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IDEA AND PERSPECTIVE

# Three questions about the eco-physiology of overwintering underground

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**Abstract**

In cold environments ectotherms can be dormant underground for long periods. In 1941 Cowles proposed an ecological trade-off involving the 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 simulated consequences of overwintering at fixed depths. In warm localities shallow ectotherms have lowest energy costs and largest reserves in spring, but in cold localities, they risk freezing. Ectotherms shifting hourly to the coldest depth potentially reduce energy expenses, but paradoxically sometimes have higher expenses than those at fixed depths. Biophysical simulations for a desert site predict that shallow ectotherms have increased opportunities for mid-winter activity but need to move deep to digest captured food. Our simulations generate testable predictions to eco-physiological questions but rely on physiological responses to acute cold rather than to natural cooling profiles. Furthermore, natural-history data to test most predictions do not exist. Thus, our simulation approach uncovers knowledge gaps and suggests research agendas for studying ectotherms overwintering underground.

**Keywords**

Biophysics, climate change, cold tolerance, digestion, dormancy, energy reserves, soil temperature, thermal ecology, winter biology.

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## INTRODUCTION

Ectotherms living in cold climates may be dormant underground or in other retreats for months. Some individuals may remain in retreats until they emerge in spring, and conditions in their retreats will affect risk of freezing or cold injury as well as energy reserves in spring (Fitzpatrick *et al.*, 2020). Others emerge, bask, and move about on sufficiently sunny days; and their mid-winter activity may enable feeding or physiological adjustments (see below); but it may also expose them to predation by endotherms. Despite a legacy of studies of the eco-physiology of cold tolerance and dormancy (Gregory, 1982; Storey, 1990; Addo-Bediako *et al.*, 2000; Costanzo *et al.*, 2008; Denlinger and Lee, 2010; Zani *et al.*, 2012; Williams *et al.*, 2014; Sinclair, 2015), an understanding of the dynamics of overwinter physiology, behaviour and ecology of ectotherms has striking gaps. As we will argue, some gaps have lain dormant for decades or have not previously been recognized.

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 underground but that some others emerge and are active on suitably warm days. He knew that soil temperatures changed with depth and were both coldest and warmest near the ground surface (Fig. S1, Supporting Information multimedia animation), and that the diurnal heat pulse on sunny days penetrated slowly downward (Smith, 1929). Cowles proposed that overwintering depth induced a trade-off: only reptiles in shallow retreats could become active on warm days in winter or spring because the diurnal heat pulse descending into the soil would reach them during daylight hours and serve as a reliable cue that surface temperatures were warm enough for activity. Nevertheless, reptiles in shallow retreats risked freezing and predation by endotherms (see p. 129 in Cowles, 1941). Thus, Cowles (1941) is not only a pioneering example of overwinter ecology, but also of "trade-off" and optimality thinking in ecology.

Testing such ideas involves determining where organisms are overwintering underground. In 1941 that was – and still is – a logistical challenge (but see Mail, 1930; Kenagy and Smith, 1973; van Gelder *et al.*, 1986; Grenot and Heulin, 1988; 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

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("brushing", photo in Fig. S2). Cowles caught any reptiles in the "dirt spill," took their body temperatures ($T_b$), and estimated their 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. S3).

Cowles's insights inspired us to ask three questions about the eco-physiological consequences of overwintering underground at various depths.

(1) How deep must a reptile go to avoid dangerously or lethally cold temperatures?
(2) Which depth best enables reptiles to detect thermal cues of sunny days 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 (Wilson and Cooke, 2001; Zani, 2008; Williams *et al.*, 2014).

Ideally, such questions should be answered with field data of known overwinter depths, $T_b$, and physiological profiles, all from geographically diverse sites. However, data for squamate reptiles are incomplete and scattered both geographically and taxonomically (e.g., Ruby, 1977; Congdon *et al.*, 1979; Bauwens, 1981; Grenot and Heulin, 1988; Christian and Weavers, 1994; Bishop and 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 overwintering communally in rocky dens at mid- to high-latitude (e.g., Gregory, 1982; Gienger and Beck, 2011; Nordberg and Cobb, 2017). Geographical field surveys of overwinter biology of squamates are rare (Tsuji, 1988; Sexton *et al.*, 1992; Christian and Weavers, 1996; Wilson and Cooke, 2001). Moreover, physiological data are typically acquired from acute-exposure assays, which may have limited relevance (Sinclair, 2001a) to ectotherms experiencing extremely slow rates of temperature change during dormancy (Patterson and Davies, 1984; Storey, 2006; Huang and Tu, 2008; Berman *et al.*, 2016; Nordberg and Cobb, 2017).

Given limitations of empirical data, we used concepts in Cowles (1941) as a narrative (Otto and Rosales, 2020) to guide simulations that explore consequences of overwintering at different depths (Fig. 1). We analysed hourly soil temperatures (instrumental) for five depths from 90 sites for 2017–2018 (Fig. S4) in the continental United States. Next, we incorporated physiological data (e.g., cold tolerance) and used simulations to predict how overwintering depth affects risk of cold injury and energy expenditure. Also, we used a biophysical model (Kearney and Porter, 2017, 2020) to simulate how retreat depths at one desert site affects opportunities for activity on warm days.

While attempting to parameterize (e.g., lethal temperature) these simulations, we discovered that the required behavioural, ecological and physiological data – as well as field data required to test their predictions – often do not exist or are of dubious relevance. These issues likely reflect the difficulty of determining depth, temporal $T_b$ profiles and behaviour of overwintering ectotherms (Sinclair, 2001a), the logistical challenge of quantifying time series of physiological responses to chronic rather than acute temperature exposures (Huang and Tu, 2008; Sinclair *et al.*, 2015), and the incomplete information on cues (e.g., environmental, innate clocks) used for mid-winter emergence (Heath, 1962; Bishop and Echternacht, 2004; Lutterschmidt *et al.*, 2006; Nordberg and Cobb, 2016). Our simulations thus rely on unvalidated parameter values, but nonetheless provide "approximate answers" (Tukey, 1962) to ecologically relevant questions. Perhaps most importantly, they help uncover what needs to be measured and thereby suggest an agenda for overwinter research.

The three questions we address are diverse (cold risk, activity opportunity, energy reserves). We summarize simulation procedures used to address these questions in Box 1 and provide details in Supporting Information. Next, we address the predictions and implications for each question. We conclude with a synthesis and research agenda. Our focus is on squamate reptiles, but our conclusions are relevant to many ectotherms.

We made several global assumptions. We assumed that ectotherms are buried in soil or in burrows (thus not under rocks or in rock crevices, which have different thermal properties) and that $T_b$ equals adjacent $T_{soil}$ (Supporting Information). We assumed that moisture (or ice), desiccation or gas (oxygen, $CO_2$) tensions did not influence results (but see, Costanzo, 1989; Burke *et al.*, 2002; Berman *et al.*, 2016; Rossi *et al.*, 2020; Yagi *et al.*, 2020). For most simulations we assumed that ectotherms did not change depths within a sample period (but see Macartney *et al.*, 2011).

