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RESEARCH ARTICLE

Cycling temperature treatments affect estimates of digestive performance in prairie lizards (*Sceloporus consobrinus*)

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ABSTRACT

In nature, many organisms experience a daily range of body temperatures. Thermal performance at stable temperatures is often extrapolated to predict function in cyclical environments. However, temperature order and cyclicity may influence physiological processes. The current study compared energy intake, digestive passage time and energy budgets at a stable temperature (33°C) and two temperature cycles in lizards (*Sceloporus consobrinus*), to determine (1) whether stable treatments adequately project performance in a cycling environment and (2) whether temperature order influences performance. Cycles had a mean temperature of 33°C, and rotated through 30°C, 33°C and 36°C daily, with equal durations of time at each temperature but differing temperature order, with warm days and cool nights in cycle 1 and cool days and warm nights in cycle 2. For analyses, performance in the stable treatment was compared with that during cycles. If temperature is the primary factor regulating performance, then performance from the stable treatment and cycles should compare favorably. However, physiological performance varied based on temperature treatment. Energy intake and budgets were similar between the stable trial and cycle 1 but not cycle 2. However, passage time did not differ. Notably, the two cycling regimes consistently varied in performance, indicating that temperature order plays a primary role in regulating performance. Physiological data collection requires careful consideration of effects of cycling versus stable temperature treatments. Stable temperatures do not consistently represent performance in cycling regimes and consideration should be paid not only to which temperatures animals experience but also to how temperature is experienced in nature.

KEY WORDS: Lizard, Energetics, Thermal performance, Constant, Passage, Consumption

INTRODUCTION

Many organisms experience a range of environmental temperatures over the course of a day or season in nature. Ectotherms in particular are susceptible to fluctuations in body temperature, as nearly all of their physiological functions exhibit thermal sensitivity (Huey, 1982). Consequently, ectotherms need to maintain adequate function in a variable thermal regime. As a result, behavioral and physiological mechanisms are implemented towards maintaining a preferred body temperature (Kefford et al., 2022). However, thermoregulation can be imprecise, and more challenging at different parts of the season or day, resulting in variation in body temperature (Huey and Pianka, 1977; Ortega and Pérez-Mellado, 2016). Understanding how organismal performance varies in response to daily temperature cycles and the order of temperature within a cycle, as opposed to just considering temperature alone, may be a critical advancement in thermal biology and in making predictions about novel environments for ectothermic organisms.

There are multiple ways in which researchers attempt to understand the relationship between performance and temperature. One common method involves collecting data on processes of interest (e.g. growth rate, tadpole or embryonic development, digestive passage time, metabolic rate, etc.) at relevant stable temperatures (e.g. Sanger et al., 2018; Plasman et al., 2019). From there, data from each temperature are integrated and a curve is fitted to create a time–temperature weighted relationship (or to generate a thermal performance curve, TPC) (Huey and Stevenson, 1979; Niehaus et al., 2012). The TPC is then used to predict organism function in a fluctuating (or cycling) environment (e.g. Hertz et al., 1983; Pinch and Claussen, 2003; Telemeco, 2014). Researchers also use TPCs to hypothesize how organisms evolve in response to environmental conditions over space, time and taxa (Huey, 1982; Gvoždík and Van Damme, 2008; Malusare et al., 2023). Quantifying performance under stable temperatures usually results in researchers finding a threshold where activity ceases, or begins to decline after reaching an optimum, as a result of chronic exposure to thermal extremes (Huey, 1975; Kaufmann and Bennett, 1989). However, stable temperatures and chronic exposure to extreme temperatures do not necessarily mimic what an organism experiences in nature.

In nature, extreme temperatures may only be experienced for short periods during the day or season, which may not have the same effect on performance as chronic exposure in lab trials (Sinclair et al., 2016; Morash et al., 2018). However, standard methods using TPCs assume function is solely based on body temperatures experienced. Therefore, TPCs fail to account for possible variation in performance throughout the day due to acute temperature fluctuations and the order in which temperature is experienced in cyclical and fluctuating environments. Such thermal variation may be important for understanding processes occurring over long periods of time (e.g. digestion or metabolic rate), while immediate temperature may be suitable for understanding short-term processes (e.g. locomotor performance). Patterns in daily body temperatures, including order of temperatures experienced within a daily cycle, could play an influential role in regulating performance. While processes occurring at specific points in time (e.g. energy consumption) may be strongly regulated by immediate body temperature, rates of processes occurring continuously (e.g. digestion) may depend heavily on temperature order and cycles as they operate at multiple points throughout the day and night. Therefore, failure to consider cumulative performance in cycling

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environments, and in particular how the organism experiences temperature throughout the day, could result in misdirected understanding and projection of natural systems. Additionally, evolution of thermal tolerance and performance is likely not a result of chronic stable temperature exposure but instead due to cumulative function across daily and seasonal ranges. As a result of the heavy reliance on TPCs, there is currently a knowledge gap regarding the influence of daily thermal cycling on the physiology and evolution of thermal performance, and whether performance is dependent on the order of temperatures experienced or if temperature alone is the primary factor regulating performance.

