

# Conserved ecophysiology despite disparate microclimatic conditions in a gecko

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## Abstract

Microscale differences in the habitats organisms occupy can influence selection regimes and promote intraspecific variation of traits. Temperature-dependent traits can be locally adapted to climatic conditions or be highly conserved and insensitive to directional selection under all but the most extreme regimes, and thus be similar across populations. The opposing slopes of Nahal Oren canyon in the Carmel Mountains, Israel, are strikingly different: the south-facing slope receives intensive solar radiation, is hot and supports mostly annual vegetation, whereas the north-facing slope is ~10°C cooler, more humid, and supports Mediterranean woodland. We examined whether these differences manifest in the thermal physiology of a common gecko species *Ptyodactylus guttatus* in controlled laboratory conditions. We predicted that geckos from the hotter south-facing slope would prefer higher temperatures, have faster gut passage times, lower metabolic and evaporative water loss rates, and start diel activity earlier compared with north-facing slope conspecifics. Contrary to these predictions, there were no differences between any of the ecophysiological traits in geckos from the opposing slopes. Nevertheless, our data showed that individuals from the north-facing slope were generally more active in earlier hours of the afternoon compared with south-facing individuals. We suggest that *P. guttatus* individuals disperse between the slopes and either gene-flow or behavioral plasticity deter local adaptation, resulting in similar physiological traits. Perhaps a stronger contrast in climatic conditions and a stronger barrier are needed to result in interpopulation divergence in temperature-dependent traits.

## KEYWORDS

evaporative water loss, Evolution Canyon, gut passage time, metabolic rate, Nahal Oren, temperature preferences

## 1 | INTRODUCTION

Important physiological processes are strongly influenced by environmental temperatures. Traits such as digestion rates and efficiency, metabolic rate and water loss rate, predator avoidance, foraging success, endurance, and reproduction are strongly affected by temperatures, especially in ectotherms (Huey, 1982; Killen, 2014;

Smith & Ballinger, 2001; Yee & Murray, 2004), including lizards (McConnachie & Alexander, 2004; Pafilis et al., 2007; Plasman et al., 2019; Van Damme et al., 1991; Watson & Burggren, 2016). Thus, climatic conditions exert selection pressures on behavior, physiology, and life history (Adolph & Porter, 1993; Hertz et al., 1983; Meiri et al., 2013; Stark et al., 2018; Stark et al., 2020). Responses to climatic conditions may include physiological alterations, for example,

shift of preferred and/or critical temperatures, reduced water loss rates, and increased nutrient absorption. Alternatively, behavioral shifts, such as altered basking frequency and/or duration, adoption of special postures, or altered substrate selection, among others, could achieve similar ends (Angilletta, 2009; Bogert, 1959; Dawson, 1975). Behavioral shifts may relax the need for physiological adaptation, and thus constrain evolution by reducing selection pressures, a phenomenon known as the “Bogert effect” (Huey et al., 2003). Temperature-sensitive traits, such as the rate of water loss, can also restrict species ranges. Studying them may therefore help understand both the evolution of species in their habitat and future ramifications of global warming on species ranges (Kolbe et al., 2014; Pontes-da-Silva et al., 2018).

Two contrasting hypotheses have been postulated to explain the thermal sensitivity of lizards to environmental conditions: the “static” and “labile” views (Hertz et al., 1983). The “static” hypothesis posits that thermal physiology is evolutionarily conservative, and is thus relatively insensitive to directional selection (Hertz et al., 1983). According to this view, closely related lizard species or conspecific populations, even those occurring in climatically distinctive habitats, show little differentiation in temperature-related traits (Osojnik et al., 2013; Rato & Carretero, 2015; Rodríguez-Serrano et al., 2009). In contrast, the “labile” view presumes that thermal physiology responds to directional selection, thus interspecific or interpopulation variation in thermal physiology is the product of adaptation to local conditions (Angilletta, 2001; Hertz et al., 1983; Scheers & Van Damme, 2002; Watson & Burggren, 2016).

Organisms' activity patterns result from a combination between an internal circadian clock and direct environmental influences (Kuhlman et al., 2018). Light cycle, which is the most reliable predictor for environmental conditions, is the dominant entrainment cue (zeitgeber) for the circadian system (Kuhlman et al., 2018; Sharma & Chandrashekar, 2005). Ambient temperature cycles, among other environmental conditions, are known to affect activity patterns (Rensing & Ruoff, 2002) and in some cases, especially in ectotherms, can entrain the circadian clock (Aschoff, 1981; Rensing & Ruoff, 2002).

