 temperature for activity plus a cue for emergence (below). Because cues that squamates
347 use to emerge in mid-winter are unknown (but see Heath 1962; Bishop & Echternacht
348 2004), we assumed that squamates would emerge only if (1) their T_b in their retreat was
349 above an arbitrary temperature threshold $T_{RB,min}$ (20 °C) and (2) their T_b was increasing
350 by at least 0.1 °C h⁻¹. Alternative thresholds and emergence models (Heath 1965) can be
351 explored.

352 Once on the surface, a squamate was simulated to forage, if its T_b was greater than
353 minimum foraging temperature $T_{F,min}$ (35.0 °C) and below the maximum foraging
354 temperature $T_{F,max}$ (43.0 °C). To achieve its preferred body temperature ($T_{pref}=39.0$ °C)
355 and to avoid overheating, the animal then shuttled between 0% and 90% shade. Once
356 potential T_b in the open was greater than T_{pref} , shade was incremented in 3% increments
357 to achieve T_{pref} up to the maximum allowed shade level of 90%, at which point the value
358 of T_{pref} was incremented until it reached $T_{F,max}$. When surface conditions were too hot or
359 cold, the animal was simulated to retreat either to a fixed depth or (for ‘Panglossy’
360 ectotherms, see below) could chose a depth that avoid extremely high [$T_{F,max} + (CT_{max} -$
361 $T_{F,max}) / 2$] or cold (freezing point of -0.63 °C, above) temperatures.

362 Metabolic rates were estimated as functions of T_b (Andrews & Pough 1985) for
363 each hour and summed for the winter (December to February) for simulations assuming
364 that lizards stayed at a fixed depth versus became active whenever possible. Activity was
365 summed from November to March.

366

367 **Results (activity)**

368 Squamates overwintering at shallow depths at Ford Dry Lake are potentially active many
369 more days in winter than are those at deep retreats (Figs. 5), as Cowles (1941) predicted.
370 Predicted total activity time declines from 603-h at -2.5 cm to 9-h at -30 cm (November-
371 March, Fig. 6A). At -50 or -100 cm, activity ceases because the warming signal does not
372 penetrate to those depths.

373 Cumulative energy cost at Ford Dry Lake is non-linearly related to depth and
374 activity. Cost is high near the surface (-2.5 or -5 cm, probably reflecting high T_{soil} on
375 warm days at this southern site), declines at moderately shallow depths (-10 to -30 cm,
376 Fig 6B), and then increases with depth. Squamates using depths shallower than about -30
377 cm can become active and have high T_b on suitable days (Fig. 5): as a result, they have
378 higher energy costs than do inactive ectotherms using the same fixed depths (Fig. 6B).
379 For example, a squamate that is active on warm days but otherwise is at -10 cm will
380 expend 2.3X more energy per winter than an inactive squamate at -10 cm. This higher
381 energy costs associated with activity suggests that surface-active squamates may need to
382 feed in winter, or risk depleting energy stores by spring emergence (see below).

383 Ford Dry Lake Depth is a relatively warm site, but very shallow squamates there
384 would still face cold risks. Squamates at -10 cm or shallower would experience below-
385 CT_{min} temperatures (Fig. 6C), and those at -2.5 cm would experience multiple below-
386 freezing events (Fig. 56D). At colder localities (Fig. 3, Table S4), these risks at shallow
387 depths will of course be elevated. The trade-off Cowles (1941) predicted is supported:

388 shallow retreats permit activity but nonetheless increase risk of exposure to cold
389 temperatures (Fig. 6).

390 **Discussion (activity)**

391 Any attempt to simulate mid-winter activity requires an environmental cue, a
392 sensory capacity, and a behavioural model. All are unknown for squamates. Field studies
393 are needed determine cues used by squamates, and neurophysiological studies can
394 evaluate organismal sensitivities to changing T_b .

395 Cowles (1941) assumed that a squamate would emerge if warming T_{soil} raised its
396 T_b above an unspecified threshold level. Our simulations used a similar approach
397 (threshold plus warming cue). Other models (or thresholds) are possible: for example,
398 emergence could be triggered by a temperature-compensated circadian clock (Heath
399 1962). However, a circadian cue might be reliable in summer, but probably not in winter,
400 when many days would be too cold for activity, at least at higher latitude and altitude
401 sites. In any case, the likely effectiveness of a circadian cue can be tested alone or in
402 combination with a behavioural model (e.g., at a given time each day, the squamate
403 moves to near the surface to evaluate conditions before deciding whether to emerge).

404 Why some squamate even emerge mid-winter is essentially unknown. Some will
405 feed (R. B. Huey and E. R. Pianka, unpublished, Congdon *et al.* 1979; Goldberg & Bursey
406 1990), but others are anorexic (Mayhew 1964). For those that feed, digestion (requires
407 warm temperatures) becomes a challenge (Question 3, below). For those that are active
408 but do not feed, their energetic expenses are increased (Fig. 6B). Presumably
409 physiological benefits override energetic costs and perhaps facilitate growth or

410 preparation for reproduction -- if reserves are sufficient. In insects, brief warming re-
411 establishes ion gradients, foraging, and removes metabolic toxins (reviewed in Lee 2010).

412 The why and how of winter activity are open areas for research. Field
413 observations are needed to explore geographic and interspecific variation winter activity
414 and in feeding, as well as predation risks while active (Wilson & Cooke 2001).

415 Physiological studies are needed to determine whether feeding is necessary in some but
416 not all regions and to clarify physiological benefits and costs associated with mid-winter
417 emergence (Stieler *et al.* 2006; Lee 2010; Zani *et al.* 2012).

418 We have focused on winter retreats, but risk of overheating will be an issue in
419 shoulder and summer months. Are optimal depths different in summer than winter?
420 Based on our simulations for Ford Dry Lake, a 20-cm depth seems suitable for both
421 summer (not too hot) and winter (not too cold). Some reptiles shift macro-habitats
422 between summer and winter (Christian *et al.* 1983; Kearney 2002), and high-latitude ones
423 often migrate to hibernacula (Parker & Brown 1974; Gregory 1982; Norberg & Cobb
424 2017). *Uta* in central California use the same retreat sites in summer and winter (B.
425 Sinervo, personal communication); but those in central Oregon move from desert scrub
426 habitat to south-facing rocky outcrops (P. Zani, personal communication).

427

428 **Question III. What depth minimizes energy expenses or**
429 **maximizes digestion?**

430 Cowles (1941) did not discuss energetics. However, we consider how T_{soil} at fixed depths
431 affects cumulative (maintenance) energy expenses. For ectotherms that feed on warm

432 days, we estimate the depth that maximizes digestion rate. Finally, we evaluate whether
433 being able to shift depths conserves energy relative to staying at one depth.