As an alternative to measuring thermal performance in a lab, researchers also study processes in the field. Studying organismal body temperature and function at field-relevant temperatures can be done in a variety of ways, including measurement of body temperature (T_b) throughout the day and season with data loggers (Kerr et al., 2004), and taking cloacal (or internal) temperature with a thermometer upon capture (Congdon et al., 1979) or through temperature-sensitive radio telemetry (Lefcourt and Adams, 1996; Nordberg and Schwarzkopf, 2019). Thus, researchers can draw conclusions on performance in a natural setting, which may not be replicable in the lab. However, field studies have limitations, including the inability to directly assess the influence of specific biotic and abiotic changes of interest and a lack of control over factors not considered in the study (e.g. specific temperatures, humidity, rainfall, predation, prey availability, etc.) (Dunham and Beaupre, 1998). Therefore, direct causation cannot always be inferred from a field study, and estimates of future temperature scenarios from field data can be hard to predict. It is evident that assessments are needed to identify whether differences occur in physiological data collected under cycling and stable temperature treatments and varying orders of temperature. Concerns about how temperature influences organisms have become of greater importance because of climate change. Climate change is expected to alter daily temperature ranges (Easterling et al., 1997), not just mean temperature. Lab experiments are critical for understanding how temperature shifts drive change in organisms because they allow researchers to identify direct causality. When modeling temperature effects, useful predictions require empirical data under realistic scenarios that organisms experience in nature. However, many studies to date, especially with regards to adult reptiles, rely on stable temperature treatments, making it difficult to interpret results in relation to realistic temperature regimes (e.g. Beaupre et al., 1993; Angilletta, 2001; Isaac and Gregory, 2007; Racic et al., 2020).

Attempts to extrapolate data from stable treatments to predict outcomes in cycling regimes have proven difficult. For example, Niehaus et al. (2012) measured metamorphic duration in striped marsh frogs (*Limnodynastes peronii*) at various stable and cycling scenarios. Measurements at stable temperatures were used to generate a temperature-dependent metamorphic rate to predict results in the daily cycling treatment. At most stages, individuals developed quicker than predicted using the stable temperature trials (Niehaus et al., 2012). Whereas some studies indicate data differ when collected under stable and cycling temperatures (Bestgen and Williams, 1994; Radmacher and Strohm, 2011), there is a lack of research on differences in physiological data. For example, experiments comparing stable and cycling temperature treatments have found differences in development, growth and maturation (Meeuwig et al., 2004; Dhillon and Fox, 2007; Kern et al., 2015; Kingsolver et al., 2015; Coulter et al., 2016), egg development (Li et al., 2013), morphology (Du and Ji, 2006; Patterson and Blouin-Demers, 2008), reproductive output (Podrabsky et al., 2008; Žák and Reichard, 2020), thermal tolerance (Arias et al., 2011) and genetics (Podrabsky and Somero, 2004). However, physiological parameters, especially those relating to energy acquisition and allocation, are critical to making mechanistic predictions about individual- and population-level processes (Dunham et al., 1989; Kearney, 2012). Comparisons of physiological performance at stable and daily cycling regimes, as well as at different orders of temperature exposure, are needed. Such information would provide meaningful data for informing methods of future physiological studies and making predictions with bioenergetic models. Comparing performance under various temperature cycles would provide additional information on how cyclicity in daily temperatures influences physiological performance.

The objectives of this study were to (1) determine whether rates of physiological processes are comparable under stable and cycling temperature regimes and (2) determine whether temperature order, as opposed to temperature alone, influences rates of physiological processes. Prairie lizards (*Sceloporus consobrinus*, Baird and Gerard) were used as a model organism for the current study, as the genus *Sceloporus* is often used in thermal biology and climate change models (e.g. Buckley, 2008; Sinervo et al., 2010), with known sensitivity to temperature change (Beaupre et al., 1993; Angilletta, 2001). The physiological parameters assessed were rate of energy consumption, digestive passage time, metabolizable energy intake (MEI) and assimilated energy (AE). These parameters were chosen because they directly relate to energy acquisition and assimilation, which influences total energy budgets. Energy budgets are important to consider because they are often affected by temperature and limit allocation to growth, reproduction, storage and maintenance, which in turn affects individual fitness and population-level processes (Congdon and Tinkle, 1982; Dunham et al., 1989; Porter et al., 1994).