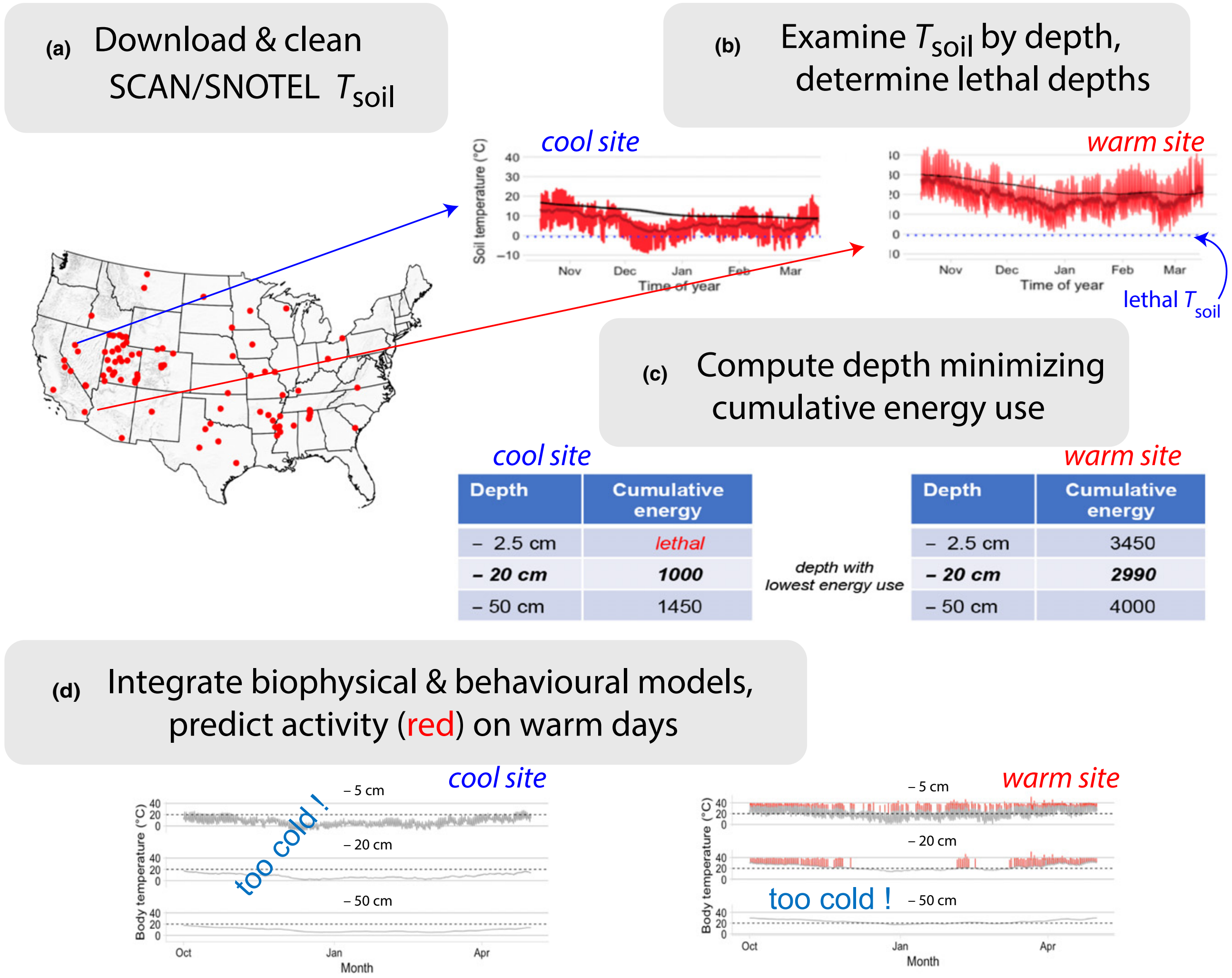
## PATTERNS OF SOIL TEMPERATURES

To provide background information on $T_{soil}$ patterns, we began by documenting empirical patterns of $T_{soil}$. We downloaded hourly $T_{soil}$ at five depths (−5, −10, −20, −50 and −100 cm) from 660 sites from the continental USA from the Soil 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. After screening we accepted 90 sites that met quality-control criteria (Table S1, see Supporting Information for procedures). Our $T_{soil}$ sites are generally close to known locality records for squamates and thus relevant to squamates (https://www.gbif.org) (see legend Fig. S4), but some regions (e.g., southeast US) with high species density of squamates had few acceptable $T_{soil}$ sites.

Monthly and hourly variation in $T_{soil}$ at various depths is shown for a representative site (Ford Dry Lake, California, Fig. 2a and b). Seasonal and daily variation in $T_{soil}$ is of course damped with depth, and the seasonal drop is slow and irregular (Fig. 2b).

Monthly variation in minimum and in maximum $T_{soil}$ (September – April) vs. depth are depicted for a random sample of 25 sites (Fig. 3a and b): each line connects minimum or maximum $T_{soil}$ with depth for one site. For most months and sites, $T_{soil}$ at shallow depths were both coldest and warmest. In mid-winter, gradients of minimum $T_{soil}$ with depth were relatively steep; and warmest $T_{soil}$ were deep.

Coldest $T_{soil}$ were usually at −5 cm in all months (between 52.0 and 93.9% of sites), except June and July, when coldest



cool site

| Depth | Cumulative energy |
|---|---|
| – 2.5 cm | *lethal* |
| ***– 20 cm*** | ***1000*** |
| – 50 cm | 1450 |

warm site

| Depth | Cumulative energy |
|---|---|
| – 2.5 cm | 3450 |
| ***– 20 cm*** | ***2990*** |
| – 50 cm | 4000 |

**Figure 1** Concept diagram of protocol for simulating consequences of overwintering underground at different depths. (a) Download and clean soil temperatures ($T_{soil}$, 5 depths, hourly) for 90 SCAN/SNOTEL sites. (b) Determine whether $T_{soil}$ dropped below freezing (–0.63 °C, assumed lethal). Here, a representative cold (Lovelock, NV: 40.18° N, 118.47° W) and a warm (Ford Dry Lake, CA: 33.65° N, 115.10° W) site are depicted. Shallow $T_{soil}$ dropped below freezing at the cold site but not the warm site. (c) $T_{soil}$ are mapped onto cumulative energy expenditure over winter (J/g), and both sites had lowest expenditures at −20 cm. (d) A biophysical and behaviour model predicts opportunities for activity. $T_{soil}$ at the cold site were too cold, but those (especially at −5 cm) at the warm site permitted activity on many winter days.

$T_{soil}$ were at −100 cm (Table S2A). Warmest $T_{soil}$ were generally at −5 cm (between 49.1 and 90.9% of sites) except November-January, when warmest $T_{soil}$ were −100 cm (Table S2B). Not surprisingly, $T_{soil}$ was least variable (interquartile range, IQR) at −100 cm in all months (34.9–93.1% of sites, Table S2C); and $T_{soil}$ was most variable at −5 cm in all months (59.8–93.9% of sites, Table S2D).

Temporal constancy of $T_{soil}$ increased with depth. The average absolute difference in median $T_{soil}$ (at each site, depth) for January 2017 vs. January 2018 was inversely correlated with depth (−5 cm = 1.17 °C, −10 cm = 1.21 °C, −20 cm = 1.13 °C, −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 (Figs 2 and 3), and within-month (Table S2D) variation in $T_{soil}$ was greatest at shallow depths.

In the spatial model, minimum $T_{soil}$ declined with latitude and elevation, but increased with depth (Table S3). Several interactions were significant. The negative effect of latitude weakened with elevation, and the negative effect of elevation weakened with depth, with a significant and negative 3-way interaction between depth, latitude and elevation. The implications of these patterns will be discussed in topic-specific sections (below).

## QUESTION I. WHICH DEPTH MINIMIZES RISK OF COLD INJURY OR DEATH?

Cowles (1941) noted that squamates overwintering at shallow depths risked death from cold. At many sites, $T_{soil}$ just below the ground surface indeed dropped below the freezing point of reptiles in winter (Fig. 3a). However, predicting whether shallow squamates are at risk from cold requires knowing which $T_b$ are damaging. Specifying such temperatures proved problematic because traditional assays are of questionable relevance to risk in winter (below).

**Box 1. Parameter values and simulation procedures**

### SIMULATING RISK OF COLD DEATH (QUESTION 1)

We used 11.2 °C as a base $CT_{\text{min}}$ (median for 40 lizard species in Grigg and Buckley, 2013; Muñoz *et al.*, 2014; Muñoz *et al.*, 2016) plus a site-specific $CT_{\text{min}}$ adjusted for latitude and elevation (Supporting Information). We used −0.63 °C for freezing point and −6.0 °C for supercooling point (medians of values for 23 species in Lowe *et al.*, 1971). We calculated the percentages of sites (by depth, month) where $T_{\text{soil}}$ dropped below each index [Supplemental Information.]

### PREDICTING MID-WINTER ACTIVITY (QUESTION 2)

To explore Cowles's prediction (1941) that only shallow squamates could detect whether winter-surface conditions were adequate for activity, we used the microclimate model in NicheMapR (Kearney and Porter, 2017) to simulate $T_{\text{soil}}$ at fixed depths (2.5, 5, 10, 15, 20, 30, 50, 100 cm) at a desert site (Ford Dry Lake, CA, 33.7° N, −115.1° W) for winter 2017-2018. [Input values are in Appendix I, Supplemental Information.] Using the 'ectotherm' function of NicheMapR (Kearney and Porter, 2020), we computed $T_b$ and hours of foraging activity by a 20-g lizard. A squamate emerged if $T_b$ was above a threshold ($T_{\text{RB,min}}$ = 20 °C) and was increasing ($\geq 0.1$ °C $h^{-1}$) (Table S10).