434 **Methods (energy, digestion)**

435 We used a metabolic temperature equation ($M \times T$) tuned for reptiles (Andrews & Pough
436 1985), converted T_{soil} to estimates of hourly metabolic rates for a 20-g lizard, and then
437 summed over time intervals. [Mean Q_{10} was 2.9 for a sample of lizard species (with at
438 least one temperature below 15°C and thus relevant to winter), and variation was
439 substantial (Table S3).]

440 We assumed that $M \times T$ relationships are geographically stable. For insects,
441 interspecific $M \times T$ relationships do steepen with latitude (Irlich *et al.* 2009), but mainly
442 at latitudes above the continental USA (M. E. Dillon, personal communication). $M \times T$ of
443 reptiles often shift (positively, negatively, or not) from active to winter periods (Halpern
444 & Lowe 1968; Aleksuik 1976; Patterson & Davies 1984; Tsuji 1988; Angilletta 2001b;
445 de Souza *et al.* 2004), but here we assume they are static within winter. When computing
446 energetic costs at each site, we excluded depths where T_{soil} dropped below freezing (-
447 0.63°C) during the sample period (month or winter), as ectotherms there would not likely
448 survive (Storey 2006).

449 Next, we relaxed the restriction that an ectotherm stayed at fixed depth and
450 allowed it to move every hour to the depth that will have the lowest T_{soil} – and thus the
451 lowest metabolic costs – in the next hour. However, we excluded depths at which T_{soil}
452 dropped below CT_{min} , simply because a lizard there be immobilized. This scenario
453 represents the optimality concept of a “Panglossy” ectotherm – one that is always in the
454 best place (Huey *et al.* 1989). We then determined how much energy a Panglossy

455 ectotherm would save compared with an ectotherm that stays at a fixed depth. We ignore
456 any costs of moving (Huey *et al.* 1989), which must be high for ectotherms moving
457 through soil (Vleck 1979), though less so if in a burrow.

458 Some reptiles feed in winter and thus require elevated temperatures to digest that
459 food (Congdon *et al.* 1979; Angilletta 2001a, R. B. Huey and E. R. Pianka, unpublished).
460 To determine how depth would affect digestion rate, we modified an equation for gut
461 passage time (hours) versus body temperature for *Sceloporus undulatus* (from table 1, in
462 Angilletta 2001a): $1/(-20.59 \times T_{\text{soil}} + 0.26 \times T_{\text{soil}}^2 + 428.85)$. We then summed hourly
463 digestion rates by month and determined the depth that maximized that sum, again
464 contingent on T_{soil} not dropping below -0.63°C (lethal, see above). [For simplicity, we
465 assumed that a lizard that captured food would immediately retreat underground to a
466 fixed depth and not emerge to bask on subsequent days.]

467 **Results (energy, digestion)**

468 *Cumulative energy costs at fixed depths.*— During cool months (October through
469 February), cumulative energy costs were generally lowest at -5 cm at many sites (\geq
470 39.3% of sites, Table S5A); but during warm months (March - September), when near-
471 surface temperatures are warm, cumulative costs were generally lowest at -50 or -100 cm
472 (Table S5A). In contrast, cumulative costs were generally highest at -100 cm in cold
473 months (October – March), but highest at -5 cm in warm months (April -
474 September)(Table S5B).

475 When cumulative energy costs were calculated over one or several months, they
476 were still lowest at -5 or -10 cm at most sites (Table 3). At many cold sites, however,
477 cumulative rates were lowest deeper in the soil, because -5 or -10 cm depths would likely

478 be lethal (Table 2).

479 *Cumulative metabolism of a 'Panglossy' ectotherm.*—Ectotherms that can change
480 depth every hour generally had lower costs than those at fixed depths (Table S6, Fig.
481 S7A). The percentage saving was very small [median saving 1.9% ($Q_{10} = 2.0$) to 3.4%
482 ($Q_{10} = 3.0$)]. Panglossy saving is small because winter energetic costs were generally
483 lowest at shallow depths (see Table 2). Moreover, Panglossy ectotherms would have to
484 make many moves totalling long distances over winter (Fig. S7B,C). For our sites, the
485 median number of depth changes was 187 (range 0 to 717). The median and maximum
486 cumulative (vertical) distances moved were 30.8 m and 149 m respectively. Were the
487 energetic costs of movement included, a Panglossy strategy is unlikely to be as
488 energetically advantageous as a fixed-depth one.

489 *Single site analysis.*--Here we contrast patterns a Panglossy vs. fixed-depth
490 strategy at Ford Dry Lake, California (33.65°N, -115.10°W, 120 m). We used
491 NicheMapR (Kearney & Porter 2017) to simulate hourly T_{soil} at eight depths (-2.5 to -100
492 cm) for two habitats (30% or 60% shade) (15 October 2013 – 15 March 2014).
493 Estimated T_{soil} profiles (Fig. 7A,F) are coded red for T_{soil} warmer than CT_{min} (arbitrarily
494 9.3°C), and blue for T_{soil} colder CT_{min} . We also estimated the depth with the lowest
495 energy costs each hour (Fig. 7B,D) and the cumulative energy costs (Fig. 7C,E).

496 Daily and seasonal variation in T_{soil} at fixed depths is shown in Fig. 7A and F.
497 Mid-winter temperatures are much colder in 60% shade (than in 30% shade) and
498 frequently drop below 9.3 °C (arbitrary CT_{min}). In 30% shade in mid-winter, Panglossy
499 squamates would achieve lowest T_b and thus minimize daily costs by shuttling between
500 shallow depths (night) and to -30 cm (day)(Fig. 7B), thus avoiding warm near-surface

501 T_{soil} during the day (Fig. 7A). In 60% shade, however, Panglossy squamates would often
502 need to go deep to find temperatures warmer than CT_{min} (Fig. 7D).

503 In 30% shade, a Panglossy strategy conserved considerable energy relative to that
504 of overwintering at fixed depths (Fig. 7C). [As above, we ignore costs of movement.] In
505 60% shade, however, a Panglossy strategy was paradoxically more expensive than fixed-
506 depth strategies. To be able to continue to move, a Panglossy ectotherm had to select T_{soil}
507 warmer than CT_{min} (9.4 °C), thus raising metabolic rate, whereas the only constraint on
508 fixed-depth ectotherms was that T_{soil} never dropped below freezing, which it never did in
509 30% shade and did only for two hours at 60% shade.