To make comparisons, performance was assessed under two temperature cycles with the same daily average temperature, and one stable temperature treatment reflecting the mean temperature of the cycles. The two cycles had the same temperatures, but different order of exposure. The first cycle (hereafter ‘cycle 1’) represented warm days and cool nights, whereas the second cycle (hereafter ‘cycle 2’) represented cool days and warm nights. Temperature cycles were selected to ensure the only variable differing between cycle 1 and 2 was order of temperatures experienced, not duration of exposure or the temperatures themselves. If stable treatments mimic performance under daily temperature cycles and temperature order is insignificant, then performance at a stable 33°C should compare favorably to both cycles, with the two cycles yielding identical results. We hypothesized that energy consumption, MEI and AE would be higher, and passage time would be lower in cycle 1 than predicted by the stable trial. The rationale is that lizards will experience warm days promoting rapid digestion and high rates of consumption prior to reaching cooler night-time temperatures in cycle 1. Meanwhile, the stable treatments is most likely going to be moderate in comparison because of processing at a single, average temperature. We also hypothesized that energy consumption, MEI and AE would be lower, and passage time would be higher in cycle 2 than predicted by the stable 33°C treatment, as a result of the cold days. We also predicted that cyclicity and temperature order play an influential role, altering rates of the physiological processes.

MATERIALS AND METHODS

Field collection

Male and female adult *S. consobrinus* were collected in northwest Arkansas in 2020–2022, ranging in size from 48 to 70 mm snout–

vent length (SVL; *n*=39). Within 1 min of capture, lizard body temperature was measured using a fine-wire thermocouple (Type K) inserted into the cloaca and read with an Omega HH12B digital thermometer (Omega Engineering, Norwalk, CT, USA) to inform lab treatments. Lizards were immediately brought to the University of Arkansas and individually housed in 37.85 l tanks. Tanks contained a natural sand substrate, heat lamp and hide box, with water provided *ad libitum*. Lizards were fed a diet of crickets, supplemented with vitamin D every 2 weeks. All necessary permits were acquired for the research conducted (IACUC #19080, Arkansas Game and Fish Commission Permit #050120211).

Temperature treatments

Lizards were randomly assigned to one of three temperature treatments, stable 33°C (*n*=12), cycle 1 (*n*=12) or cycle 2 (*n*=15), with males and females as evenly divided as possible. The first cycling temperature treatment (referred to as cycle 1) consisted of a night-time temperature of 30°C and a daytime temperature of 36°C, with a sunrise and sunset temperature of 33°C during the transition between the low and high temperatures. To compare performance under different orders of temperature cycles, the second cycling temperature treatment (referred to as cycle 2) consisted of a night-time temperature of 36°C and a daytime temperature of 30°C, with a sunrise and sunset temperature of 33°C during the transition between the low and high temperatures. By flipping the timing of warm and cool temperatures, we can determined whether the order of temperature within a cycle alters performance, or whether temperatures experienced daily is the primary factor of influence. An additional reason for flipping temperatures in the cycle is to allow assessment of the dependence of digestion on day–night cycles. The typical circadian cycle for *S. consobrinus* follows a diurnal pattern, where lizards are active during the day, and inactive at night.

The amount of time spent at each temperature during cycling trials was identical for a 24 h period. One full daily cycle totaled 6 h and 40 min at each temperature, with a 1 h transition period for the environmental chamber to reach the next set point (Fig. 1). The average body temperature experienced by lizards in both cycles was 33°C, which is why it was selected for comparison as the stable temperature treatment. The temperature treatments were selected based on body temperature profiles in the field, quantified by inserting an Omega thermocouple (K) into the cloaca immediately upon capture. Body temperatures were maintained during trials using an environmental chamber.

