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## Abstract

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

## INTRODUCTION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## **Patterns of soil temperatures**

### **Methods (soil temperatures)**

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

Downloaded  $T_{\text{soil}}$  data were messy (Kearney 2020). We numerically scanned  $T_{\text{soil}}$  and examined plots of  $T_{\text{soil}}$  versus date for obvious errors. Questionable sites were omitted (blind as to location), as were sites missing data for more than 5% of hours at any depth from October-March. This left 90 sites (table S1, black dots in Fig. S5). The minimum completeness (by depth and month) was 97.3%, and 71.9% of samples were 100% complete. To characterize  $T_{\text{soil}}$  at each site, depth, and month, we computed the median  $T_{\text{soil}}$  and the 2.5% and 97.5% quantiles for  $T_{\text{soil}}$ . We chose quantiles as estimates of minimum and maximum  $T_{\text{soil}}$  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>).  $T_{\text{soil}}$  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 (glms) to analyse how minimum  $T_{\text{soil}}$  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 (glms) 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  $T_{\text{soil}}$  (September – April) versus  
173   depth are depicted for a random sample of 25 sites (Fig. 3A,B): each line connects  
174   minimum or maximum  $T_{\text{soil}}$  with depth for one site. In most months and sites, the coldest  
175   and warmest  $T_{\text{soil}}$  are both at shallow depths. In mid-winter, gradients of minimum  $T_{\text{soil}}$   
176   with depth are relatively steep; and warmest  $T_{\text{soil}}$  are deep.

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



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

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

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

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

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

## **Methods (cold risk)**

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

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

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

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

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

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

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

## Results (cold risk)

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

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

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

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

## Discussion (cold risk)

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

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

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

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

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

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

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

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

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

survival experiments are feasible only with invertebrates or microorganisms.

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

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

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

## **Methods (activity)**

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

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

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

Metabolic rates were estimated as functions of  $T_b$  (Andrews & Pough 1985) for each hour and summed for the winter (December to February) for simulations assuming that lizards stayed at a fixed depth versus became active whenever possible. Activity was 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  $T_{\text{soil}}$  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  $CT_{\text{min}}$  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:

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

## **Discussion (activity)**

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

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

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

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

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

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

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

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

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

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

## **Methods (energy, digestion)**

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

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

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

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

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

## Results (energy, digestion)

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

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

be lethal (Table 2).

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

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

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

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

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

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

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

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

## **Discussion (energy, digestion)**

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

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



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

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

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

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

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

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

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

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

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

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

## **Concluding remarks**

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

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

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

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

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

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

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

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

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

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We dedicate this paper to the memory of Raymond B. Cowles, a pioneer in thermal 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
<b>Intercept</b>	26.1647	2.2104	11.8371	< 0.0000
<b>Latitude (°N)</b>	-0.7025	0.0598	-11.7412	< 0.0000
<b>Elevation (m)</b>	-0.0169	0.0024	-71749	< 0.0000
<b>Depth (cm)</b>	0.1040	0.0433	2.4024	0.0167
<b>Latitude:Elevation</b>	0.0004	0.0001	6.7943	< 0.0000
<b>Latitude:Depth</b>	-0.0005	0.0012	-0.4399	0.6602
<b>Elevation:Depth</b>	0.0001	< 0.0000	2.1435	0.0326
<b>Latitude:Elevation:Depth</b>	< 0.0000	< 0.0000	-2.3435	0.0194