Once emerged, a squamate foraged if its $T_b$ was within a foraging range ($T_{\text{F,min}}$ = 35.0 °C; $T_{\text{F,max}}$ = 43.0 °C). To achieve its preferred body temperature ($T_{\text{pref}}$ = 39.0 °C) and avoid overheating, it shuttled between 0% and 90% shade. Once $T_b$ in sun exceeded $T_{\text{pref}}$, the lower shade level was increased (3% increments, from 0% to 90%) to enable $T_{\text{pref}}$; thereafter $T_{\text{pref}}$ was incremented to $T_{\text{F,max}}$. When surface conditions were too hot or cold, the animal retreated to a fixed depth or (for 'Panglossy' ectotherms, below) to a depth that avoided extremely high [$T_{\text{F,max}} + (CT_{\text{max}} - T_{\text{F,max}})/\ 2$] and cold (−0.63 °C) temperatures.

Using a metabolic temperature equation (M × T, $Q_{10} \sim 2.4$) for reptiles (Andrews and Pough, 1985), we mapped empirical $T_{\text{soil}}$ to estimates of hourly metabolic rates, assuming the M × T relationship was constant during dormancy. [Table S6 provides sample $Q_{10}$ for lizards tested at low $T_b$ and thus relevant to winter).] Hourly metabolic rates were summed for December–February. Foraging activity was summed for November–March.

### PREDICTING ENERGETIC LOSSES AND DIGESTION (QUESTION 3)

We mapped empirical $T_{\text{soil}}$ (all sites) to hourly metabolic rates for a 20-g lizard and summed over time, assuming that M × T relationships are geographically and temporally stable (see text). We excluded depths where $T_{\text{soil}}$ dropped below freezing (−0.63 °C), as ectotherms would not likely survive there (Storey, 2006).

Next, we relaxed the restriction that ectotherms stay at fixed depths and allowed them to move every hour to the depth that would have the lowest $T_{\text{soil}}$ (thus lowest metabolic costs) in the next hour. This scenario represents the optimality concept of a 'Panglossy' ectotherm – one that is always in the best thermal environment (Huey *et al.*, 1989). To enable Panglossy ectotherms to move, we excluded depths with $T_{\text{soil}}$ below $CT_{\text{min}}$. When determining energy costs for Panglossy ectotherms, we ignored locomotion costs (Wu *et al.*, 2015).

Reptiles feeding require elevated temperatures for digestion. To determine how depth affects digestion rate, we calculated the reciprocal of an equation for gut passage time (hours) vs. 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 determined the depth that maximized cumulative costs, contingent on $T_{\text{soil}}$ not dropping below −0.63 °C (lethal, see above). [We assumed that a lizard that captured food would immediately retreat underground to a fixed depth and not emerge to bask on subsequent days.]

Three methods have been used to measure squamate cold tolerance, but their relevance to overwinter survival is unclear. The most frequent method involves acclimating animals and then rapidly cooling them until they lose a righting response ('critical thermal minimum,' $CT_{\text{min}}$) (Cowles and Bogert, 1944; Bennett *et al.*, 2018). Such acute exposures to $CT_{\text{min}}$ are non-lethal. Other assays measure survival duration after a sudden drop to a fixed $T_b$ (e.g., Heatwole *et al.*, 1969; Gregory, 1982; Burke *et al.*, 2002; Storey, 2006; Olson *et al.*, 2013; Rukke *et al.*, 2016; but see Huang and Tu, 2008; Berman *et al.*, 2016), freezing temperature (Lowe *et al.*, 1971) or super-cooling temperature (Lowe *et al.*, 1971; Storey, 2006). Exposure to freezing or supercooling temperatures for a few hours or day is typically lethal (Storey, 2006; but see Costanzo *et al.*, 1995; Berman *et al.*, 2016), but whether prolonged exposure to cold but above-freezing temperatures is lethal is unclear.

The rapid cooling of the above assays is problematic. In nature, $T_b$ of squamates underground drops slowly over months (Nordberg and Cobb, 2017) (Figs 1b, S6). Rapid assays are experimentally convenient but of uncertain relevance to overwinter cold tolerance (Sinclair, 2001a; Sinclair *et al.*, 2015).

Given these concerns, we explored patterns for three acute measures (see Box 1), hoping to bracket cold risk. $CT_{\text{min}}$ can vary geographically, and so we used a median value as well as

**Table 1** Percentage of depths (by site) having the lowest cumulative energy cost over several winter periods, contingent on $T_{\mathrm{soil}}$ never dropping below freezing at that depth (−0.63 °C). Depth with lowest cumulative energy cost at most sites is boldfaced.

| Depth (cm) | Jan only | Dec–Feb | Nov–Mar | Oct–Apr |
|---|---|---|---|---|
| 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 |

a value adjusted for elevation and latitude of each site (Supporting Information). Freezing and supercooling temperatures do not vary geographically, and we used single values for each (Supporting Information).

We calculated the percentage of sites (by month, depth) where temperatures dropped below base or adjusted $CT_{\mathrm{min}}$, median freezing temperature (−0.63 °C), or below supercooling temperature (−6.0 °C). Also, because repeated cold exposures can be damaging (Sinclair and Chown, 2005; Marshall and Sinclair, 2012, 2018), we calculated the number of times (and duration of runs) when $T_{\mathrm{soil}}$ dropped below freezing.

## Predictions and results

$CT_{\mathrm{min}}$ is the most commonly measured cold-tolerance index of reptiles (Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997; Bennett *et al.*, 2018), but $T_{\mathrm{soil}}$ in January actually dropped below the median $CT_{\mathrm{min}}$ for lizards (Box 1) 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 $CT_{\mathrm{min}}$ adjusted for latitude and elevation (Table S4B). At −5 cm, 64.3% of sites experienced sub-freezing temperatures (Table S4C), and 17.4% experienced sub-supercooling temperatures (Table S4D). Thus, risk of cold injury or death appears geographically widespread for squamates in the USA.

Two-thirds of sites at −5 cm had sub-freezing runs (multi-hour) of $T_{\mathrm{soil}}$ at least once over winter (Table S5). The median and maximum number of cold runs were highest at −5 cm, but declined with depth, as did the median length of the longest freezing event (Table S5). The single longest sub-freezing run (−5 cm) was 131 days (Crow Creek, WY, 2539 m).

Geographical patterns in the proportion of hours with sub-freezing temperatures by depth in January (coldest month) are in Fig. 4. Many southern sites never experienced sub-freezing

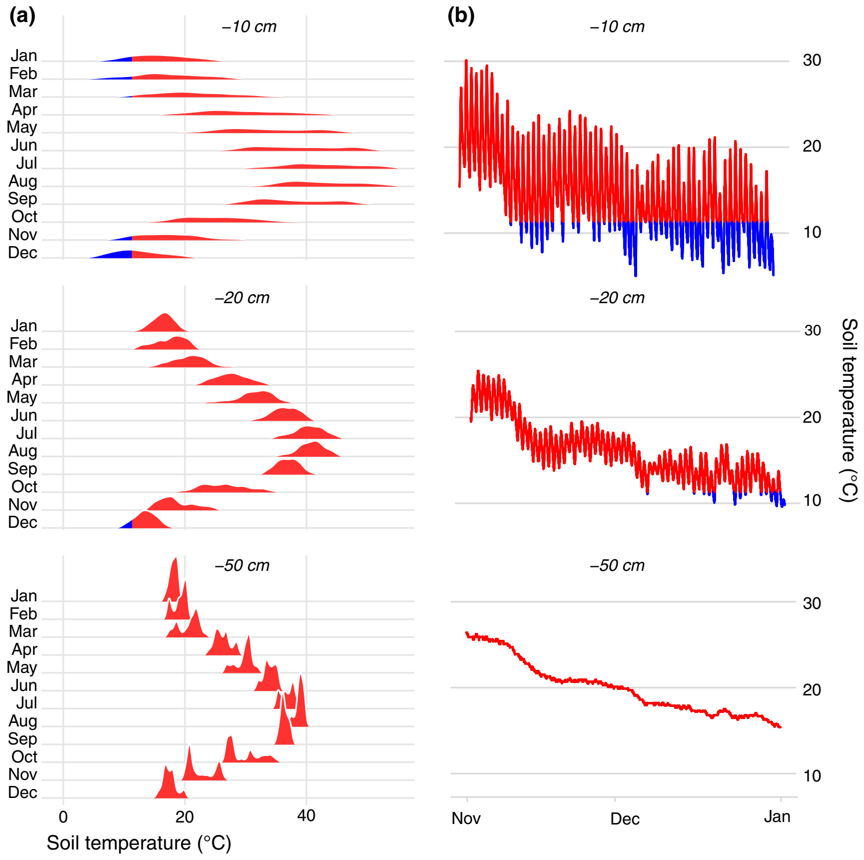


**Figure 2** Soil temperatures in 2018 at Ford Dry Lake, CA (33.65° N, 115.10° W, 120 m). (a) Ridgeline plots (Wilke,2020) of monthly soil temperatures at three representative depths. Monthly and seasonal variation decreases with depth. (b) Hourly soil temperatures from November through December. Blue colours represent temperatures at or below the median critical thermal minimum for lizards (11.3 °C).