Physiological data collection

For lab trials, lizards were housed in plastic containers (41.9 cm×33 cm×16.8 cm) lined with heavy duty, uncoated paper (butcher paper), with a hide box and water provided *ad libitum*. Prior to beginning trials, lizards were acclimated to their respective temperature treatment for 5 days. At the beginning of the acclimation period, lizards were fed one meal to allow for digestion at the treatment temperature, and then fasted to ensure the gut was clear. Temperature was maintained using two environmental chambers (±0.5°C). During trials, live Fluker's 2 and 3 week old crickets were weighed and then fed to lizards *ad libitum* every morning. The uneaten crickets were removed after ∼3 h, and their mass was subtracted from the mass offered to determine mass of crickets consumed (weighed to the nearest 0.1 mg). Digestive passage time (hours) represents the time it takes to pass food from consumption to excretion. MEI is a measure of the maximum potential energy to be allocated to growth, maintenance, storage and reproduction, and was calculated using the formula:

$$\mathrm{MEI} = C - F - U, \tag{1}$$

where C is energy consumed, F is energy lost as feces and U is energy lost as uric acid, measured in kilojoules. Assimilated energy represents digestible energy, and was calculated using the formula:

$$\mathrm{AE} = C - F. \tag{2}$$

To begin trials, a single cricket was injected with a marker, which was a slurry made by mixing inert UV-fluorescent powder (Scientific Marking Materials Inc., Seattle, WA, USA) with water (Beaupre et al., 1993; Beaupre and Zaidan, 2012). The fluorescent powder associates with feces and does not influence the edibility of crickets, so lizards consumed the marked crickets voluntarily. Trials began when lizards ate the first marked cricket, and the time of feeding was noted. Lizard tanks were then monitored every 2–4 h during the day for feces until the fluorescent powder was identified using a UV blacklight, indicating passage time (amount of time from marker consumption to first appearance in feces). After the first marker was excreted, typically 10 or more days were allotted to feed lizards and collect feces and urate for adequate measurement of consumption and bomb calorimetry. Afterwards, a second marker was fed, and tanks were monitored again every 2–4 h. Once the second marker appeared in feces, the trial was considered complete.

During trials, all feces and urates were collected, separated, frozen and freeze dried. To quantify energy ingested (consumption rate), wet mass was measured for 10 crickets, which were then freeze

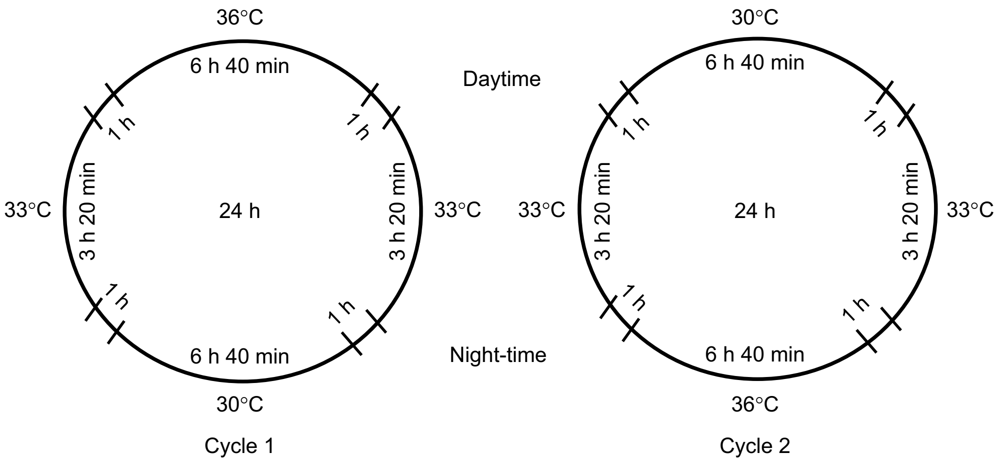

Fig. 1. Cycling thermal regimes. The diagrams depict the temperatures and durations experienced by lizards in the two regimes: cycle 1 (*n*=12) represented warm days and cool nights, while cycle 2 (*n*=15) represented cool days and warm nights. Within a 24 h period, the temperature cycled through 30°C, 33°C and 36°C for both cycles, with an equal duration for each temperature. A 1 h period was allotted for transition between temperatures.

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dried, and re-weighed. The relationship between wet and dry cricket mass allowed for conversion of wet cricket mass consumed to dry cricket mass consumed. Using a Parr Semimicro Calorimeter, the energy density of crickets was determined, which was used to convert dry cricket mass consumed to kilojoules consumed for each lizard. To determine fecal and urate production (kJ), excrement samples collected during the trials were pooled for individual lizards, weighed, homogenized, and analyzed using a Parr Semimicro Calorimeter. MEI and AE could then be calculated.