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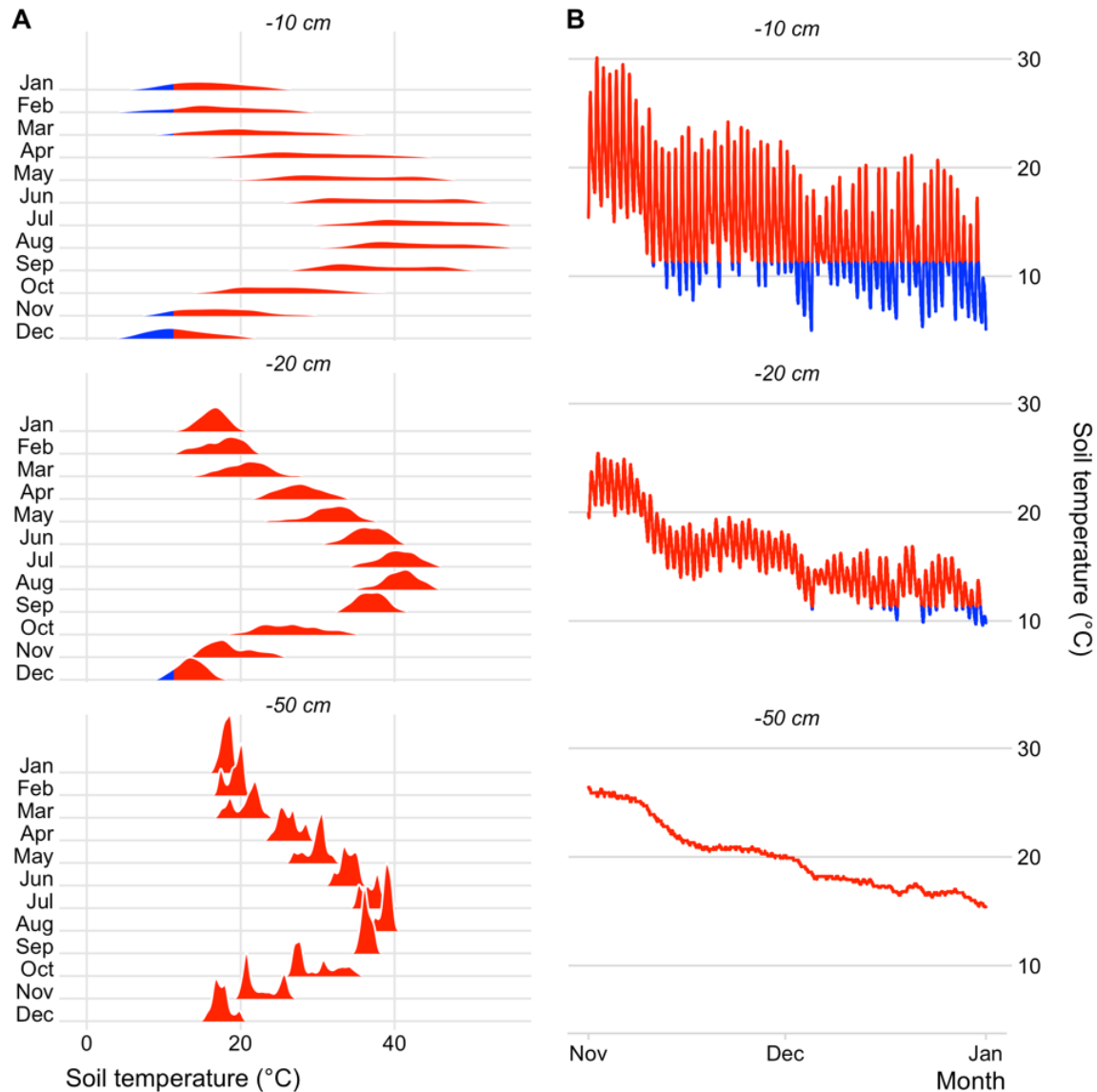
943 **Table 2.** Percentage of sites (by depth) with runs of  $T_{\text{soil}}$  below freezing ( $-0.63^{\circ}\text{C}$ ),  
 944 number (minimum, median, and maximum) of runs and length of runs below freezing in  
 945 hours (minimum, median, maximum), compiled for 90 sites for October 2017 through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