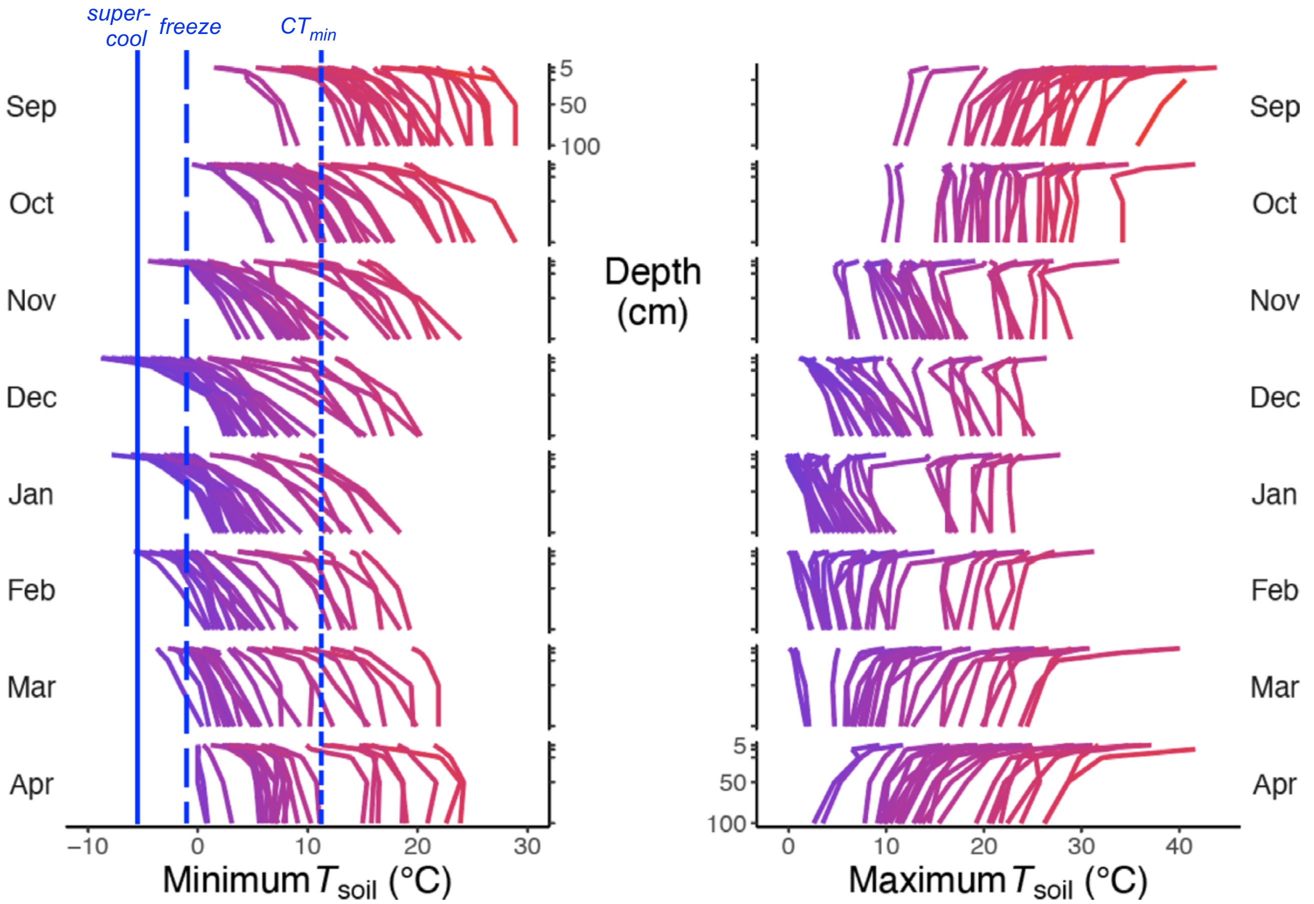


**Figure 3** Soil temperature extremes by month, depth and site. (a) Minimum $T_{soil}$ by month and depth (cm) for a random sample of 25 sites. Each line represents one site. Supercooling temperature (−6.0 °C), freezing temperature (−0.63 °C) and median $CT_{min}$ temperature (11.2 °C, short-dashed blue line) are indicated (blue dashed lines). (b) Maximum $T_{soil}$ by month and depth. Within sub-panels, depths (y-axis) are arranged from shallow to deep.

temperatures at any depth (red dots, Fig. 4). At northern and montane sites, freezing was common at shallow depths (blue dots). Freezing was uncommon at −50 cm, and did not occur at −100 cm.

## Implications

Cowles (1941) predicted that reptiles overwintering at shallow depths potentially face cold injury or death. Not surprisingly,

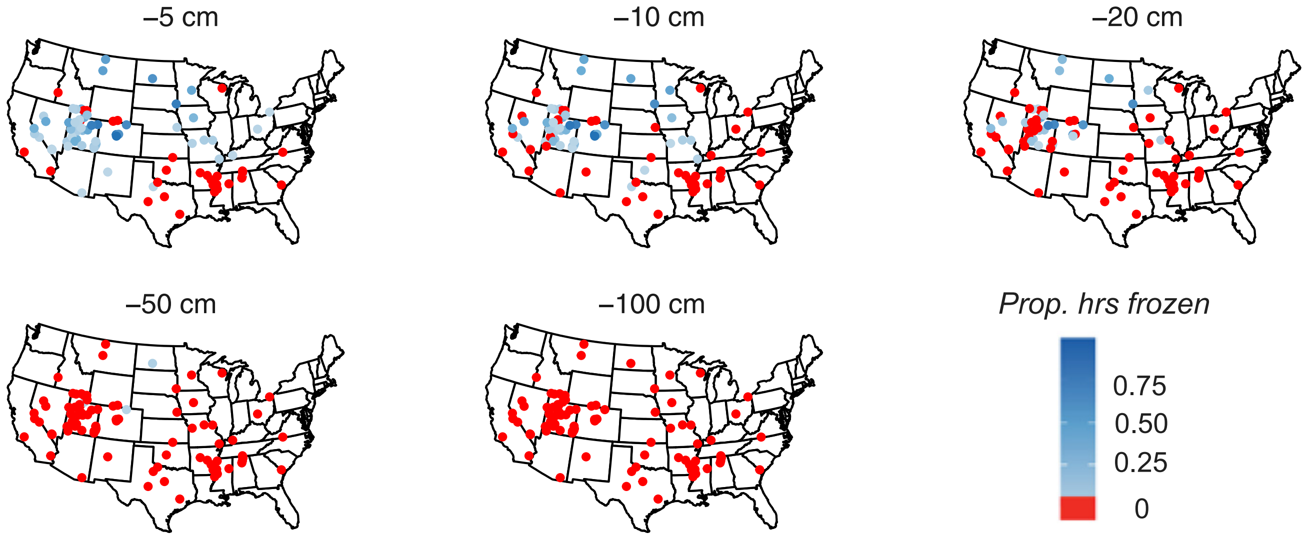


**Figure 4** Proportion of hours in January having sub-freezing (−0.63 °C) temperatures as function of depth. Depths that never froze are red.