Statistical analyses

Analyses of covariance (ANCOVA) were performed to determine the effect of temperature treatment on energy consumption (kJ), digestive passage time, MEI (kJ) and AE (kJ). Comparisons were made among performance at stable 33°C, cycle 1 and cycle 2, as 33°C represents the mean temperature experienced by lizards in each cycle. When assessing energy consumption, SVL was also included as a covariate and an interaction term of SVL×treatment was included to test for heterogeneous slopes. For MEI and AE, energy consumption was included as a covariate and an interaction term of energy consumption×treatment was included to test for heterogeneity of slopes. *Post hoc* analyses were made by comparing adjusted means and 95% confidence intervals (CI) generated from adjusted means (Day and Quinn, 1989). Significance among treatments was determined based on non-overlapping CIs with adjusted treatment means, assuming the probability of a type 1 error is 0.05. The residuals of analyses were examined to determine whether the assumptions of parametric statistics were met. All analyses were conducted using R Statistical Programming Software (http://www.R-project.org/).

RESULTS

The analyses of residuals indicated slight variation from normality in some instances; however, all followed a hump-shaped distribution and were deemed suitable for the robust procedures of ANCOVA (Blair, 1981). The average body temperature of *S. consobrinus* in the field was 32.4°C and ranged from 22.9 to 36.7°C. Therefore, the temperature treatments implemented were within the range of active body temperatures. Energy consumption significantly differed among treatments (ANCOVA, F=9.157, P<0.001, d.f.=2,33; Fig. 2A), with no effect of lizard SVL (covariate, F=1.234, P=0.275, d.f.=1,33). The *post hoc* Tukey test indicated that energy consumption was significantly lower in cycle 2 compared with cycle 1 (CI: −1.465, −15.291; P=0.015) and stable 33°C (CI: −4.577, −18.402; P<0.001). Passage time did not differ among treatments (ANCOVA, F=2.649, P=0.085, d.f.=2,36; Fig. 2B).

When comparing MEI among all trials, there was a significant interaction between temperature treatment and energy consumed (ANCOVA, F=657.667, P<0.001, d.f.=2,33), preventing further combined analyses of all treatments. Through utilization plots (Fig. 3A), it was determined that the slope for cycle 2 relating MEI with energy consumption was lower than the slopes for cycle 1 and stable 33°C. There was also a positive effect of energy consumed on