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

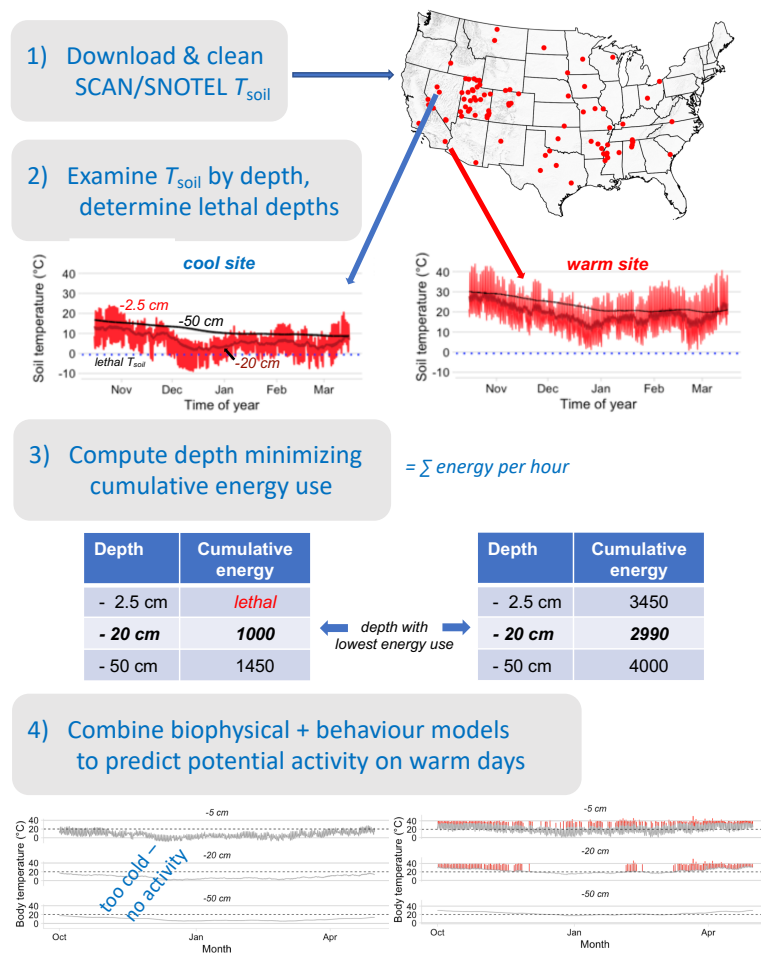
947

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

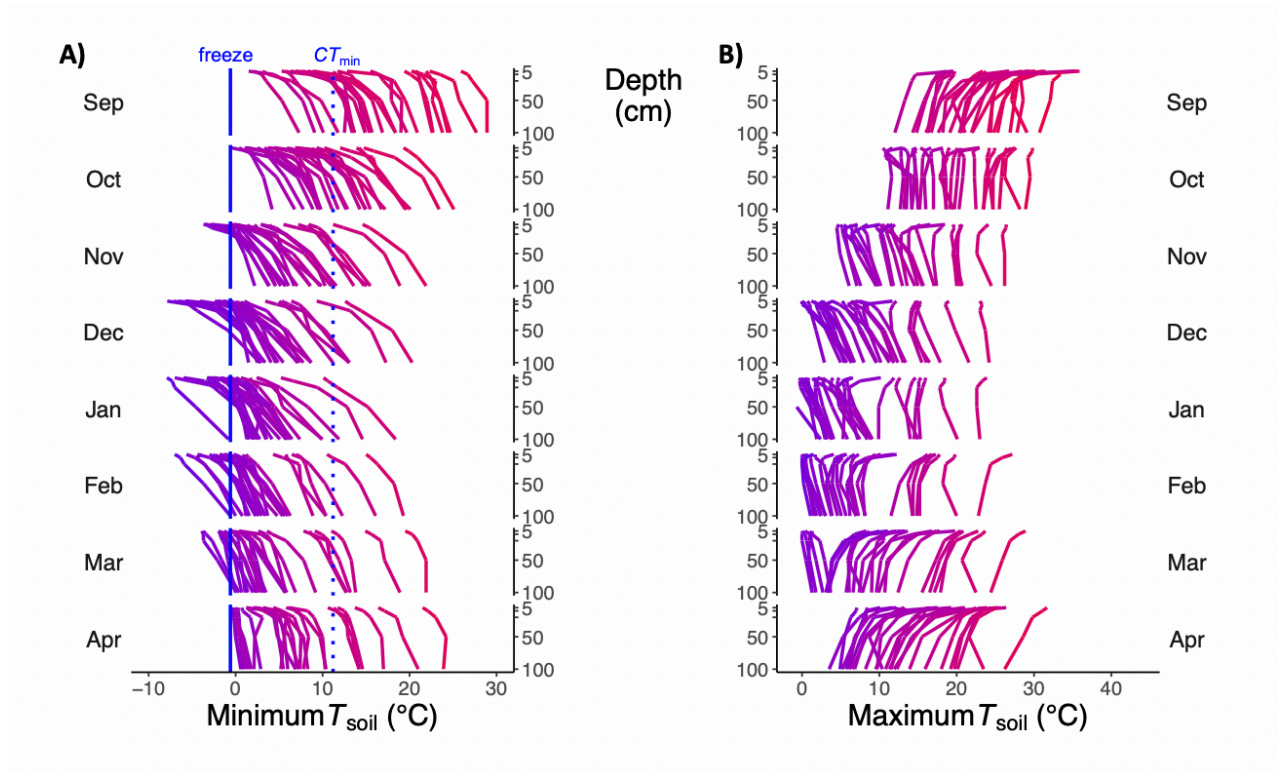
<i>Depth (cm)</i>	<i>Jan only</i>	<i>Dec-Feb</i>	<i>Nov-Mar</i>	<i>Oct-Apr</i>
5	<b>33.0</b>	<b>41.8</b>	<b>60.2</b>	<b>69.4</b>
10	19.6	28.6	27.6	24.5
20	21.6	14.3	11.2	5.1
50	19.6	14.3	1.0	1.0
100	6.2	1.0	0.0	0.0