Traits such as metabolic rate, water loss rates, gut passage time, and activity pattern have been found to vary between species (Frankenberg, 1979; García-Muñoz & Carretero, 2013; Li et al., 2017; Pafilis et al., 2007; Scheers & Van Damme, 2002; Watson & Burggren, 2016) and populations (Angilletta, 2001; Belmont, 1977; Rato & Carretero, 2015; Tocher & Davison, 1996) occurring in habitats differing in temperatures. In contrast, studies focusing on trait differences between microhabitats with different microclimates within the same habitat are scarce.

The northern slopes in canyons north of the equator face southwards, and therefore receive higher solar radiation than do the southern (north-facing) slopes (Pavlicek et al., 2003). The south-facing slopes are thus often hotter and drier than north-facing slopes, because of the higher solar radiation (Nevo, 2012). Even though the rocks, soils, and topography are similar among slopes, the thermal conditions lead to differences in the biotic characteristics of the

opposing slopes (Kutiel & Lavee, 1999; Nevo, 1995, 2006; Pen-Mouratov et al., 2009). Therefore, the opposing slopes of a canyon present an opportunity to test the ecophysiological trait difference at a small spatial scale, and whether traits evolve to adapt organisms to prevailing conditions.

Genetic polymorphism, mutation rates, and recombination among others, were claimed to be higher in populations from south-facing slopes of East-West oriented canyons compared to populations from the North-facing slope (Nevo, 2012). In a long series of studies, Nevo et al. hypothesized that selection drives local adaptation to the microclimate of each slope in organisms belonging to various taxa, at this microgeographic scale (e.g., Hadid et al., 2014; Hübner et al., 2013; Kang et al., 2019; Li et al., 2016; Nevo, 2006). Various attributes such as morphology (Debat et al., 2008; Lyman et al., 2002; Nevo et al., 2000; Raz et al., 2011), biochemical composition (Grishkan et al., 2018; Sikorski et al., 2008; Singaravelan et al., 2008), sexual behaviors (Iliadi et al., 2001; Iliadi et al., 2009; Korol et al., 2000; Palavicino-Maggio et al., 2019), life-history traits (Rashkovetsky et al., 2000), and physiology (Nevo et al., 1998; Rashkovetsky et al., 2006) were found to differ between populations from the opposing slopes across taxa (but see Gefen & Brendzel, 2011).

The gecko *Ptyodactylus guttatus* (Heyden, 1827; Squamata: Phyllodactylidae) is a cathemeral saxicolous lizard (Bar et al., 2021; author personal observations). Its range spans from Northern Israel in the north, through Western Jordan in the east and including the Sinai Peninsula in the south (Bar et al., 2021; Frankenberg, 1978; Metallinou et al., 2015; Roll et al., 2017), and it is very abundant in Israel. In Nahal Oren in the Carmel 32.714°N, 34.977°E; Figure 1) it is found under large boulders, and on cave walls and rock walls on both slopes. We examined the thermal ecology, metabolic rates and water loss rates, digestive efficiency, and activity patterns of geckos from both slopes in controlled laboratory experiments. We tested whether they follow the “labile” view of ecophysiology in a microclimate scenario and feature locally adapted traits, or whether traits are similar, as predicted by the “static” view, or because gene-flow prevents local adaptation to microclimates.

Following evidence of genetic and trait local adaptation on other species in this study system (e.g., Grishkan et al., 2018; Hadid et al., 2014; Hübner et al., 2013; Kang et al., 2019; Li et al., 2016; Nevo, 2006; Palavicino-Maggio et al., 2019; Raz et al., 2011; Singaravelan et al., 2008), we hypothesized that gene-flow is minimal and local adaptation is common. We predicted that results would support the “labile” hypothesis. Specifically, we predicted that: 1. Individuals from the hotter and drier south-facing slope would be adapted to higher temperatures and would thus select higher temperatures in the laboratory and start activity earlier in the morning, to reach higher body temperatures earlier. 2. When tested in temperatures higher than the mean annual temperature (>20°C), individuals from the south-facing slope would have lower metabolic and evaporative water loss rates than individuals from the north-facing slope (Watson & Burggren, 2016). 3. Because higher temperatures enable physiological processes (e.g., nutrient absorption



**FIGURE 1** Nahal Oren canyon site. The north-facing slope (southern slope) is on the right and the south-facing slope (northern slope) is on the left. Photo was taken on 03.04.2021

and assimilation) to occur faster (Huey, 1982), gut passage time of geckos would decline with increasing temperature. We predicted this decline to be steeper in the heat-adapted individuals from the south-facing slope than in cold-adapted individuals from the north-facing slope. This is because individuals from the south-facing slope would be able to assimilate energy efficiently enough even with faster passage time (McConnachie & Alexander, 2004).