510

511 *Digestion.*-- Some squamates that are active on warm winter days may capture food
512 (Mayhew 1964R. B. Huey and E. R. Pianka, unpublished; Congdon *et al.* 1979; Goldberg
513 & Bursey 1990) and will need high T_b for digestion (Angilletta *et al.* 2002): this will
514 basking (Norberg & Cobb 2016) or moving to a warm retreat. Consequently, we
515 calculated the depths at which digestive rates (see above) were highest (Table S7) by site
516 and by month, excluding depths where T_{soil} dropped below freezing (above). We assumed
517 a squamate with food would move to (then stay at) a depth until digestion was
518 complete. [Alternatively, a spectral analysis of surface T_e would inform the possibility of
519 being able to bask on the surface in subsequent days.]

520 In cold months (October-February), digestion rates were maximal at -100 cm at
521 almost all sites (Table S7), reflecting relatively warm T_{soil} at depth. In warmer months
522 (April - September), however, digestion rates were instead maximal at - 5 cm (Table S7),

523 except at hot sites, where T_{soil} at -5 cm would too hot (above CT_{max}), forcing lizards to
524 have deeper retreats).

525 **Discussion (energy, digestion)**

526 For individuals that do not feed in winter, a depth with cold (but not lethally cold)
527 temperatures minimizes cumulative energy costs and thus reduces starvation risk and
528 maximizes energy reserves on emergence in spring. Predicting optimal depth is
529 challenging because $M \times T$ relationships are non-linear (Ruel & Ayers 1999) and because
530 near-surface depths have both the lowest and highest temperatures (Fig. 3). Our
531 simulations suggest that cumulative energy costs (October- February) were generally
532 lowest at shallow depths (Table 2), though not at every site (Fig. 6). Shallow depths can
533 be warm during the day, but winter days are short and often inclement. Nevertheless,
534 ectotherms at very cold sites will need to move deeper to avoid cold injury or death
535 (Table 2B, Fig. 4) (Tucker & Packard 1998).

536 For Panglossy ectotherms at Ford Dry Lake (Fig. 7), the depth that minimizes
537 cumulative metabolic costs changes seasonally and even within a 24-h day (Fig. 7B,D).
538 Moreover, Panglossy ectotherms using retreats with different shading will use very
539 different depths (Fig. 7B,D). Over the entire winter, Panglossy ectotherms can generally
540 save energy by moving to the coolest (but $> CT_{\text{min}}$) depth, but the magnitude is generally
541 small (median saving = 1.9% to 3.4%, see Supplementary Table S6), and this small
542 benefit would likely be swamped if the cost of movement were deducted (Wu *et al.*
543 2015). Paradoxically, a fixed-depth strategy can potentially result in lower energy costs
544 over winter than does a Panglossy strategy, primarily because Panglossy ectotherms must
545 restrict themselves to depths warm enough to permit movement (Fig. 7E), whereas fixed-

546 depth ectotherms can benefit energetically if T_{soil} drops below CT_{min} but stays above the
547 freezing point.

548 For winter-active ectotherms that feed, digestion will require warm temperatures.
549 Depths that enhance digestion in winter are typically deep (Table S7), simply because
550 that is where temperatures are warmest (Fig. 1, Table S2B). We will return to this in the
551 CONCLUSIONS section.

552 ‘*Unknowns*’ for metabolism.— We used a $M \times T$ relationships tuned for reptiles
553 (Andrews & Pough 1985), and assumed that $M \times T$ relationships were fixed and thus
554 independent of latitude, altitude, and time of winter. However, $M \times T$ curves can shift
555 geographically (Irlich *et al.* 2009) and seasonally. For example, $M \times T$ curves of winter-
556 active species are sometimes elevated (“compensation,” see Tsuji 1988), but those of
557 winter-inactive species are often lowered (“reverse acclimation,” Patterson & Davies
558 1978; Tsuji 1988). Incorporating known sources of variation (seasonal, ontogeny, local
559 adaptation, drift) in simulations is feasible, but only if actual patterns are known. They
560 are not.

561 What is an ecologically realistic protocol in the laboratory for estimating
562 cumulative energetic expenses during winter? To our knowledge, one does not exist. A
563 realistic one should mimic specific conditions hibernating ectotherms experience
564 underground. Therefore, temperature profiles should drop very slowly and incorporate
565 fluctuations (Figs. 3, S6) and not follow traditional ‘drop and hold’ exposures. Short
566 photoperiods might seem appropriate, but ectotherms underground will actually be in full
567 darkness (unless they emerge during the day), as light rarely penetrates even 5 mm into
568 the soil (Tester & Morris 2006). Housing conditions should allow ectotherms to bury

569 themselves in the soil, and $M \times T$ relationships should be measured multiple times over
570 winter. Thus, traditional laboratory protocols, which are designed to partition
571 thermodynamic from acclimation effects (Havird *et al.* 2020), do not match natural
572 environmental exposures and are thus are questionable for simulating overwinter
573 expenses.

574 For a field approach, one could release animals into field enclosures in the
575 autumn, allow the them to bury themselves or bury them at fixed depths (Tucker &
576 Packard 1998), if one were interested in depth effects. One could dig them up at intervals
577 and acutely measure their $M \times T$ (Kevin Roberts, personal communication).

578 A very different approach would be to simulate whether known variation in M
579 $\times T$ is large enough to alter designation of optimal depths or even to compute how large
580 $M \times T$ shifts would have to be to alter conclusions about optimal depths (thus a
581 sensitivity analysis). Ideally such calculations should be made in context of the complete
582 energy budget across the whole life-cycle and its consequences for time to maturity and
583 reproduction (Kearney 2012; Levy *et al.* 2016; Schwarzkopf *et al.* 2016).

584 ‘*Unknowns*’ for winter activity.— Cowles (1941) observed that some reptiles are
585 active on warm days in winter, but whether winter activity and feeding vary
586 geographically is known for few widespread species. *Sceloporus occidentalis* are winter
587 active in southern California but not in Washington (Tsuji 1988). Low-latitude
588 populations of *Uta stansburiana* can be active in winter (Wilson & Cooke 2001) and will
589 feed (B. Sinervo, personal communication), whereas higher-latitude ones can be active
590 (Wilson & Cooke 2001) but do not feed (P. Zani,, personal communication). Winter
591 feeding can be assayed by field observations or retrospectively by examining gut contents

592 of winter-captured individuals (e.g., in museum collections). Alternatively, one could test
593 whether individuals observed active in nature accept food when offered.