**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).

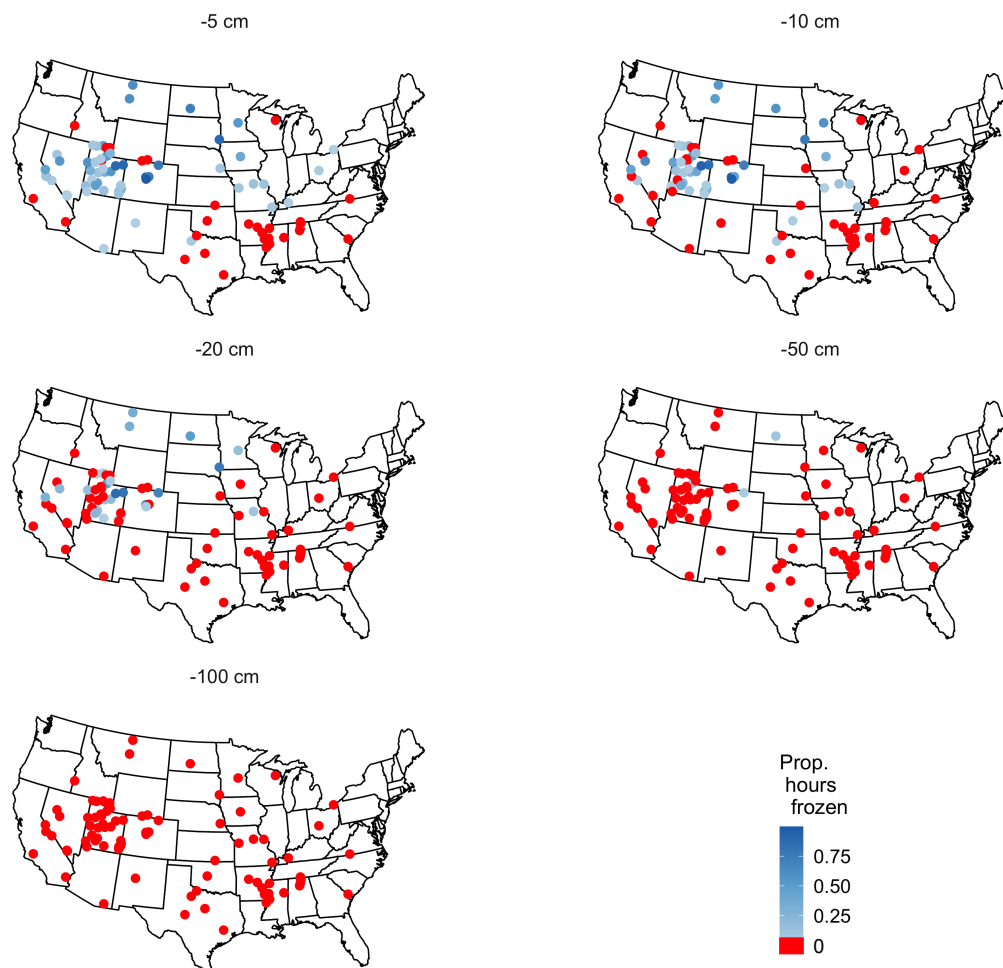


**Figure 2** Concept diagram of protocol for simulating consequences of overwintering underground at different depths. (1) Download and clean soil temperatures ( $T_{\text{soil}}$ , 5 depths, hourly) for 90 SCAN/SNOTEL sites. (2) Determine whether  $T_{\text{soil}}$  dropped below freezing ( $-0.63^{\circ}\text{C}$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



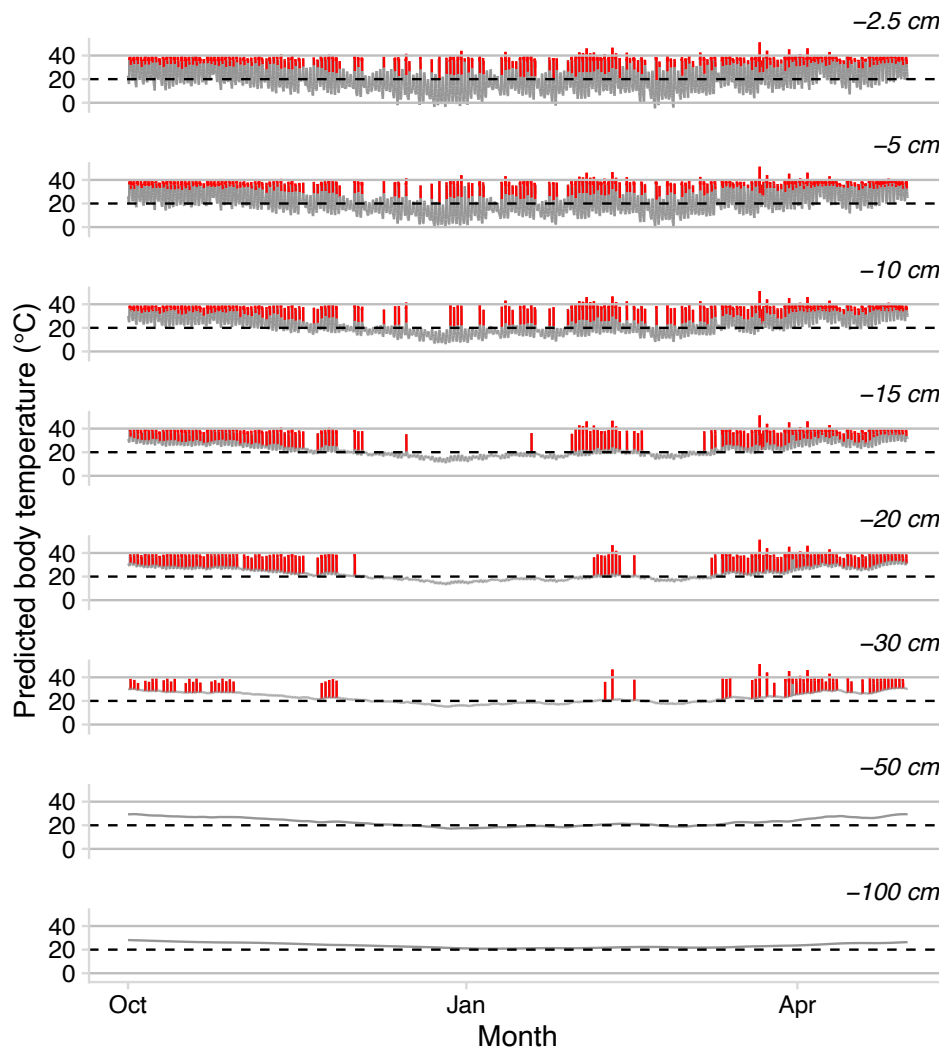
**Figure 3 Soil temperature extremes by month, depth, and site.** (A) Minimum  $T_{\text{soil}}$  by month and depth (cm) for a random sample of 25 sites. Each line represents one site. Freezing temperature (-0.63 °C, solid blue line) and median  $CT_{\text{min}}$  temperature (11.2 °C, dotted blue line) are indicated. (B) Maximum  $T_{\text{soil}}$  by month and depth. Within sub-panels, depths (y-axis) are arranged from shallow to deep.