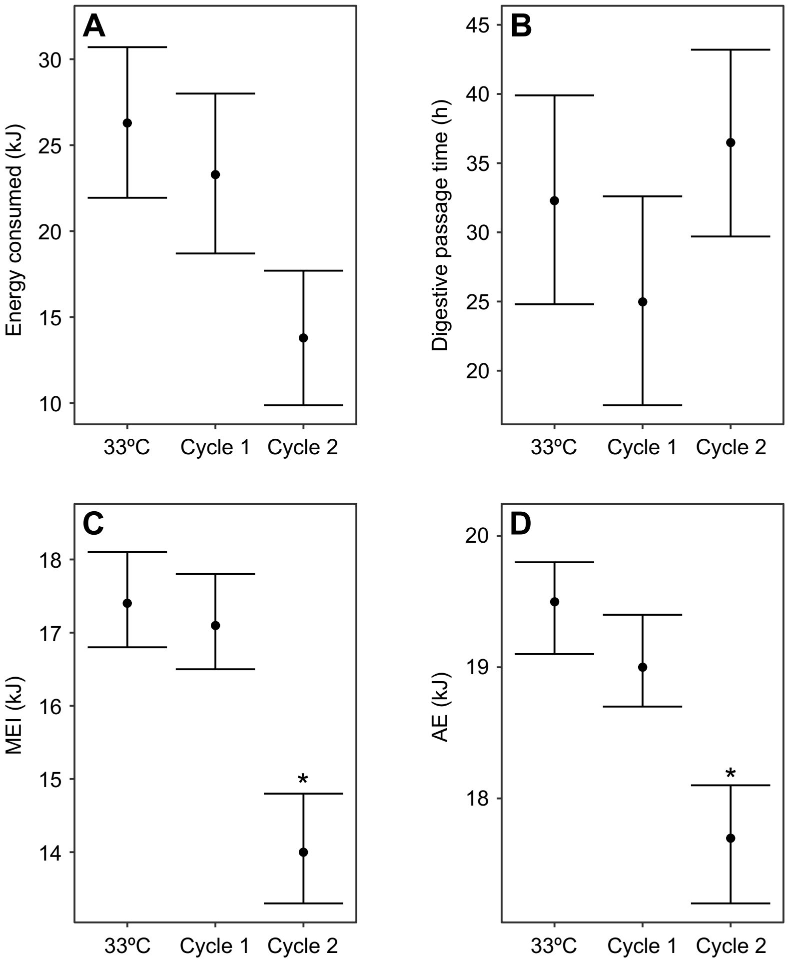

Fig. 2. Physiological performance of prairie lizards (*Sceloporus consobrinus*) under stable and cycling thermal regimes. Lizards were exposed to a constant temperature of 33°C (*n*=12), or to cycle 1 (*n*=12) or cycle 2 (*n*=15) with an average temperature of 33°C but differing temperature patterns. (A) Energy consumption was significantly lower in cycle 2 where lizards experienced cool days and warm nights, compared with that in cycle 1 (warm days and cool nights) and stable 33°C (ANCOVA, F=9.157, P<0.001, d.f.=2,33). (B) Digestive passage time was not significantly affected by temperature treatment (ANCOVA, F=2.649, P=0.085, d.f.=2,36). (C,D) There was an interaction between energy consumption and temperature treatment in relation to metabolizable energy intake (MEI; C) and assimilated energy (AE; D), where MEI and AE increased at a slower rate with energy consumption in cycle 2 (ANCOVA, F=657.667, *P<0.001, d.f.=2,33). However, MEI and AE were higher under stable 33°C and cycle 1, compared with cycle 2. The plot depicts means adjusted for energy consumption and error bars representing 95% confidence intervals calculated from adjusted means, for all treatments and the pooled average.

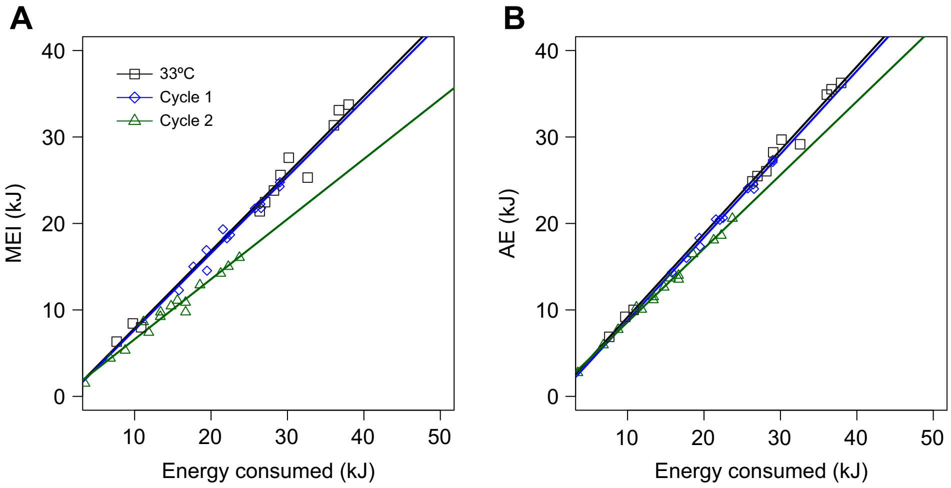

Fig. 3. Energy utilization of prairie lizards under stable and cycling thermal regimes. MEI (A) and AE (B) increased with energy consumption at a slower rate in cycle 2 (*n*=15), compared with stable 33°C (*n*=12) and cycle 1 (*n*=12). The points indicate metabolizable energy intake (MEI) for lizards experiencing a stable 33°C (black squares), a cycling regime with warm days and cool nights (cycle 1, blue diamonds) or cool days and warm nights (cycle 2, green triangles), with linear trend lines.

MEI (covariate, *F*=1575.379, *P*<0.001, d.f.=2,33). Despite the inability to statistically compare the temperature treatments with regards to MEI, it can be observed when viewing the adjusted means for energy consumed that MEI was overall higher in cycle 1 and at a stable 33°C, than in cycle 2 (Fig. 2C). AE also had a significant interaction between energy consumption and treatment (ANCOVA, *F*=7.653, *P*=0.002, d.f.=2,33; Fig. 3B). Again, the slope relating AE to energy consumption was lower for cycle 2 than for cycle 1 and at a stable 33°C. There was also a positive effect of energy consumption (covariate, *F*=5709.367, *P*<0.001, d.f.=1,33) on AE. AE followed the same pattern as MEI, where AE was overall higher in cycle 1 and stable 33°C treatments, compared with that in cycle 2 (Fig. 2D).