594 Reptiles that are active and basking in winter – but not feeding – will of course
595 deplete metabolic reserves (Case 1976), and thus presumably must gain compensatory
596 benefits (e.g., establishing physiological homeostasis, priming reproduction) (Stieler *et*
597 *al.* 2006; Lee 2010; Zani *et al.* 2012) or reduce risk of freezing from food particles in guts
598 that can catalyse ice formation (Bale 2002). Little is known about such benefits and
599 whether those benefits vary ontogenetically, geographically, and interspecifically in
600 squamates. In any case, if non-feeding emergence and basking is beneficial to some
601 species and populations, why is it not beneficial to all? The occurrence and physiological
602 consequences of activity versus inactivity and feeding versus anorexia in winter are
603 essentially ‘unknown unknowns’ and thus offer diverse opportunities for field and
604 laboratory research.

605 **Concluding remarks**

606 Winters present ecological and physiological challenges for ectotherms in cold
607 environments. Our project was inspired by Raymond B. Cowles’s (1941) novel insights
608 on the winter biology of ectotherms. We explored three basic questions involving how
609 overwinter depth affects risk of cold death, metabolic expenses, and opportunities for
610 activity. We used a simulation approach to address these questions. In every case,
611 however, we discovered that the field and laboratory data necessary to ‘map’ T_{soil} onto
612 physiological and ecological consequences – as well as to test our predictions – are
613 inappropriate or even non-existent. In winter biology, there are both ‘known unknowns’
614 plus ‘unknown unknowns.’ Accordingly, we outline a research agenda for the winter

615 biology of ectotherms (Box 1). This agenda is not exhaustive but focuses specifically on
616 issues germane to our paper's themes. In all cases we encourage studies that incorporate
617 seasonal, ontogenetic, geographic, and interspecific variation.

618 Implementing this agenda will be challenging and sometimes impossible for
619 ethical reasons. But some solutions are evident. The technique that Cowles (1941; fig.
620 S3) used to find reptiles in winter was opportunistic (see also Broadley 1972; DeNardo *et*
621 *al.* 2018). However, body temperature and depth data can be estimated by implanting
622 (attaching) temperature-sensitive loggers in ectotherms prior to hibernation and decoding
623 them after emergence. If other temperature recorders are placed in likely retreat sites
624 (depths), the overwinter positions and movements of individuals can be inferred. Above-
625 ground activity can be indicated by rapid T_b shifts (Harris *et al.* 2015), or from
626 dataloggers that record light level (Davis *et al.* 2008). These are indirect methods, but
627 they should be accompanied by direct natural-history observations in winter.

628 As regards physiological studies of metabolism and of cold tolerance, we have
629 argued (above) that acclimation regimes and conditions should approximate natural ones:
630 in contrast, traditional 'drop and hold' protocols do not match the dynamic thermal
631 profiles seen in nature (Fig. 1, S6, Sinclair 2001b). We appreciate that these designs may
632 be feasible primarily invertebrates (e.g., *C. elegans*, arthropods), especially the cold
633 tolerance assays.

634 A major puzzle involves animals that are active on warm days but do not feed
635 (above). Does activity (and elevated T_b) enable active ectotherms to clear accumulated
636 and toxic by-products (Stieler *et al.* 2006), recover from infections (Harris *et al.* 2015),

637 prime reproductive capacity in anticipation of spring, or readjust metabolic stores (e.g.,
638 replenish glycogen, Zani *et al.* 2012)?

639 Similarly, experiments are needed to evaluate what cues prompt mid-winter
640 activity. Are animals using a temperature-compensated clock (Heath 1962), or a
641 threshold temperature (above), a change in sign in the derivative of T_b vs time (Heath
642 1965), or some combination thereof? Do cues vary geographically? Critical experiments
643 remain to be done, and simulations studies can guide experiments.

644 On a personal level, we concede that we have focused our own field studies on the
645 thermal biology of ectotherms in warm seasons. But despite our personal ‘dormancy’
646 from the field in winter, ectotherms in nature experience winter – sometimes long
647 winters. As others have argued, the overwinter ecology of ectotherms offers rich
648 opportunities for exploration, including impacts of climate change (Bradshaw &
649 Holzapfel 2006; Bradshaw & Holzapfel 2009; Williams *et al.* 2014).

650 In concluding, we return to Cowles (1941). Almost eight decades after its
651 publication, this paper is remarkably contemporary. To us it is an early classic in
652 behavioural and physiological ecology, as well as an early example of trade-off thinking
653 in ecology. But according to Web of Science (accessed 2020-04-22), this paper has been
654 cited only 54 times. None of those citing papers draws attention to Cowles’s recognition
655 of trade-offs involving overwintering depth. This oversight needs to be corrected.

656

657 **Acknowledgements**

658 We dedicate this paper to the memory of Raymond B. Cowles, a pioneer in thermal
659 biology (Turner 1984).

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939 **Table 1** Estimated effects of latitude, elevation and soil depth on minimal soil
 940 temperatures during winter according to the gls model.

Coefficients	Value	Std. error	t-value	P
Intercept	26.1647	2.2104	11.8371	< 0.0000
Latitude (°N)	-0.7025	0.0598	-11.7412	< 0.0000
Elevation (m)	-0.0169	0.0024	-71749	< 0.0000
Depth (cm)	0.1040	0.0433	2.4024	0.0167
Latitude:Elevation	0.0004	0.0001	6.7943	< 0.0000
Latitude:Depth	-0.0005	0.0012	-0.4399	0.6602
Elevation:Depth	0.0001	< 0.0000	2.1435	0.0326
Latitude:Elevation:Depth	< 0.0000	< 0.0000	-2.3435	0.0194

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943 **Table 2.** Percentage of sites (by depth) with runs of T_{soil} below freezing (-0.63°C),
 944 number (minimum, median, and maximum) of runs and length of runs below freezing in
 945 hours (minimum, median, maximum), compiled for 90 sites for October 2017 through
 946 April 2018. A ‘run’ is defined as 2 or more consecutive hours below freezing.