$T_{soil}$ – especially at shallow depths – are often low (Figs 2–4, Table S4), especially at high latitudes and elevations (Table S3). But are those $T_{soil}$ low enough to cause physiological stress or death?

Although $CT_{min}$ is the most common cold-tolerance assay, $T_{soil}$ at almost all sites and depths dropped below the median $CT_{min}$ of lizards during winter (Table S4A), and most still did so even with site-adjusted $CT_{min}$ (Table S4B). Because squamates live near most sites (Fig. S4), many probably experience and survive sub-$CT_{min}$ temperatures in winter. Thus, $CT_{min}$ is likely not a hard boundary for cold survival: its relevance to survival and geographic range limits must be indirect at best (Lowe *et al.*, 1971; Huang and Tu, 2008; Calosi *et al.*, 2010; Andersen *et al.*, 2015).

Freezing and supercooling temperatures are more likely hard boundaries for survival, as exposure to these temperatures for a few hours or days is likely lethal for most squamates (see Table 1 in Storey, 2006). If squamates tolerate only above-freezing temperatures, they must overwinter at or below −20 cm at most sites (Fig. 3, Table S4C). If, however, they survive to near super-cooling temperatures, they can survive at most sites – except perhaps at very shallow depths at the coldest sites (Table S4D).

The question "how cold is too cold for reptiles?" cannot presently be answered, because traditional cold-tolerance assays are of uncertain relevance to overwinter survival. Traditional assays use rapid temperature drops for experimental convenience (Sinclair *et al.*, 2015). For example, the median cooling rate for 51 $CT_{min}$ studies with squamates is −1.0 °C/min (Bennett *et al.*, 2018). However, that rate is roughly four orders of magnitude faster than natural cooling rates at depth (Fig. 2b, S6; see also Sinclair, 2001b). At −50 cm at Ford Dry Lake (Fig. 2b), $T_{soil}$ drops only about 11.8 °C from October through December. At −20 or −10 cm, $T_{soil}$ also drops slowly but has large daily fluctuations (Fig. 2b). Thus, rapid and monotonic cooling protocols of $CT_{min}$ assays are incongruent with natural cooling patterns (Sinclair, 2001a) and thus yield point estimates of uncertain relevance to overwinter survival or cold injury in nature (but see Andersen *et al.*, 2015).

Another protocol involves scoring survival (or length of survival) after sudden transfer to one or more low and fixed $T_b$ (e.g., Storey, 2006; Olson *et al.*, 2013; but see Huang and Tu, 2008). 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 and Tu, 2008; Olson *et al.*, 2013), but still do not approximate natural $T_b$ profiles (Fig. 2b, Figs S2 & S6, Sinclair, 2001a; Marshall and Sinclair, 2012, 2015; Nordberg and Cobb, 2017). Do such temporal differences render traditional assays unreliable for predicting overwinter survival? That is unknown. 'Drop-and-hold assays' probably underestimate natural cold tolerance, as natural and slow cooling trajectories (Fig. 2b, Nordberg and Cobb, 2016, 2017) offer considerable time for acclimatization (Kelly and Lee, 1999) or potentially for injury, dehydration or starvation. This issue may never be resolved for squamates, for which 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. 2b, Fig. S5), varying exposure duration, and repeated cold-exposure bouts – and does so for multiple populations and species – is logistically daunting (Sinclair, 2001a; Huang and Tu, 2008; Nordberg and Cobb, 2017; Marshall and Sinclair, 2018) and potentially practical only with insects or microorganisms. Moreover, any derived statistical model will not be predictive for other sites and depths. A more pragmatic approach would be to try to determine whether cold tolerance estimates from 'drop-and-hold' or even 'slow-drop' experiments (Huang and Tu, 2008) are acceptably close to those derived from natural patterns of cooling thermal profiles (Figs. 2b & Fig. S5) (Marshall and Sinclair, 2015, 2018) or from field experiments (below).

Perhaps asking what temperature is too cold is an 'inappropriate question' (sensu Hertz *et al.*, 1993) because no single temperature likely exists. Lethal and damaging temperatures may shift over winter and depend on exposure duration as well as each individual's retreat site (Bishop and Echternacht, 2004; Hall and Warner, 2017), age (Kingsolver *et al.*, 2011) and physiological state. Individual (Dowd *et al.*, 2015) and season variation (Layne *et al.*, 1985; Hu and Appel, 2004; Pingor *et al.*, 2016) in cold tolerance is rarely assayed, even for a single species at a single site.

Rather than ask what temperature is too cold, 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 organisms in the laboratory to empirical $T_{soil}$ profiles measured over winter at different sites and depths (e.g., Fig. 2b, Fig. S5) and score survival at intervals through spring emergence. Alternatively, bury organisms at various depths, monitor their thermal profiles and score survival (Mail, 1930; Tucker and Packard, 1998; Kevin Roberts, personal communication); and then determine whether acute measurements of cold tolerance correctly predict overwinter survival. Either approach would illustrate how deep an organism needs to go to survive, but any conclusions would be descriptive and only locally applicable. Again, such survival experiments are feasible mainly 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 cold-tolerance indices have yet to be evaluated against cold tolerance in nature. The need for developing ecologically relevant protocols has been recognized (Kelly and Lee, 1999; Sinclair, 2001a; Williams *et al.*, 2014; Andersen *et al.*, 2015; Marshall and Sinclair, 2015), but deriving and implementing these protocols will be conceptually and logistically challenging.

## QUESTION II. WHAT DEPTH MAXIMIZES OPPORTUNITIES FOR ACTIVITY ON WARM DAYS IN WINTER?

Cowles (1941) observed that some reptiles in California deserts were active on warm winter days. But how can buried reptiles detect whether a given day was warm enough for activity? Cowles suspected that the heat pulse moving slowly down into the soil on sunny days was a cue of warm conditions on the surface, but only for shallow reptiles. For those at depth, the signal would be muted and delayed (Campbell and Norman, 1998). To explore Cowles's prediction, we used the microclimate model (Kearney and Porter, 2017) and the steady state heat-budget model in NicheMapR (Kearney and Porter, 2020) and predicted $T_{\mathrm{soil}}$, activity times, and metabolic rates for lizards (20-g) using fixed-retreats at a California desert site (Ford Dry Lake, 33.7° N, −115.1° W). [R code is in Supporting Information.]

Cowles (1941) did not address why some squamates emerge mid-winter. Activity might yield benefits such as energetic gains (feeding) or various physiological benefits, but activity will also induce risks (predation) and costs (increased energy expenditure). We address these issues below.

## Predictions

Squamates overwintering at shallow depths at Ford Dry Lake are potentially active many more days in winter than are those at deep retreats (Fig. 5), as Cowles (1941) predicted. Predicted total activity time declines from 603-h at −2.5 cm to 9-h at −30 cm (November-March, Fig. 6a) (see Box 1). At −50 or −100 cm, activity ceases because warming signals (Box 1) do not penetrate that deep.

Cumulative energy cost at Ford Dry Lake is non-linearly related to depth and activity. Cost is high near the surface (−2.5 or −5 cm, probably reflecting high $T_{\mathrm{soil}}$ on warm days), declines at moderately shallow depths (−10 to −30 cm, Fig. 6b), and then increases with depth. Squamates using depths shallower than about −30 cm can become active and have high $T_b$ on suitable days (Fig. 5), but have higher energy costs than do inactive ectotherms using the same depths (Fig. 6b). For example, a squamate active on warm days but otherwise is at −10 cm expends 2.3X-fold more energy per winter than a squamate staying a −10 cm. Thus, surface-active squamates may need to feed in winter, or risk depleting energy stores (see below).