## 2 | METHODS

### 2.1 | Site description and collection of specimens

Lower Nahal Oren is the best-studied E-W-oriented canyon in Israel. Situated in the Mediterranean biome, the mean annual temperatures in Nahal Oren are  $\sim 20^{\circ}\text{C}$  (across both slopes during 2019; the Israel Meteorological Service archive) and it receives on average  $\sim 550$  mm of annual precipitation (Malkinson & Wittenberg, 2007). Formed around 3–5 Mya, the opposite slopes are separated by only 200 m at the valley bottom (with a road crossing through), to 500 m at the top (Nevo, 1995). Its substrate consists primarily of carbonate rocks, mainly limestone, dolomite, chalk, and marl, covered by terra Rossa soil (Malkinson & Wittenberg, 2007). Solar illuminance is 2.3–8 times higher on the more exposed south-facing slope than on the more shaded north-facing slope, directly affecting the surface rock temperatures, deriving a difference of  $\sim 10^{\circ}\text{C}$  between equally sun-exposed rocks across slopes (Pavlíček et al., 2003). Relative humidity on the north-facing slope was higher than that of the south-facing slope, especially during the colder months (1%–7% difference; Pavlíček et al., 2003). As a result, vegetation cover is 35% in the south-facing slope but 150% (stratified layers, e.g., annuals growing on top of perennials) on the north-facing slope (Nevo et al., 1999). The vegetation of the north-facing slope is a typical Mediterranean maquis comprised of oak (*Quercus*) and carob (*Ceratonia siliqua*) trees and tall *Pistacia* shrubs, while that of the south-facing slope is dominated by annuals with numerous carob trees, low *Pistacia* shrubs, and low perennial bushes such as *Pennisetum ciliare* and *Stachys palaestina* (Nevo, 1995; Nevo et al., 1998, 1999).

We collected 15 adult individual geckos from the north-facing slope, and 16 from the south-facing slope of Nahal Oren between March and July 2019. On the north-facing slope geckos were collected between 07:24 and 15:00 (66% between 08:00 and 10:00), and on the south-facing slope between 08:15 and 11:30. Upon capture, we measured the gecko's body temperature by using a cloacal probe attached to a K-type thermometer (Hyelec<sup>®</sup> MS6501) to the nearest  $0.1^{\circ}\text{C}$ . We successfully obtained temperatures from 28 individuals. We also measured the temperature of the substrate upon which the gecko was caught and the air temperature at a height of 5 cm above that substrate. To exclude the effects gravity may have on the preferred temperatures, we only performed experiments on gravid females after they laid their eggs (the eggs can be easily observed through the semi-transparent abdomen; Carretero et al., 2005).

After capture, animals were housed individually in terraria for acclimation to laboratory conditions ( $25^{\circ}\text{C}$ ) at the Zoological Research Garden at Tel Aviv University, and fed ad libitum, until 48 h before initiation of the experiments. Out of the 31 geckos caught, eight (four from each slope) were taken for the activity time experiment (see below) after measuring their oxygen consumption and evaporative water loss rates, and therefore all other experiments were performed on a maximum of 23 geckos. Within 5 months of collection, we performed the following experiments:

### 2.2 | Oxygen consumption and evaporative water loss rates

$\text{O}_2$  consumption ( $\dot{V}\text{O}_2$ , a proxy for metabolic rate) and evaporative water loss were measured using flow-through respirometry system (Sable Systems) between April 10 and May 29, 2019. We placed each individual (16 from the south-facing slope and 12 from the north-facing slope) in a 0.25 L cylindrical plexiglass metabolic chamber, which was then placed horizontally in a controlled-temperature cabinet (MIR-554, Panasonic) at  $25 \pm 2^{\circ}\text{C}$ . Each individual was measured for 1 h after a 1-h acclimation period to metabolic chamber conditions. Dry,  $\text{CO}_2$ -free air (by passing through silica gel and ascarite columns) was passed through the chamber at  $50 \text{ ml}\cdot\text{min}^{-1}$  using

mass flow controllers (Alicat). Excurrent air was then passed through a CO<sub>2</sub>/H<sub>2</sub>O analyzer (LI-7000, LiCor Biosciences). Subsequently the excurrent passed through a column of ascarite and magnesium perchlorate to remove CO<sub>2</sub> and water vapor, to an Oxzilla-II oxygen analyzer (Sable Systems International). The first and last five minutes of every recording were used for baselining, passing air directly to the gas analyzers through an empty metabolic chamber. Animals were weighed to the nearest 0.1 mg (CPA224S, Sartorius) after each measurement.

Data acquisition and analyses were carried out using ExpeData software (Sable Systems International). Calibration of the H<sub>2</sub>O detector was carried out by injecting microliter amounts of water into a stream of CO<sub>2</sub>-free dry air passing through the analyzer at 50 ml·min<sup>-1</sup> at 25°C and integrating the area under the resulting H<sub>2</sub>O trace.