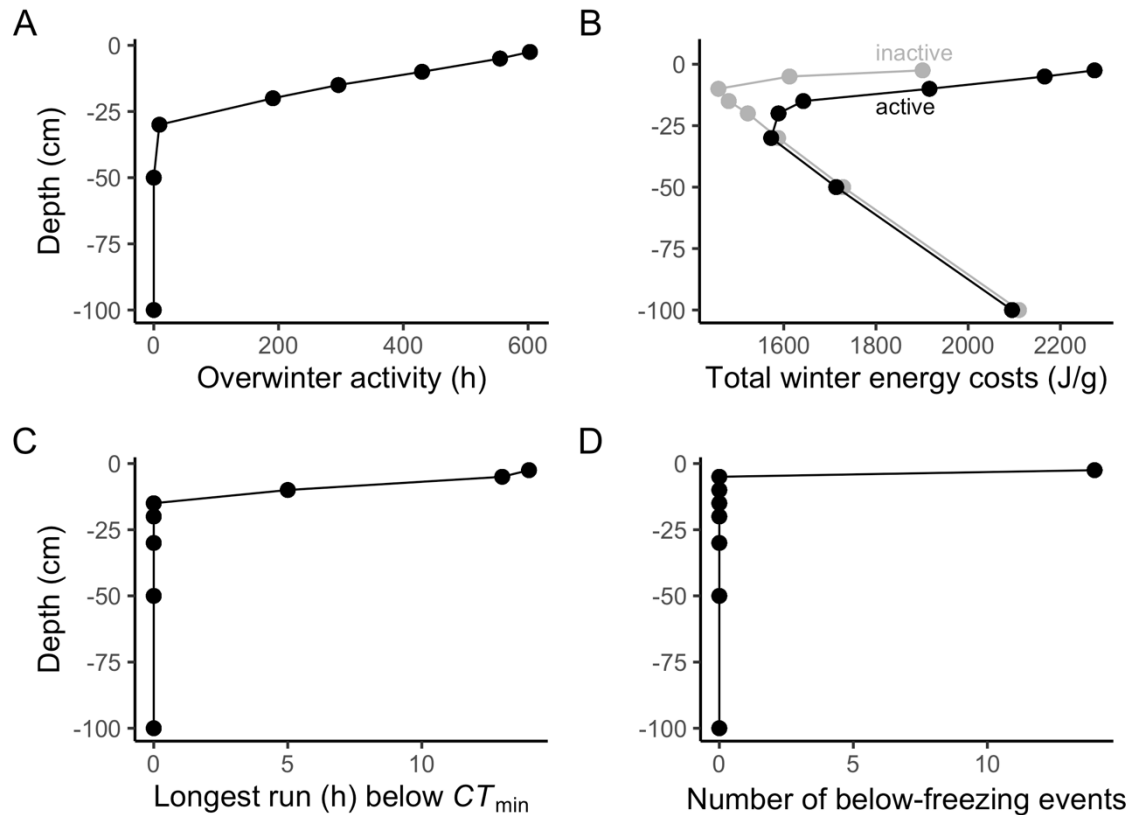


**Figure 4** Proportion of hours in January having below-freezing ( $-0.63^{\circ}\text{C}$ ) temperatures as function of depth. Depths that never freeze are red.