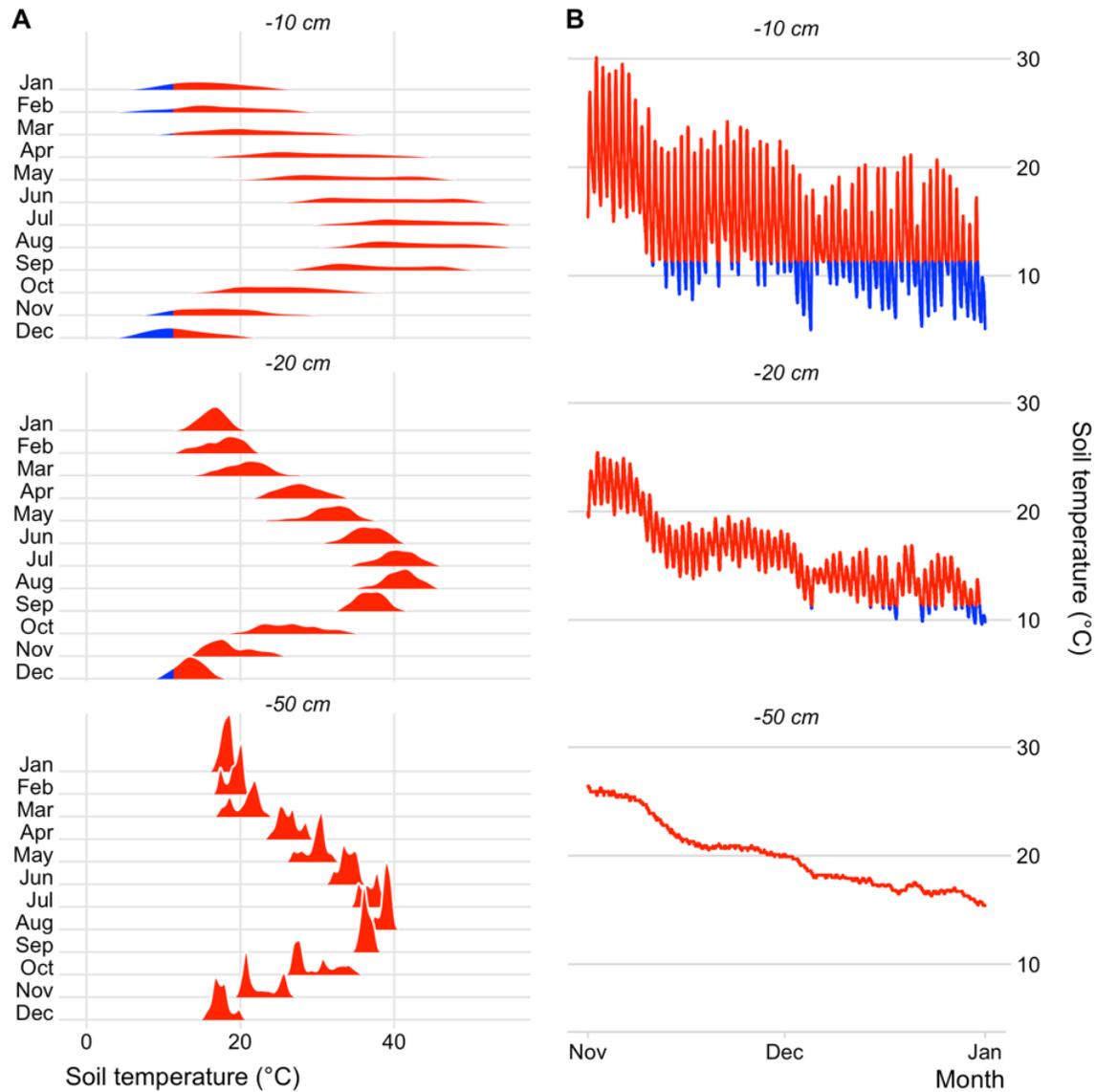
<i>Depth</i> (<i>cm</i>)	Pcnt. sites frozen	Number of runs			Length of runs (hours)		
		Min.	Median	Max.	Min.	Median	Max.
-5	65.6	0	6.5	93	0	17.5	2791
-10	55.6	0	2.5	59	0	10	2804
-20	33.3	0	0	23	0	0	1700
-50	5.6	0	0	16	0	0	655
-100	0	0	0	0	0	0	0

947

948 **Table 3.** Percentage of depths (by site) having the lowest cumulative energy cost over
 949 several winter periods, contingent on T_{soil} never dropping below freezing at that depth (-
 950 0.63°C). Depth with lowest cumulative energy cost at most sites is boldfaced.

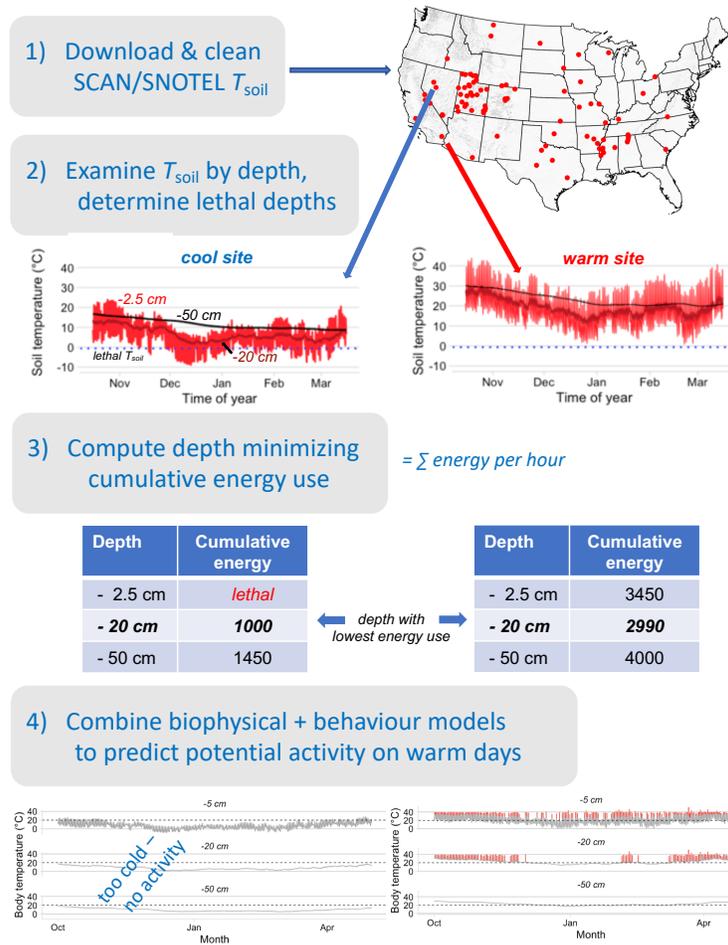
<i>Depth (cm)</i>	<i>Jan only</i>	<i>Dec-Feb</i>	<i>Nov-Mar</i>	<i>Oct-Apr</i>
5	33.0	41.8	60.2	69.4
10	19.6	28.6	27.6	24.5
20	21.6	14.3	11.2	5.1
50	19.6	14.3	1.0	1.0
100	6.2	1.0	0.0	0.0