Resting metabolic rate was calculated as the mean  $\dot{V}O_2$  value of the lowest and most level 15-min period recorded (which always coincided). Evaporative water loss rate was calculated by integrating the area under the H<sub>2</sub>O trace of the lowest most level 5-min period in the recording of each individual.

## 2.3 | Temperature preference experiment

We tested the preferred body temperature of 19 geckos (nine from the south-facing slope and 10 from the north-facing slope) by placing each lizard on a thermal gradient of 18–50°C. The thermal gradient was created by placing two circulating cold and hot water-filled baths at opposite ends of an arena. The baths were connected to copper pipes running underneath the arena, thus heating one edge and cooling the other, creating a gradient in the middle. The gradient arena was then completely covered with a lid, to which we fixed LED stripes that provided light between 06:00 and 18:00. We then put each individual separately in the middle of the thermal gradient and recorded their body temperatures every 10 s for 21 h using a data logger (Besag K thermometer SD logger 88598, DANIU Portable professional tools) connected to a thermocouple inserted and fixed in its cloaca with medical tape (Vetrap bandaging tape). For each individual, we discarded the first hour of measurement, in which readings may have been influenced by handling, and calculated the mean preferred temperature in the laboratory over 20 h, and the mean temperature for 30 min before and after the time it was collected in the field (1 h total, for 20 individuals).

## 2.4 | Gut passage time experiment

To measure gut passage time, we inserted small (~1 × 1 mm<sup>2</sup>) red plastic tags in the interior of mealworms weighing 0.05–0.1 g using forceps, after cutting off their heads. We then fed 19 geckos (8 from the south-facing slope and 11 from the north-facing slope) one tagged mealworm each, and made sure they swallowed it by observing the movement of their gullet. We then placed the individual in its

terrarium, lined with a white piece of paper, in a room with the air temperature set to the experimental temperature (18°C, 25°C, and 35°C, each individual repeating the experiment in all three temperature regimes). Geckos were acclimated to experimental temperatures 24 h before the experiment. Individuals were supplied with water ad libitum throughout the duration of the experiment, but not fed additional mealworms. We recorded the terraria with video cameras and checked every few hours whether there were feces in the terraria. When we found feces we collected them and disintegrated them with water on a petri dish, and when we found the red plastic tag, we recorded the time that passed from feeding to excreting using the time-stamped videos. All geckos were first tested at 25°C, then at 35°C, and finally at 18°C.

## 2.5 | Activity pattern experiment

This experiment started after 18 days of acclimation to the laboratory conditions (30 ± 1.5°C, 12L:12D; lights were on between 06:00 and 18:00) during July and August 2019. Eight individuals (four from each slope) were placed in clear 44 × 28 × 17 cm<sup>3</sup> plastic terraria lined with white chiffon that enabled geckos to climb and cling to it, and supplied with a flat rock hiding place, water bowl, and a food plate (filled without opening the terraria lids, to minimize stress). The light was emitted by LED tapes (emitting white light) placed above the terraria, creating an intensity of 805–1240 lux. Geckos' movements were photographed by a webcam (through the clear lid) and monitored using Active WebCam software (PY Software© 1997) with motion detection triggering capture of photos. Active IR lighting was used for photographing in the dark. After the acclimation period, we monitored the geckos in two light regimes to characterize the daily and circadian activity pattern of the geckos: 1. 12L:12D, neutral light:dark conditions for 15 days; 2. Constant darkness (D:D) for 14 days. We used the number of movements of each individual in 6-min periods throughout the day as an index for the activity level. Using CTools 7.0 by Daan van der Veen we calculated the center of gravity, which represents the central tendency of the distribution of activity along the day, as the time in each day at which the median of activity is registered (individuals active earlier in the day would have an earlier center of gravity; Kenagy, 1980; Refinetti et al., 2016; Wicht et al., 2014). The circadian (free-running) period ( $\tau$ ; Kuhlman et al., 2018) was calculated using the ClockLab software (Actimetrics Ltd.) over the 14 consecutive days of the constant darkness regime. For one individual no  $\tau$  could be calculated due to poor reception of movements in the darkness.

## 2.6 | Data analysis

To test whether individuals from the south- and north-facing slopes differed in their O<sub>2</sub> consumption rate we constructed an ANCOVA including the rate of O<sub>2</sub> consumption [ml·min<sup>-1</sup>] as the response, the slope [south-facing/north-facing] and sex as main effects, the mass

[g] as covariate, and the interactions between mass and slope and slope and sex. We also used an ANCOVA including the rate of water loss [ $\mu\text{L}\cdot\text{h}^{-1}$ ] as the response, the slope [south-facing/north-facing] and sex as main effects, the mass [g] as a covariate, and the interaction between mass and slope and slope and sex to test whether individuals from the south and north-facing slopes differed in evaporative water loss.