Ford Dry Lake is relatively warm, but squamates overwintering there at shallow depths still face cold risks. Squamates

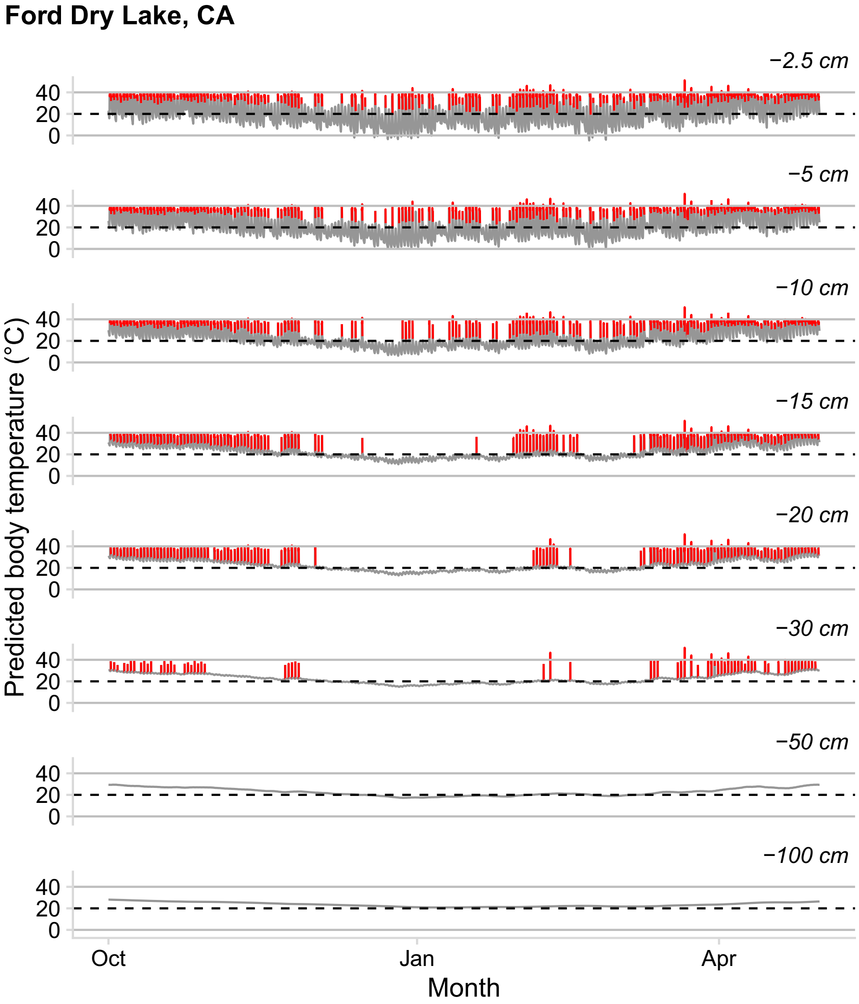


**Figure 5** Ectotherms that choose shallow retreats are active much more often in winter than are those overwintering deep in the soil. Plotted are predicted body temperature of 20-g ectotherms that retreat to various depths at Ford Dry Lake, CA. If $T_{\mathrm{soil}}$ at a given depth rises 0.1 °C/h and is above an assumed threshold (20 °C), the ectotherm emerges and becomes active on the surface if surface $T_e$ is ≥ 35 °C (see Box 1). Otherwise, it remains underground and has a $T_b = T_{\mathrm{soil}}$. Predicted $T_b$ is red for active animals, otherwise grey.

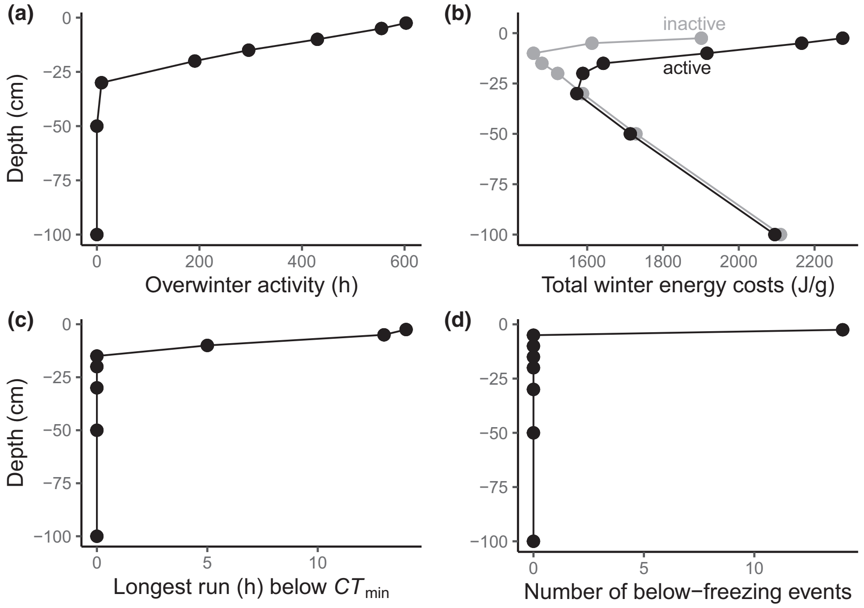


**Figure 6** (a) Cumulative hours of activity (potential) for a lizard spending inactive periods at various depths at Ford Dry Lake, CA for November – March (see legend Fig. 4). (b) Total energy costs for winter for ectotherms that remain inactive at fixed depths (grey symbols) or become active on warm 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.

at −10 cm or shallower experience sub-$CT_{min}$ temperatures (Fig. 6c), and those at −2.5 cm multiple sub-freezing events (Figs 5 and 6d). At colder localities (Fig. 3, Tables S4, S5), such risks will of course be elevated. The trade-off Cowles (1941) proposed is supported: shallow retreats permit activity but increase risk of cold death (Fig. 6).

## Implications

Which species are active in winter and where is largely unknown. In fact, Cowles (1941) is one of the few examples where activity of a community of squamate species is evaluated. Is mid-winter activity restricted to relatively cryophilic species in relatively warm areas? To our knowledge this question has not been asked.

Simulations of mid-winter activity require an environmental cue, a sensory capacity and a behavioural model. All are essentially unknown for squamates. Field studies are needed to determine cues that appear to trigger activity (Bishop and Echternacht, 2004; Nordberg and Cobb, 2016). Neurophysiological studies can evaluate whether squamates use temperature-compensated clocks (Heath, 1962) and whether they can detect and respond to temperature cues (Viitanen, 1967) when dormant.

Cowles (1941) assumed that squamates would emerge if warming $T_{soil}$ raised $T_b$ above some threshold level. Our simulations used a similar approach (a threshold plus warming cue, Box 1). Other models are possible (Heath, 1965). For example, emergence could be triggered by a temperature-compensated circadian clock (Heath, 1962). Such a clock 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 elevation sites. In any case, simulations can evaluate the reliability of likely thermal cues, either tested alone or in combination with a behavioural model (e.g., each day squamates move up to evaluate conditions before deciding to emerge).

Why some squamates even emerge mid-winter is essentially unknown. Some will feed (Congdon *et al.*, 1979; Goldberg and Bursey, 1990), but others are anorexic (Mayhew, 1964). For those that feed, digestion (which requires warm temperatures) becomes a challenge (Question 3, below). For those that are active but anorexic, their energetic expenses are increased (Fig. 6b) (Congdon *et al.*, 1979; Christian and Weavers, 1994; but see Nordberg and Cobb, 2016). Presumably some physiological benefits override energetic costs, perhaps by facilitating growth, repair, immune function, or future reproduction – if reserves are sufficient. In insects, brief warming re-establishes ion gradients, foraging, restores metabolic homeostasis and removes metabolic toxins (reviewed in Lee, 2010).

Thus, the ‘who, why, when and how’ of winter activity are open areas for research. Field observations can explore variation in winter activity and feeding, as well as predation risks while active (Wilson and Cooke, 2001). Physiological studies can determine whether feeding is necessary in some regions as well as identify physiological benefits and costs associated with 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? At Ford Dry Lake, a 20-cm depth seems suitable for both summer (not too hot) and winter (not too cold). However, some reptiles shift macro-habitats between summer and winter (Christian *et al.*, 1983; Kearney, 2002), and high-latitude ones often migrate to hibernacula (Parker and Brown, 1974; Gregory, 1982;

Nordberg and Cobb, 2017). *Uta stansburiana* (Baird & Girard, 1852) in central California use the same retreat sites in summer and winter (B. Sinervo, personal communication); but those in central Oregon may seasonally migrate between desert scrub habitat and 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 cumulative (maintenance) energy expenses relate to depth (see Box 1). Also, for ectotherms that feed mid-winter, we estimate depths that maximize digestion rate. Finally, we evaluate whether being able to shift depths conserves energy relative to staying at one depth.