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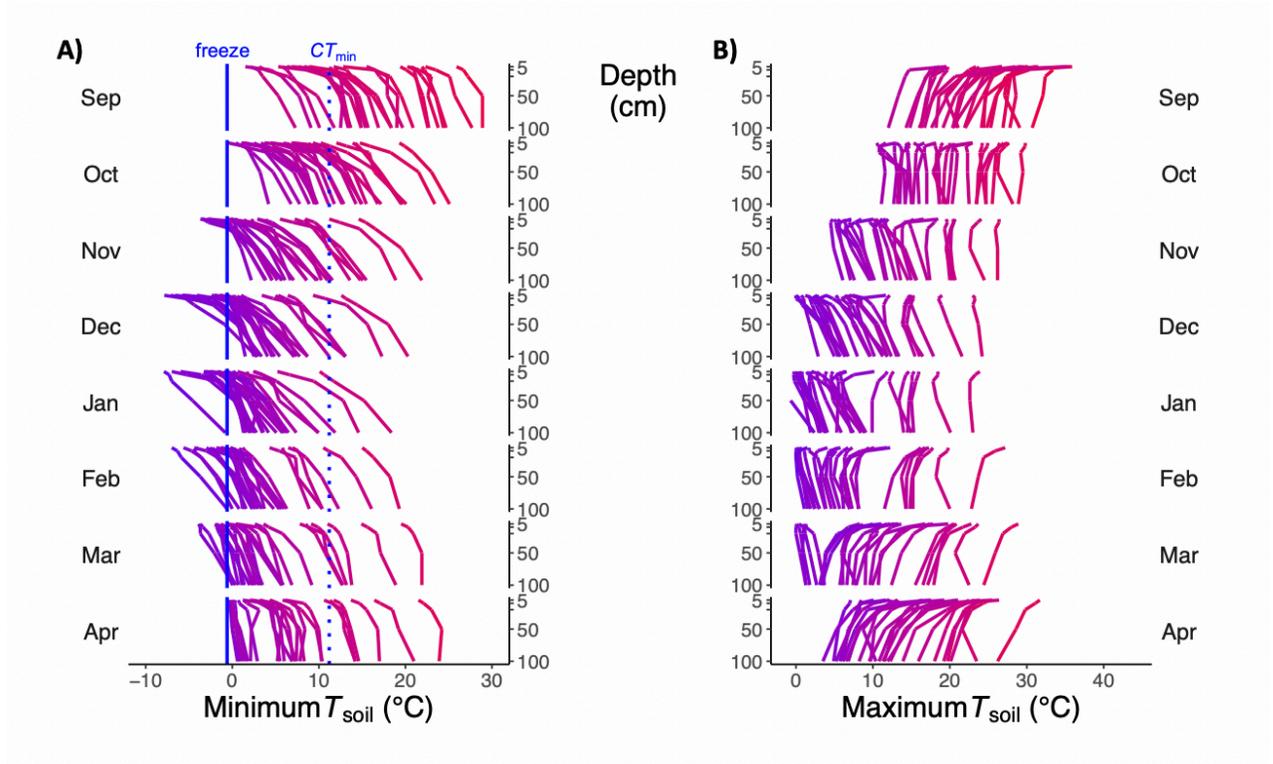


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Figure 1 Soil temperatures in 2018 at Ford Dry Lake, CA. (A) Ridge plots (Wilke 2020) of monthly soil temperatures at three representative depths. Monthly and seasonal variation decreases with depth. (B) Hourly soil temperatures from November through December. Blue colours represent temperatures at or below the median critical thermal minimum for lizards (11.3 °C).



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 963 **Figure 2** Concept diagram of protocol for simulating consequences of overwintering
 964 underground at different depths. (1) Download and clean soil temperatures (T_{soil} , 5
 965 depths, hourly) for 90 SCAN/SNOTEL sites. (2) Determine whether T_{soil} dropped below
 966 freezing (-0.63°C , assumed lethal). Here a representative cold (Lovelock, NV) and a
 967 warm (Ford Dry Lake, CA) site are depicted. Shallow T_{soil} dropped below freezing at the
 968 cold site but not the warm site. (3) T_{soil} are mapped onto cumulative energy expenditure
 969 over winter, and both site had minimal expenditures at -20 cm. (4) A biophysical and
 970 behaviour model predict opportunities for activity. T_{soil} at the cold site were too cold, but
 971 those (especially at -5 cm) at the warm site permitted activity on many winter days.



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975 **Figure 3 Soil temperature extremes by month, depth, and site.** (A) Minimum T_{soil} by

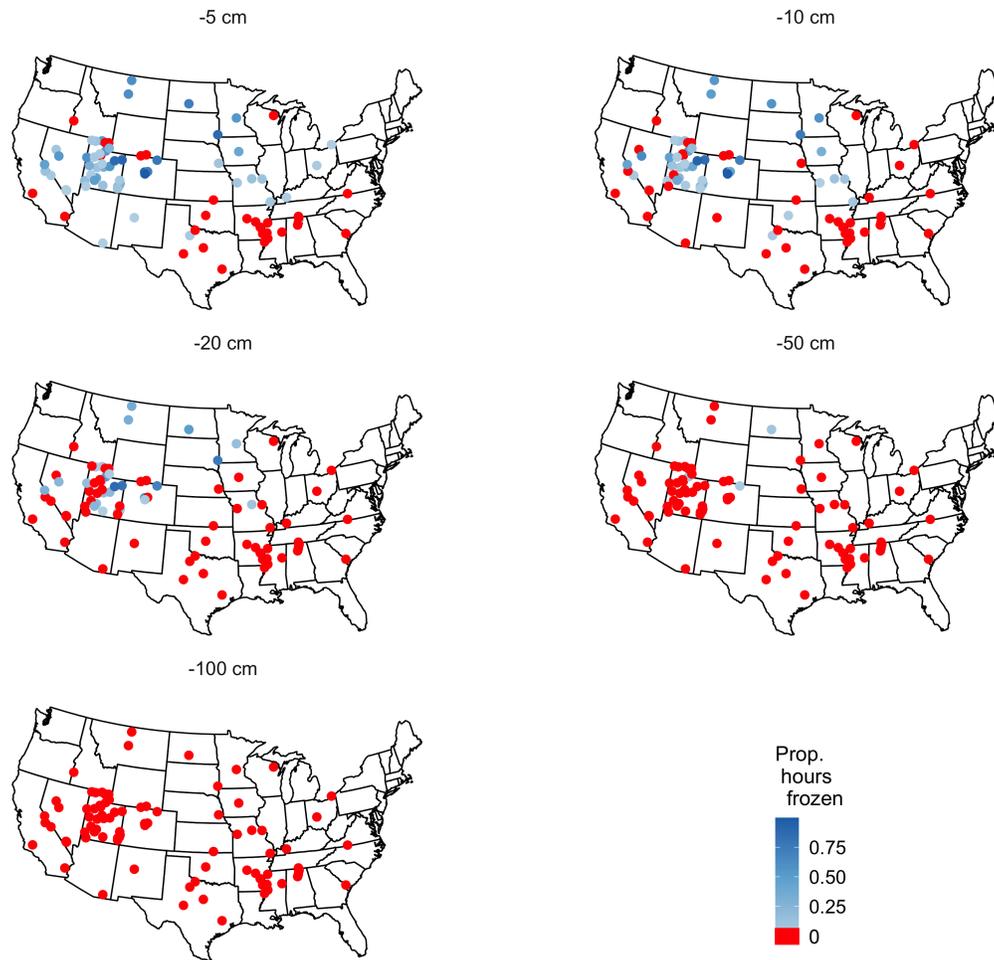
976 month and depth (cm) for a random sample of 25 sites. Each line represents one site.