A two-way ANCOVA was used to test whether preferred temperatures differed between individuals from the south- and north-facing slopes. We included the mean preferred temperature ( $^{\circ}\text{C}$ , over a period of 20 h) as the response, the slope [south-facing/north-facing] and sex as main effects, the mass [g] as the covariate, and the interactions between mass and slope and sex and slope. To test whether the preferred body temperatures of geckos in the laboratory differed from their measured body temperatures in the field we constructed a mixed-effects model with the body temperature of each individual in the field and the laboratory (measured at the time the individual was collected in the field) as the response, and the place where the temperature was measured (laboratory/field), the sex and the slope, and their interaction as fixed main effects. We used the identity of each gecko as a random effect to correct for the fact that the same individual was measured both in the field and the laboratory. To test whether substrate temperature or air temperature in the field affected body temperature in the field we performed a mixed-effects model using the field body temperature as the response and the ground and air temperatures as fixed covariates, and the slope as a fixed main effect, with an interaction between the slope and each of the covariates. We included the identity of each individual as a random factor, to control for the fact that body, ground, and air temperatures were not independent and belonged to the same individual.

To test whether gut passage time differed between individuals from the south- and north-facing slopes we ran a mixed-effects model including the time the plastic tag took to pass through the digestive tract as the response, the experimental temperature (low— $18^{\circ}\text{C}$ , medium— $25^{\circ}\text{C}$ , and high— $35^{\circ}\text{C}$ ) and slope as fixed main effects, the larvae and gecko mass as covariates and the interaction between temperature and slope as a fixed effect. We included the identity of the individual as a random effect to correct for the fact that the same individual was measured at three temperature regimes (observations are not independent—the model considers different intercepts for different individuals).

To compare activity patterns of geckos across slopes, we used a Generalized Additive Mixed Model (GAMM), using the “`gamm`” function from the “`mgcv`” package (Wood, 2011). We constructed two models, for the light and dark phases separately, using the mean number of movements of each individual over each 6 min during each phase as the response and the slope and sex as fixed main effects. We included the identity of the individual as random effect to account for variation among individuals and the difference in sensitivity of the system to different individuals. Because the response is count data, we used a Poisson error structure and considered

autocorrelation in time capturing temporal dependency of the movements in the model. We also set the soothing term “*k*” (the upper limit on the degrees of freedom associated with the time) to 8, after validation simulation (“`gam.check`” function in “`mgcv`” package), to help flag up terms in which “*k*” is too low.

To test the difference between the centers of gravity of individuals from the opposing slopes we performed a two-sample *t*-test. To check if there is a correlation between the center of gravity and  $\tau$  (and if so, what is the trend), we performed a Pearson's correlation test using “`cor.test`” function from the “`stats`” R package, with “`pearson`” as the method in the function.

All analyses were conducted with R version 1.3.1073 (R Core Team, 2020). The statistical analysis R script is provided in full in the Supporting Information (Appendix S1).

### 3 | RESULTS

All means for experiment results are presented in Table 1 and the full data on all experiments are available in Table S1.