For these analyses we assumed that M × T relationships are geographically and temporally stable (Box 1). For insects, interspecific M × T relationships steepen with latitude (Irlich *et al.*, 2009), but mainly at latitudes above the continental USA (M. E. Dillon, personal communication). M × T of reptiles can shift (positively, negatively or not) from active to dormant periods (Halpern and Lowe, 1968; Patterson and Davies, 1984; Tsuji, 1988; Christian *et al.*, 1999; Angilletta, 2001b; de Souza *et al.*, 2004), but we assumed that they were static within dormancy. Geographical and temporal variation could be incorporated in simulations – where known.

### Predictions

*Cumulative energy costs at fixed depths*

During cool months (specifically, October through February), cumulative energy costs (by month) were generally lowest at −5 cm at many sites (≥ 39.3% of sites, Table S7A); but during warm months (March–September), when near-surface temperatures are warm, cumulative costs were generally lowest at −50 or −100 cm (Table S7A). In contrast, cumulative costs were generally highest at −100 cm in cool months (October–March), but highest at −5 cm in warm months (April–September) (Table S7B).

When cumulative energy costs were calculated over one or several months, they were still lowest at −5 or −10 cm at most sites (Table 1). At many cold sites, however, cumulative rates were lowest deeper in the soil (Table S7), because 5 or 10 cm depths would likely be lethal and thus excluded (Table S4C).

*Cumulative metabolism of a "Panglossy" ectotherm*

Ectotherms that can change depth every hour [always moving to the coldest (above-$CT_{\min}$) depth, see Box 1] generally had lower costs than those at fixed depths (Table S8, Fig. S6a). The percentage saving was very small [median saving 1.9% ($Q_{10}$ = 2.0) to 3.4% ($Q_{10}$ = 3.0)]. Panglossy saving was small because winter energetic costs were generally lowest at shallow depths (see Table S7), and thus costs of a fixed-shallow-depth strategy are low. Moreover, Panglossy ectotherms would have to make many moves totalling long distances over winter (Fig. S6b and c). For our sites, the median number of depth changes was 187 (range 0–717). 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. We used NicheMapR (Kearney and 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 and f) are coded red for $T_{\text{soil}}$ warmer than $CT_{min}$ (assumed 9.3 °C for this analysis), and blue for $T_{\text{soil}}$ colder than $CT_{min}$. We also estimated the depth with the lowest energy costs each hour (Fig. 7b and d) and the cumulative energy costs (Fig. 7c and e). [Details are in Box 1 and Supporting Information.]

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 ($CT_{\min}$). 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 $CT_{min}$ (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 $T_{\text{soil}}$ always warmer than $CT_{\min}$ (9.3 °C), thus raising its metabolic rate. In contrast, 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, 1964; Congdon *et al.*, 1979; Goldberg and Bursey, 1990) and will need high $T_b$ for digestion (Angilletta *et al.*, 2002): this will require either basking (Regal, 1966; Nordberg and Cobb, 2016) or access to a warm retreat. Consequently, we calculated the depths at which digestive rates (see above) were highest (Table S9) 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. [Note: a spectral analysis of surface $T_e$ would indicate whether basking on the surface would likely be possible on subsequent days.]

In cold months (October-February), digestion rates were maximal at −100 cm at almost all sites (Table S9), reflecting relatively warm $T_{\text{soil}}$ at depth. In warmer months (April - September), however, digestion rates were instead maximal at −5 cm (Table S9), except at hot sites, where $T_{\text{soil}}$ at −5 cm would too hot (above $CT_{\max}$), forcing lizards to have deeper retreats).

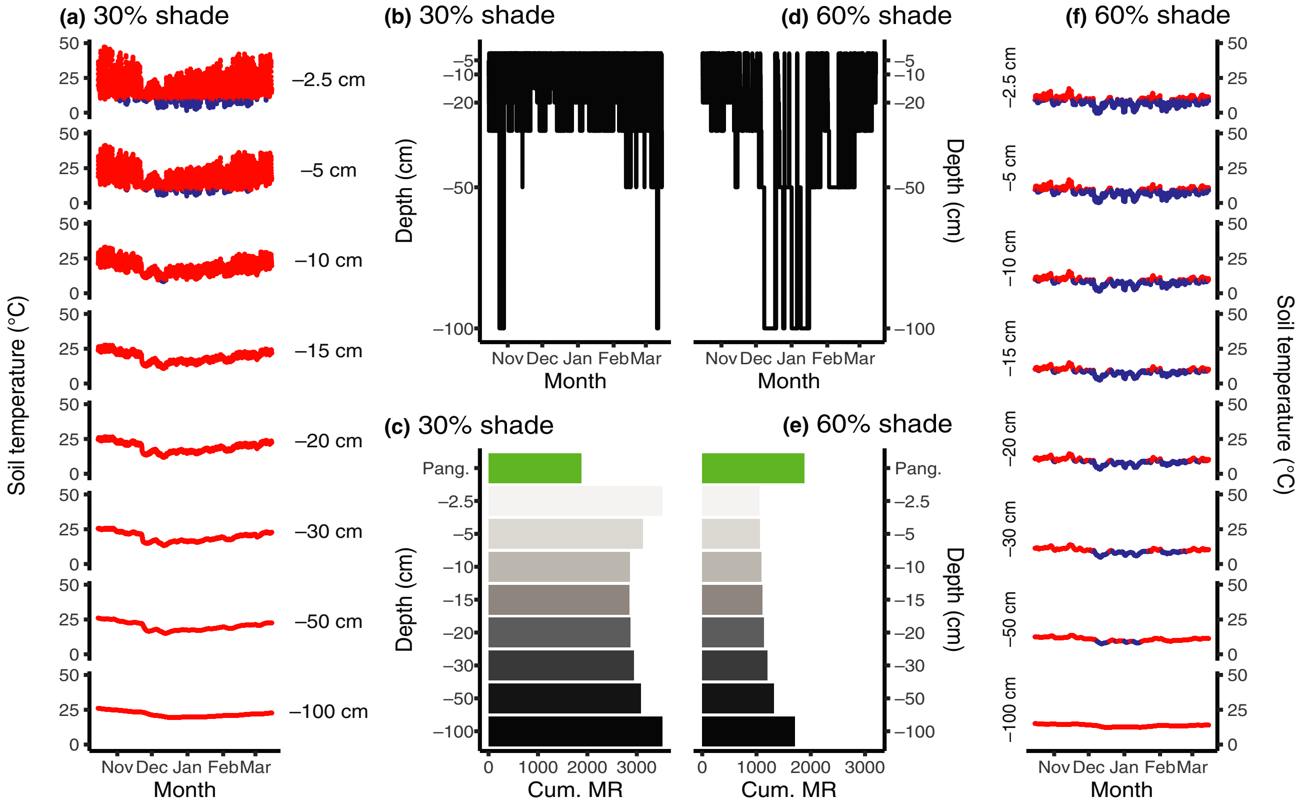


**Figure 7** Simulated soil temperatures at various depths for Ford Dry Lake, CA for 30% shade coverage (a) or 60% coverage (f). Red indicates $T_{soil} > 9.3$ °C. (b & d) Depths minimizing metabolic rate each hour over winter, contingent on $T_{soil} > 9.3$ °C). (c and e) Cumulative metabolic rate (ml $O_2$/h) for a Panglossy ectotherm (green) and for ectotherm at fixed depths (−2.5 cm to −100 cm: darker lines indicate deeper in soil).

## Implications

For individuals that do not feed in winter, a depth with low (but not lethally low) 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 × T relationships may shift seasonally, are non-linear (see Fig. 1 in Williams *et al.*, 2012), and because near-surface depths have both the lowest and highest temperatures (Fig. 3). Here, we used a M × T relationships tuned for reptiles (Andrews and Pough, 1985), and assumed that M × T relationships were fixed and thus independent of latitude, elevation and time during winter. However, M × T curves can shift geographically (Irlich *et al.*, 2009) and seasonally (Christian *et al.*, 1999; Guppy and Withers, 1999). For example, M × T curves of winter-active populations may be elevated ("compensation," see Tsuji, 1988), those of winter-inactive ones may be lowered ("reverse acclimation" or "metabolic depression," Patterson and Davies, 1978; Tsuji, 1988; Guppy and Withers, 1999), but those in tropical areas may not shift (Tsuji, 1988). Incorporating these and other sources of variation (seasonal, ontogeny, local adaptation, drift) in simulations is feasible, but only if actual patterns are known for diverse taxa and regions. Currently they are not.