DISCUSSION

Physiological performance can differ when collected under stable and cycling temperature treatments, and variation is process dependent. To compare performance under stable temperature versus cycling regimes, physiological data were collected on *S. consobrinus* lizards when experiencing two temperature cycles with a mean temperature of 33°C, and at a stable 33°C. A common assumption is that performance under stable temperatures accurately represents rates in cycling regimes, with no regard to temperature pattern (Sinclair et al., 2016). If true, the rate of processes when lizards experienced 33°C should not have differed from the cycling regimes, in the current study. However, not all processes responded similarly to temperature cycling, and unique results were found based on temperature order. Specifically, in cycle 1 (warm days, cool nights), energy consumption, MEI and AE were comparable to performance at 33°C. Therefore, extrapolation of such variables from stable treatments to cycling regimes may be possible. However, this may only be the case when the cycling regime has the same mean temperature as stable treatments and performance occurs near the overall mean temperature. Lizards were fed in the morning, when experiencing temperatures from 33°C (sunrise) to 36°C (daytime) for cycle 1. One possible explanation of the observed trend is that in cycle 1, lizards were consuming food when their body temperature was transitioning from 33°C to 36°C, and therefore consumption rates were high, and comparable to those for the stable 33°C treatment. As a result, MEI and AE followed the same pattern because they are heavily influenced by energy consumption. However, in cycle 2, lizards were cooling down near the time of energy consumption, resulting in lower performance. We expected to find that passage time and energy consumption follow similar patterns, as food cannot be consumed if the gut is full. However, digestive passage time did not differ among temperature treatments. With passage being a continuous process, it is possible that passage occurred at nearly all temperatures within a cycle, and is therefore comparable among cycles and with the stable 33°C (a reflection of the mean temperature within cycles).

In addition to cycling regimes, the order of temperature within a cycle may play a crucial role in rates of physiological processes over the course of a day. Cycle 2 was designed to reverse the temperature order of cycle 1 to assess the influence of temperature pattern and the potential dependence on day–night cycles for digestion. Temperature order played an important role in digestion, because physiological performance drastically differed between cycles, but this was likely due to temperature and not circadian cycles. Energy consumption was lower in cycle 2 than in the other treatments. In cycle 2, lizards were fed when body temperatures were undergoing the transition to the cooler temperature, 30°C. Additionally, there was an interactive effect of temperature and energy consumption on MEI and AE. The assimilation of energy in cycle 2 was lower than when lizards were in cycle 1 or at a stable 33°C. While the heterogeneity of slopes prevented statistical comparisons of MEI and AE, the overall values were consistently lower in cycle 2 (slopes never intersected). These findings indicate that temperature order plays a critical role in the efficiency of digestive processes. Moreover, the relationships among variables are not static across temperature regimes for stable and cycling treatments, and therefore extrapolation cannot be reliably made from stable temperature treatments to predict performance in a cycling environment, especially when cycles differ in temperature order.

While the current study used drastically different temperature cycles to determine whether temperature order influences physiological performance, it is likely that differing orders in nature exist that could influence performance. In particular, organisms may experience different daily temperature patterns with regards to duration of exposure, seasonal changes, variation in habitat and the thermal landscape, and the changing thermal climate. Similar differences among temperature cycles have also been observed in growth and developmental rates (Kern et al., 2015; Kingsolver et al., 2015; Verheyen and Stoks, 2018; Vajedsameil et al., 2021) and thermal tolerance and survival (Bozinovic et al., 2016). Therefore, the timing of temperatures experienced by organisms in nature plays a highly influential role in dictating performance. Consequently, temperature experienced is not the sole factor that should be considered when aiming to understand performance in a natural, cyclical environment.

Energy consumption is an essential component for predicting energy budgets (e.g. Kitchell and Windell, 1972; Hudson et al., 2021). It was found that energy consumption is the most important determinant of MEI. One approach to *ad libitum* feeding in lab studies on lizards is to offer food all at once (e.g. Van Damme et al., 1991; Levy et al., 2017) and then to remove uneaten food after a certain amount of time. The method of offering food at a single time was implemented in the current study, and therefore lizards primarily ate at a single point in the temperature cycle (typically sunrise or early in the daytime temperature cycle). As a result, energy consumption reflected the body temperature at which lizards were offered food and may not have been influenced by the cycle itself. The cycles used here had a single daytime temperature. In nature, lizards experience a range of body temperatures throughout the day (Light et al., 1966; Bauwens et al., 1999). It is possible that animals do not just consume food in the morning or all at once, and instead eat throughout the day. However, few data are available on feeding frequency of lizards in nature. If consumption does occur throughout the day, natural fluctuations in body temperature may alter rates of energy consumption based on the body temperature when food is encountered, with potential effects for resulting energy budgets. However, when estimating the influence of temperature cycles on brown trout (*Salmo trutta*), food was also offered at one temperature within the cycle and variation was still observed (Flodmark et al., 2004). A better approach to informing lab methods may be to first estimate frequency of feeding in the field to be replicated in lab trials. By doing so, researchers could better understand how cycling regimes and temperature influence consumption.