#### 3.1 | O<sub>2</sub> consumption

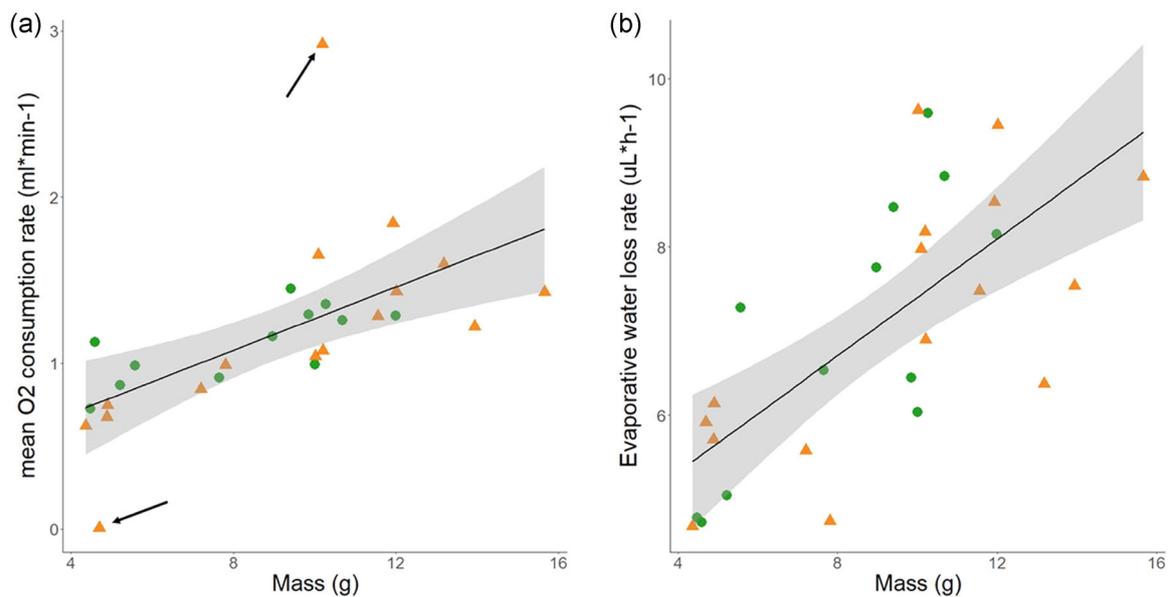
O<sub>2</sub> intake rate increased with increasing body mass (best model: slope =  $0.10 \pm 0.03$ , intercept =  $0.32 \pm 0.24$ ,  $t = 3.86$ ,  $p = 0.0007$ ,  $R^2 = 0.36$ ,  $n = 28$ ), but there was no significant difference between individuals from the south-facing slope (full model: mean O<sub>2</sub> consumption rate =  $0.37 \pm 0.27 \text{ ml}\cdot\text{min}^{-1}$ ) compared to that of individuals from the north-facing slope (mean O<sub>2</sub> consumption rate =  $0.45 \pm 0.26 \text{ ml}\cdot\text{min}^{-1}$ ,  $t = 0.44$ ,  $p = 0.66$ ; Figure 2a). There was also no difference between males (mean =  $0.25 \pm 0.25 \text{ ml}\cdot\text{min}^{-1}$ ) and females (mean =  $0.37 \pm 0.27 \text{ ml}\cdot\text{min}^{-1}$ ,  $t = 1.14$ ,  $p = 0.27$ ), and no interaction between mass and slope ( $t = 1.10$ ,  $p = 0.28$ ) and sex and slope ( $t = -1.76$ ,  $p = 0.09$ ). Results did not change qualitatively when we omitted two outliers from the south-facing slope (GSh23419 with very low O<sub>2</sub> consumption rate and GSc19319 with very high consumption rate, Figure 2a; Table S1), thus the results we present are of the model for the entire sample.

#### 3.2 | Evaporative water loss

Evaporative water loss increased with increasing body mass (best model: slope =  $0.35 \pm 0.07$ , intercept =  $3.94 \pm 0.66$ ,  $t = 5.03$ ,  $p < 0.0001$ ,  $R^2 = 0.49$ ,  $n = 28$ ), but there was no significant difference between individuals from the south-facing slope (full model: mean water loss rate =  $4.13 \pm 0.88 \mu\text{L}\cdot\text{h}^{-1}$ ) compared to that of individuals from the north-facing slope (mean water loss rate =  $3.06 \pm 1.29 \mu\text{L}\cdot\text{h}^{-1}$ ,  $t = 0.70$ ,  $p = 0.49$ ; Figure 2b). There was also no difference between males (mean =  $3.08 \pm 1.17 \mu\text{L}\cdot\text{h}^{-1}$ ) and females (mean =  $3.06 \pm 1.29 \mu\text{L}\cdot\text{h}^{-1}$ ,  $t = 0.05$ ,  $p = 0.97$ ), and also no interaction between mass and slope ( $t = -0.97$ ,  $p = 0.34$ ) and sex and slope ( $t = -0.17$ ,  $p = 0.86$ ).

**TABLE 1** Mean values and SE of physiological parameters from experiments performed on *Ptyodactylus guttatus* females (F) and males (M) collected from the north-facing (NFS) and south-facing slope (SFS) of Nahal Oren