977 Freezing temperature (-0.63 °C, solid blue line) and median CT_{min} temperature (11.2 °C,

978 dotted blue line) are indicated. (B) Maximum T_{soil} by month and depth. Within sub-

979 panels, depths (y-axis) are arranged from shallow to deep.

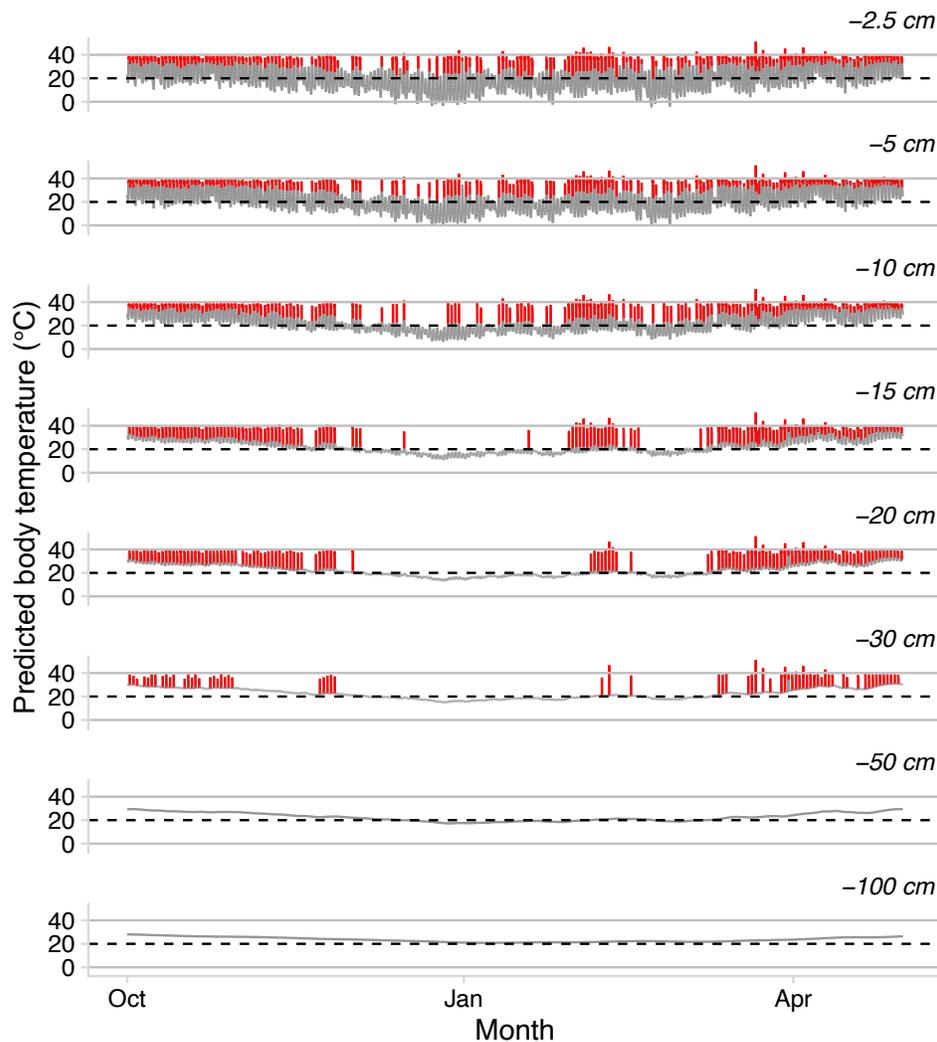
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Figure 4 Proportion of hours in January having below-freezing (-0.63°C) temperatures as function of depth. Depths that never freeze are red.