In contrast to energy consumption, digestive passage time was not restricted to a single temperature within the cycle, as it is a continuous process that occurs until completion. While consumption can be influenced by passage time as gut clearance is required to create room to eat, this did not appear to be heavily influential in our study as we identified differences in consumption rate but not passage time across treatments. Different patterns may be observed if lizards experience even colder temperatures, when digestion and consumption begin to halt (Alexander et al., 2001). However, the temperature ranges implemented are within realistic body temperatures that *S. consobrinus* experiences in nature throughout parts of the season, although lizards likely experience night-time body temperatures colder than 30°C. The results suggest that for processes occurring continuously throughout the day, stable temperature treatments do not accurately predict cycles. Moreover, the underlying relationships, such as the slopes between consumption and MEI and AE, differ in cycle 2, complicating the ability of researchers to extrapolate and compare data. Such findings would not have been identifiable had the study used ratios, such as apparent digestive coefficient (e.g. Chen et al., 2003; Pafilis et al., 2007), as opposed to ANCOVA, to compare digestive efficiencies among temperature treatments. Therefore, the current study contributes more evidence that using ratios to interpret digestive assimilation data is inappropriate (Raubenheimer, 1995; Beaupre and Dunham, 1995), and prevents adequate assessment of such complex processes.

The cycle 1 results suggest it may be reasonable to predict certain physiological processes in a cycling regime based on stable temperature data. However, making such extrapolations should be done with great caution, as passage time did not follow the predicted trend, and the order of temperatures altered results. Additionally, mechanisms underlying the differences identified are not fully understood. The consistency of temperatures in cycle 1 and the stable treatment, and the equal duration of time spent at each temperature within the cycle, are likely the reason for comparable findings for consumption, MEI and AE. If lizards experienced different durations at each temperature within a cycle, a different approach would be required where weights are applied based on hours at each temperature. The order of temperatures in a cycle plays an important role in organismal performance. The temperature when food was consumed was a critical factor because it influenced individual appetite and satiation. Consequently, temperatures experienced when consuming food have cascading effects on other digestive processes, as MEI and AE are heavily influenced by consumption, and passage does not begin until food is consumed. Therefore, the timing and duration of feeding relative to specific temperature cycles is also an important determinant of performance.

The influence of temperature on organismal performance has long been an area of interest (Porter and Gates, 1969). Recently, focus has increased on temperature effects due to climate change. Performance under daily temperature cycles is of particular concern because they more accurately represent nature, and daily cycles are beginning to shift (Morash et al., 2018). Evidence from multiple fields of study indicates that stable temperature treatments do not always accurately represent responses to daily temperature cycles (Meeuwig et al., 2004; Du and Ji, 2006; Dhillon and Fox, 2007; Podrabsky et al., 2008; Arias et al., 2011; Li et al., 2013; Kern et al., 2015; Kingsolver et al., 2015; Coulter et al., 2016). There is currently little information on how temperature cycles influence digestion and energy budgets. However, bioenergetics offers important insight into variation in life history, survival, reproduction and population dynamics (Dunham et al., 1989). Future studies should aim to increase understanding of how temperature patterns and cycles influence digestion and energy budgets. Additionally, studies of the interactive effects of feeding and temperature cycles as they influence digestive performance are needed. In conclusion, extrapolating rates of physiological processes from stable temperatures to daily cycles may provide inaccurate results. Additionally, underlying relationships between energy consumption and energy assimilation can differ based on temperature order. Directly collecting data under cycles of interest and aiming to represent patterns and behaviors used by organisms in the field will offer the most accurate estimate of performance. Such estimates will be invaluable for understanding how climate influences organisms, for making accurate predictions for future scenarios, and for understanding the influence of temperature on performance and the evolution of thermal tolerance.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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Data availability

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ECR Spotlight

This article has an associated ECR Spotlight interview with Allison Litmer.

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