Our simulations suggest that cumulative energy costs (October- February) were generally lowest at shallow depths (Table 1), though not at every site (Fig. 6b). Shallow depths can be warm during the day, which will elevate metabolism, but winter days are short and often inclement. Nevertheless, ectotherms at relatively cold sites will need to move deep to avoid cold injury or death (Fig. 4, Table S4) (Tucker and 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 and d). Moreover, Panglossy ectotherms that use retreats with different shading will use very different depths (Fig. 7b and d). Over the entire winter, Panglossy ectotherms can generally save energy by moving to the coolest (but > $CT_{min}$) depth, but the magnitude is surprisingly small (median saving = 1.9% to 3.4%, see Table S8), and this benefit would likely be swamped if the cost of movement were deducted (Wu *et al.*, 2015). Paradoxically, a fixed-depth strategy sometimes results 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 $T_{soil}$ 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 S9), simply because temperatures are warmest there (Fig. 2, Table S2B). If below-ground $T_{soil}$ are insufficient for digestion, fed ectotherms will need to bask when feasible or perhaps regurgitate when not (Regal, 1966; Nordberg and Cobb, 2016). We will return to digestion in the Synthesis and Research agenda section.

What is an ecologically realistic protocol in the laboratory for estimating cumulative energetic expenses during winter? To our knowledge, such a model does not exist. Available M × T data are usually based on acute temperature shifts, but an ecologically realistic one should mimic specific conditions ectotherms experience underground throughout dormancy (Nordberg and Cobb, 2017). Therefore, temperature profiles should drop very slowly and incorporate fluctuations (Fig. 3, Fig. S5) and not follow traditional “drop-and-hold” exposures. Also shifting photoperiods would seem appropriate (Tsuji, 1988), but ectotherms buried underground will be in full darkness (unless they emerge during the day), as light rarely penetrates even 5 mm into the soil (Tester and Morris, 2006). Housing conditions should allow ectotherms to bury themselves in the soil (or use burrows), and M × T relationships should be measured multiple times over winter. Thus, traditional laboratory protocols, which are usually designed to partition thermodynamic from acclimation effects (Havird *et al.*, 2020), do not match natural environmental exposures and are thus have questionable for estimates of overwinter expenses.

For a field approach, one could release animals into field enclosures in the autumn, allow them to bury themselves or manually bury them at fixed depths (Tucker and Packard, 1998), if one were interested in depth effects. One could dig them up at intervals and acutely measure their M × T patterns (Bartlett, 1976, Kevin Roberts, personal communication).

A very different approach would be to simulate whether known variation in M × T is large enough to alter designation of optimal depths or even to simulate how large M × T shifts (e.g., depression, compensation) 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).

The extent of winter activity and winter feeding is understudied, as geographic patterns are known only for few species. *Sceloporus occidentalis* (Baird & Girard, 1852) are winter active in southern California but not in Washington (Tsuji, 1988). Low-latitude populations of *Uta stansburiana* can be active in winter (Wilson and Cooke, 2001) and will feed (B. Sinervo, personal communication), whereas higher-latitude ones can also be active (Wilson and 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 bask in winter – but not feed – will of course deplete metabolic reserves (Case, 1976; but see Nordberg and Cobb, 2016). Presumably basking brings compensatory benefits (Tinkle and Hadley, 1973; Stieler *et al.*, 2006; Lee, 2010; Zani *et al.*, 2012) or reduces risk of freezing from ice-nucleating agents in guts (Bale, 2002). Little is known about such benefits for squamates and whether those benefits vary ontogenetically, geographically and interspecifically. In any case, if non-feeding emergence and basking are beneficial to some species and populations, why are they not beneficial to all? The occurrence and physiological consequences of activity vs. inactivity and feeding vs. anorexia in winter are essentially unexplored in squamates and thus offer diverse opportunities for field and laboratory research.

## SYNTHESIS AND RESEARCH AGENDA

Winter dormancy presents ecological and physiological challenges. Our project was inspired by Cowles's (1941) insights on the winter biology of squamates. We explored three questions involving how overwinter depth affects risk of cold death, metabolic expenses, and opportunities for activity. Because empirical field data do not exist, we used simulations to address these questions. In every case, however, we discovered that the field and laboratory data necessary to ‘map’ $T_{\mathrm{soil}}$ onto physiological and ecological consequences – and to test derived predictions – are either non-existent or of uncertain relevance. In winter biology, there are both previously unrecognized gaps as well as gaps that are recognized but that have lain dormant for decades. Accordingly, we outline a research agenda for the winter biology of ectotherms (Box 2). This agenda is neither exhaustive nor always original (Williams *et al.*, 2014), but focuses on issues germane to themes discussed herein. In all cases we encourage studies that incorporate seasonal, ontogenetic, geographic, interspecific and individual variation.

Implementing this agenda will be challenging. But some solutions are evident. The technique that Cowles (1941; Fig. S2) 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 with temperature-sensitive loggers (Davis *et al.*, 2008; Bernstein and Black, 2009; Nordberg and Cobb, 2017). If temperature recorders are also placed at likely retreat positions and depths, overwinter positions and movements can be inferred (Macartney *et al.*, 2011; Moore *et al.*, 2018). Above-ground activity can be indicated by rapid $T_b$ shifts (Harris *et al.*, 2015; Nordberg and Cobb, 2016) or from dataloggers that record light level (Davis *et al.*, 2008). Mesocosm experiments are feasible (Elfström and Zucker, 1999; Gao *et al.*, 2015). These are indirect methods, and they should be accompanied by direct natural-history observations where feasible.

As regards physiological studies of metabolism and of cold tolerance, we have argued (above) that acclimation regimes and conditions should approximate natural ones; traditional rapid cooling or ‘drop and hold’ protocols do not match natural thermal profiles (Fig. 2, Fig. S6, Sinclair, 2001b; Nordberg and Cobb, 2016). Ecologically relevant designs – especially cold tolerance assays – are most feasible with invertebrates.

A major puzzle involves animals that are active on warm days but do not feed (see above). Does activity (and elevated $T_b$) enable active ectotherms to clear accumulated and toxic by-products (Stieler *et al.*, 2006; Lee, 2010), recover from infections (Harris *et al.*, 2015), prime reproductive capacity (Tinkle and Hadley, 1973), readjust metabolic stores (Zani *et al.*, 2012) or synthesize vitamin D (Ferguson *et al.*, 2003)?

**Box 2. An agenda for studies of overwinter biology of ectotherms**

FIELD STUDIES

(1) Document body temperature profiles throughout winter.
(2) Determine overwintering microsites used by ectotherms. Do animals shift depths or positions during winter or stay at fixed sites?
(3) Quantify winter patterns of activity and of feeding. Explore ecological (condition, growth, life history) consequences.
(4) Measure variation in overwinter mortality and causes thereof. Determine whether animals die of cold, starvation, desiccation, 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 dormancy. Relate metabolic shifts (e.g., compensation, depression) to ecology and distribution.
(2) Quantify physiological costs and benefits of winter activity with and without feeding.
(3) Determine environmental and internal cues that initiate and terminate activity in winter. Do these vary seasonally, geographically, ontogenetically and interspecifically?

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 the sign of the derivative of $T_b$ vs. time (Heath, 1965; Viitanen, 1967), or some combination thereof? Do cues vary geographically and phylogenetically? Experiments are needed, and simulations can guide designs for strong-inference experiments.

On a personal level, we concede that we have largely focused our own field studies on the thermal biology of ectotherms in warm seasons. But despite our personal ‘dormancy’ from the field in winter, many ectotherms in nature experience winter – sometimes long winters. We join others in arguing that the overwinter ecology of ectotherms offers rich opportunities for exploration, including impacts of climate change and of evolutionary shifts (Bradshaw and Holzapfel, 2006; Zani, 2008; Bradshaw and Holzapfel, 2009; Williams *et al.*, 2014). Simulations studies will be useful here.

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 and an early example of trade-off thinking in ecology. However, only 56 papers have cited Cowles (1941) (Web of Science, accessed 2020-09-11), and none mentions Cowles’s recognition of trade-offs involving overwintering depth. This oversight needs to be corrected.

## ACKNOWLEDGEMENTS

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## AUTHORSHIP

RBH and MRK conceived the study, LM and OM added concepts, all authors performed analyses, RBH and MRK wrote the first draft of the manuscript. All authors contributed to the ideas and writing of the manuscript.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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