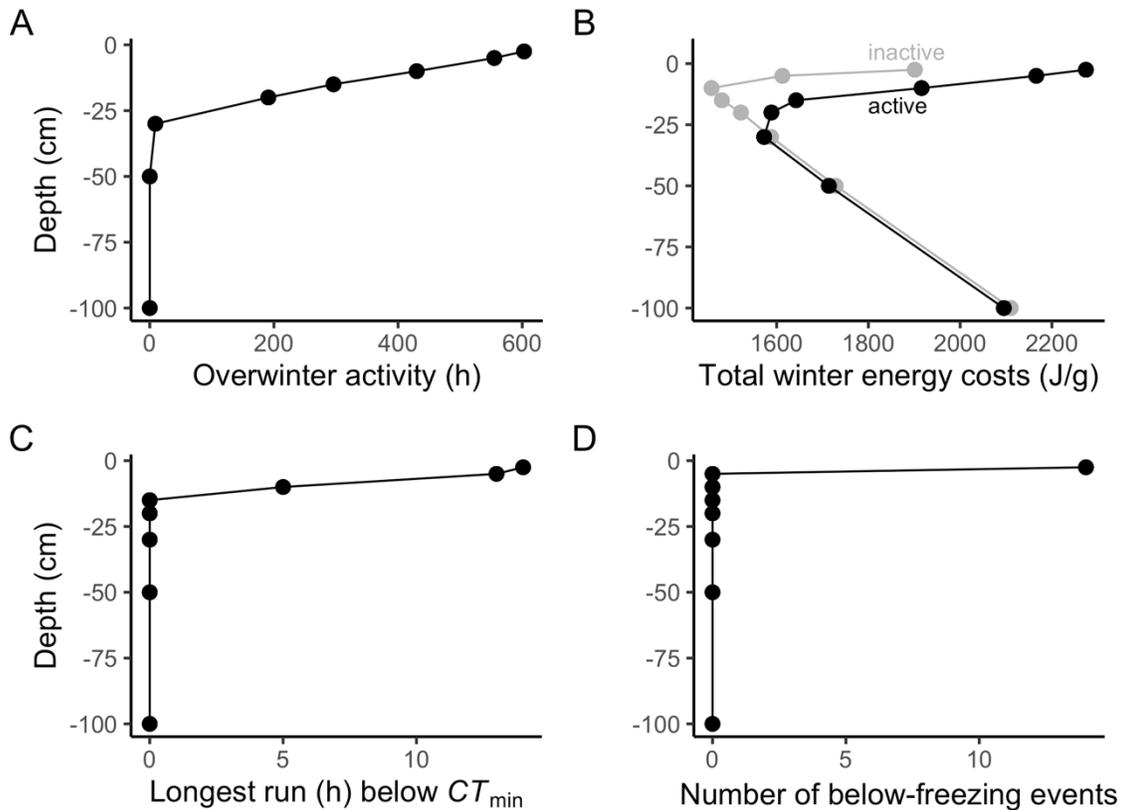
Ford Dry Lake, CA



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989 **Figure 5** Ectotherms that choose shallow retreats are active much more often in winter
990 than are those overwintering deep in the soil. Plotted are predicted body temperature of
991 20-g ectotherms that retreat to various depths at Ford Dry Lake, CA. If T_{soil} at a given
992 depth rises $0.1\text{ }^{\circ}\text{C}/\text{h}$ and is above an arbitrary threshold ($20\text{ }^{\circ}\text{C}$), the ectotherm emerges
993 and become active on the surface if surface T_e is $\geq 35\text{ }^{\circ}\text{C}$ (see Methods). Otherwise, it
994 remains underground and has a $T_b = T_{\text{soil}}$. Predicted T_b is red for active animals,
995 otherwise grey.



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999 **Figure 6** (A) Cumulative hours of activity (potential) for a lizard spending inactive

1000 periods at various depths at Ford Dry Lake, CA for November – March (see legend Fig.

1001 4). (B) Total energy costs for winter for ectotherms that remain inactive at fixed depths

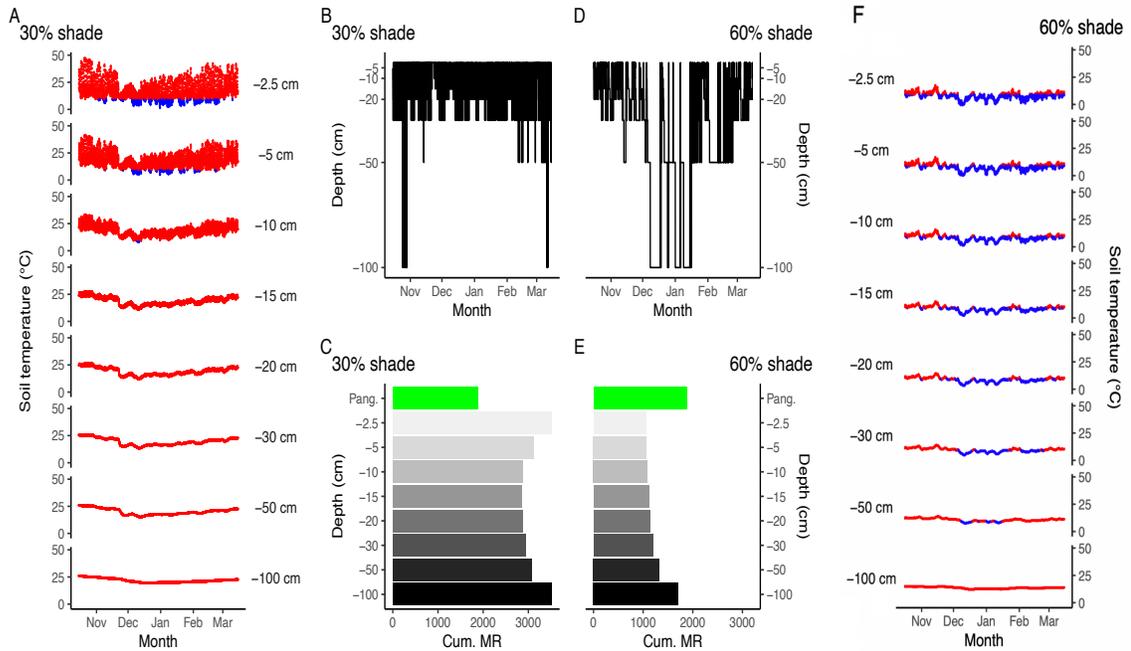
1002 (grey symbols) or become active on warm winter days (December – February) (black).

1003 (C) Maximum run-lengths below the critical thermal minimum (CT_{min} , here 7.3 °C) with

1004 depth in winter. (D) Number of freezing events (< -0.63 °C) in winter by depth. All

1005 calculations assume a warm signal of 0.1 °C/h.

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1012 **Figure 7** Simulated soil temperatures at various depths for Ford Dry Lake, CA for 30%

1013 shade coverage (A) or 60% coverage (F). Red indicates $T_{\text{soil}} > 9.3$ °C. (B & D) Depths

1014 minimizing metabolic rate each hour over winter, contingent on $T_{\text{soil}} > 9.3$ °C). (C & E)

1015 Cumulative metabolic rate (ml O₂/h) for a Panglossy ectotherm (green) and for ectotherm

1016 at fixed depths (-2.5cm to -100 cm: darker lines indicate deeper in soil).

1017

1018 **Box 1. An agenda for studies of overwinter biology of ectotherms**

1019 *Field studies*

1020 (1) Document body temperature profiles throughout winter.

1021 (2) Determine microsites used by ectotherms. Do animals shift depths during winter or
1022 stay at fixed sites? Do they use burrows or crevices, or are they buried?

1023 (3) Quantify patterns of activity and of feeding in winter, and explore ecological
1024 (condition, growth, life history) consequences.

1025 (4) Measure variation in overwinter mortality and causes thereof. Do animals die of
1026 starvation, cold, suppressed immune responses, or predation?

1027 *Physiological studies*

1028 (1) Derive and implement ecologically relevant assays (using natural cooling patterns) of
1029 cold tolerance and of metabolic-temperature relationships before, during, and after
1030 winter.

1031 (2) Quantify physiological costs and benefits of winter activity with and without feeding.

1032 (3) Determine environmental and internal cues that initiate activity in winter. Do these
1033 vary seasonally and geographically?