Trait	NFS			SFS			Grand total
	F	M	Total	F	M	Total	
<i>n</i>	6	9	15	7	9	16	31
Mass [g]	10.11 ± 0.81	7.86 ± 0.96	8.55 ± 0.72	8.51 ± 1.10	10.34 ± 1.32	9.54 ± 0.89	9.10 ± 0.58
Mean O <sub>2</sub> consumption rate [ml·min <sup>-1</sup> ]	1.09 ± 0.10	1.13 ± 0.08	1.12 ± 0.06	1.30 ± 0.27	1.11 ± 0.18	1.20 ± 0.15	1.16 ± 0.09
Mean evaporative H <sub>2</sub> O loss rate [μl·h <sup>-1</sup> ]	7.39 ± 0.79	6.83 ± 0.55	6.97 ± 0.42	6.81 ± 0.69	7.34 ± 0.48	7.10 ± 0.40	7.05 ± 0.28
Mean preferred temperature [°C]	34.2 ± 0.2	32.4 ± 0.5	32.9 ± 0.4	32.2 ± 0.2	31.9 ± 0.5	32.0 ± 0.3	32.5 ± 0.3
Mean field body temperature [°C]	24.9 ± 1.7	24.6 ± 1.8	24.8 ± 1.2	21.0 ± 1.9	22.9 ± 2.2	22.0 ± 1.5	23.3 ± 1.0
Mean field substrate temperature [°C]	19.5 ± 2.0	20.7 ± 1.5	20.2 ± 1.1	20.0 ± 2.0	18.4 ± 1.3	19.1 ± 1.1	19.6 ± 1.0
Mean field air temperature [°C]	19.4 ± 2.0	18.5 ± 1.0	18.9 ± 1.0	20.5 ± 1.7	18.4 ± 1.3	19.3 ± 1.0	19.1 ± 0.7
Mean gut passage time at 18°C [h]	170 ± 19	200 ± 15	185 ± 12	175 ± 31	193 ± 13	186 ± 14	186 ± 9
Mean gut passage time at 25°C [h]	78 ± 13	89 ± 7	84 ± 7	97 ± 11	92 ± 16	95 ± 10	90 ± 6
Mean gut passage time at 35°C [h]	143 ± 42	110 ± 4	125 ± 18	158 ± 29	152 ± 25	155 ± 18	141 ± 13
Mean onset [h]	8.4 ± 0.0	8.3 ± 0.5	8.3 ± 0.3	10.3 ± 0.3	9.9 ± 0.6	10.1 ± 0.3	9.2 ± 0.3
Mean centre of gravity [h]	11.1 ± 0.0	11.6 ± 0.2	11.4 ± 0.2	13.3 ± 0.1	13.4 ± 0.5	13.3 ± 0.2	12.4 ± 0.2
Mean Tau [h]	23.4 ± 0.0	23.5 ± 0.1	23.5 ± 0.1	24.0 ± 0.0	24.0 ± 0.0	24.0 ± 0.0	23.8 ± 0.1

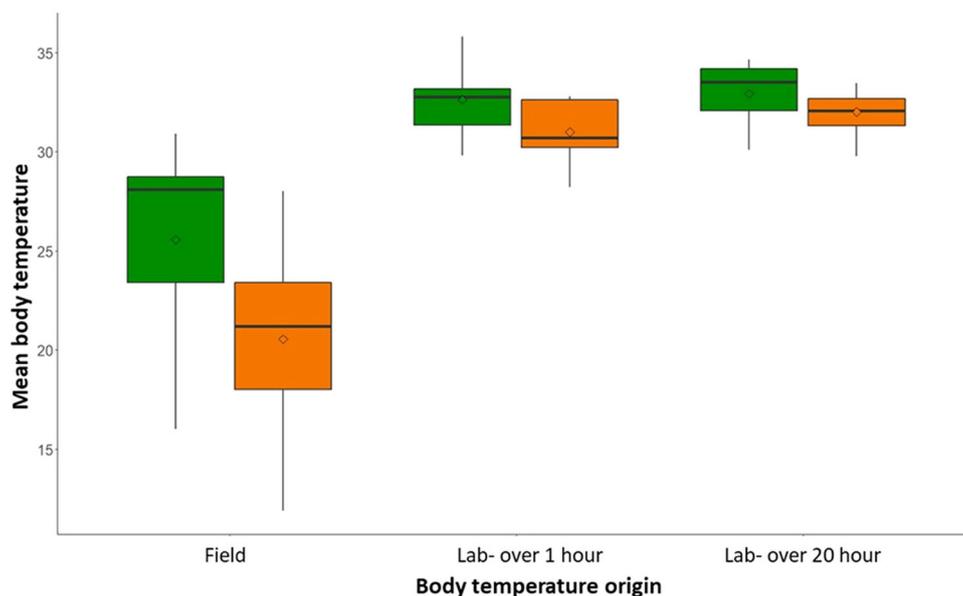
**FIGURE 2** (a) Mean O<sub>2</sub> consumption rate [ml·min<sup>-1</sup>] and (b) mean evaporative water loss rate [μl·h<sup>-1</sup>] against body mass [g] of individuals from the south-facing slope (orange triangles) compared to that of individuals from the north-facing slope (green circles). The outliers are indicated with an arrow

### 3.3 | Preferred temperatures and field temperatures

The mean preferred temperatures of geckos from the opposing slopes did not differ significantly (south-facing slope = 31.4 ± 1.0°C, *n* = 9; north-facing slope = 33.2 ± 1.3°C, *n* = 10; *t* = 1.71, *p* = 0.11; Figure 3a), there was no effect of mass (slope = 0.1 ± 0.1, *t* = 0.95,