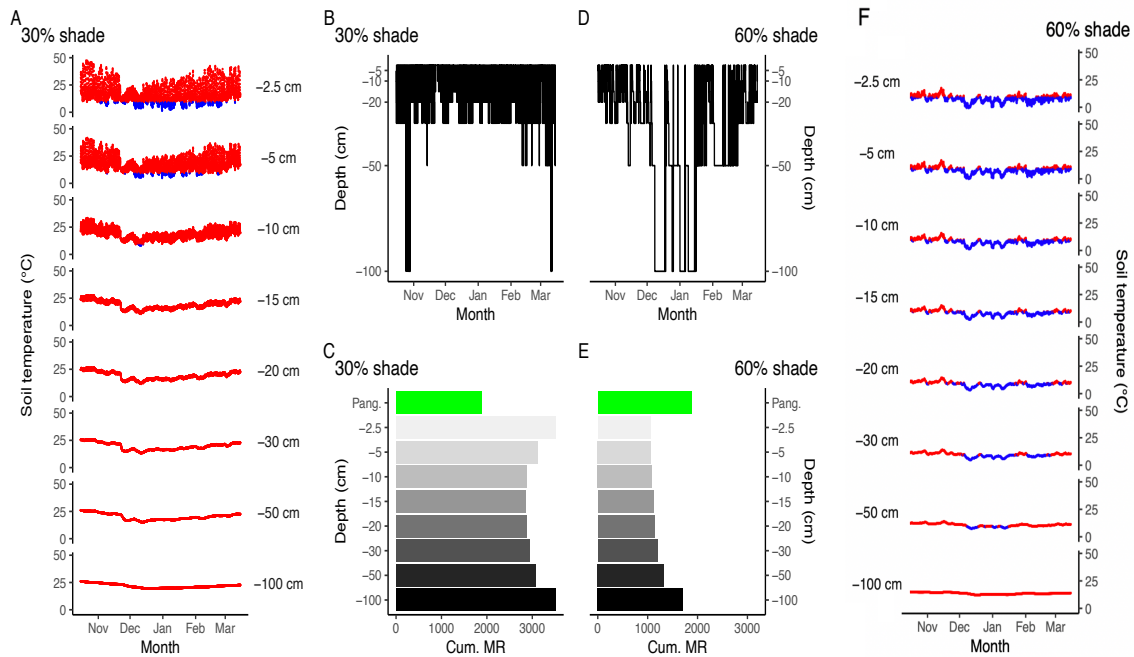
## Ford Dry Lake, CA



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



**Figure 6** (A) Cumulative hours of activity (potential) for a lizard spending inactive periods at various depths at Ford Dry Lake, CA for November – March (see legend Fig. 4). (B) Total energy costs for winter for ectotherms that remain inactive at fixed depths (grey symbols) or become active on warm winter days (December – February) (black). (C) Maximum run-lengths below the critical thermal minimum ( $CT_{min}$ , here 7.3 °C) with depth in winter. (D) Number of freezing events (< -0.63 °C) in winter by depth. All calculations assume a warm signal of 0.1 °C/h.



**Figure 7** Simulated soil temperatures at various depths for Ford Dry Lake, CA for 30% shade coverage (A) or 60% coverage (F). Red indicates  $T_{\text{soil}} > 9.3$  °C. (B & D) Depths minimizing metabolic rate each hour over winter, contingent on  $T_{\text{soil}} > 9.3$  °C). (C & E) Cumulative metabolic rate (ml O<sub>2</sub>/h) for a Panglossy ectotherm (green) and for ectotherm at fixed depths (-2.5cm to -100 cm: darker lines indicate deeper in soil).

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

*Field studies*

- (1) Document body temperature profiles throughout winter.
- (2) Determine microsites used by ectotherms. Do animals shift depths during winter or stay at fixed sites? Do they use burrows or crevices, or are they buried?
- (3) Quantify patterns of activity and of feeding in winter, and explore ecological (condition, growth, life history) consequences.
- (4) Measure variation in overwinter mortality and causes thereof. Do animals die of starvation, cold, suppressed immune responses, or predation?

*Physiological studies*

- (1) Derive and implement ecologically relevant assays (using natural cooling patterns) of cold tolerance and of metabolic-temperature relationships before, during, and after winter.
- (2) Quantify physiological costs and benefits of winter activity with and without feeding.
- (3) Determine environmental and internal cues that initiate activity in winter. Do these vary seasonally and geographically?