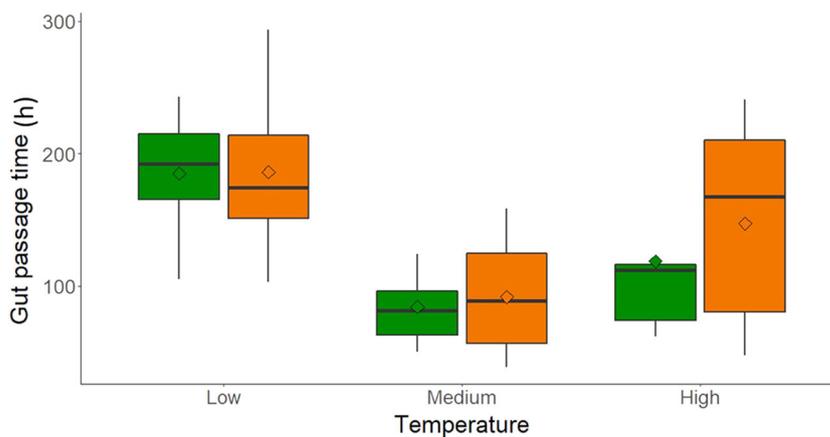
*p* = 0.36) and sex (*t* = 0.54, *p* = 0.60), and there was no interaction between sex and slope (*t* = 0.63, *p* = 0.54) and mass and slope (*t* = -0.70, *p* = 0.50).

The temperatures preferred in the laboratory at the time of day the geckos were collected in the field (mean preferred temperatures over 1 h = 32.5 ± 1.7°C) were similar to those measured over 20 h (mean preferred temperatures = 31.9 ± 0.8°C, *t* = 0.61, *p* = 0.55) and



**FIGURE 3** Boxplots of mean preferred body temperature between the south-facing slope (orange) and north-facing slope (green) measured in the field, and calculated over 1 and 20 h in the laboratory

**FIGURE 4** Gut passage time of individuals from the north-facing slope (green) and the south-facing slope (orange), in high (35°C), medium (25°C), and low (18°C) temperature regimes



were higher by 7°C and 10°C than those measured in the field for geckos from the south-facing slope ( $20.6 \pm 1.6^\circ\text{C}$ ) and north-facing slope ( $25.8 \pm 1.7^\circ\text{C}$ ,  $t = 3.80$ ,  $p = 0.002$ ; Figure 3b), respectively. There was no difference in body temperatures measured in the field between females (mean =  $22.6 \pm 1.7^\circ\text{C}$ ) and males (mean =  $23.4 \pm 1.5^\circ\text{C}$ ;  $t = 0.19$ ,  $p = 0.85$ ). The body temperatures measured in the field were significantly higher on average by 5°C in geckos from the north-facing slope ( $25.8 \pm 1.7^\circ\text{C}$ ) compared to that of geckos from the south-facing slope ( $20.6 \pm 1.6^\circ\text{C}$ ;  $t = 2.64$ ,  $p = 0.02$ ). Body temperatures measured in the field increased by 0.9°C with every 1°C increase in the substrate temperatures on which individuals were collected (best model: intercept =  $5.3 \pm 3.8^\circ\text{C}$ , slope =  $0.9 \pm 0.2$ ,  $t = 4.70$ ,  $n = 28$ ,  $p < 0.0001$ ; Figure S1a) but not with the air temperatures (full model: intercept =  $7.3 \pm 4.4^\circ\text{C}$ , slope =  $-0.2 \pm 0.4$ ,  $t = -0.36$ ,  $n = 28$ ,  $p = 0.72$ ; Figure S1b). These results did not differ between slopes ( $t = 1.09$ ,  $p = 0.29$ ; Figure S1a,b), and we found no

interaction between slope and either substrate ( $t = 0.69$ ,  $p = 0.50$ ) or air ( $t = 0.92$ ,  $p = 0.37$ ) temperatures.

### 3.4 | Gut passage time

The gut passage time of individuals from both slopes was longest in the low (18°C) temperature regime (mean =  $186 \pm 12$  h) followed by the high (35°C) temperature regime (mean =  $135 \pm 12$  h), and shortest in the medium (25°C) temperature regime (mean =  $89 \pm 12$  h; Figure 4). This means that on average, it took the plastic tag twice the time to pass through the digestive system in the low-temperature regime compared to the medium-temperature regime (Best model:  $t = -6.35$ ,  $p < 0.0001$ ), but only 25% longer than in the high-temperature regime ( $t = -3.05$ ,  $p = 0.004$ ). There was no interaction between temperature and slope (Full model = low:south-facing slope;

$t = 0.98$ ,  $p = 0.34$ ; medium: south-facing slope;  $t = 0.98$ ,  $p = 0.52$ ). Neither gecko body mass (slope =  $3.8 \pm 3.2$ ,  $t = 1.19$ ,  $p = 0.25$ ) nor meal size (slope =  $-1167.6 \pm 816.5$ ,  $t = 1.43$ ,  $p = 0.16$ ) significantly affected gut passage time. There was no difference in the gut passage time of individuals from the different slopes ( $t = 0.97$ ,  $p = 0.35$ ) or sexes ( $t = 0.79$ ,  $p = 0.44$ ).

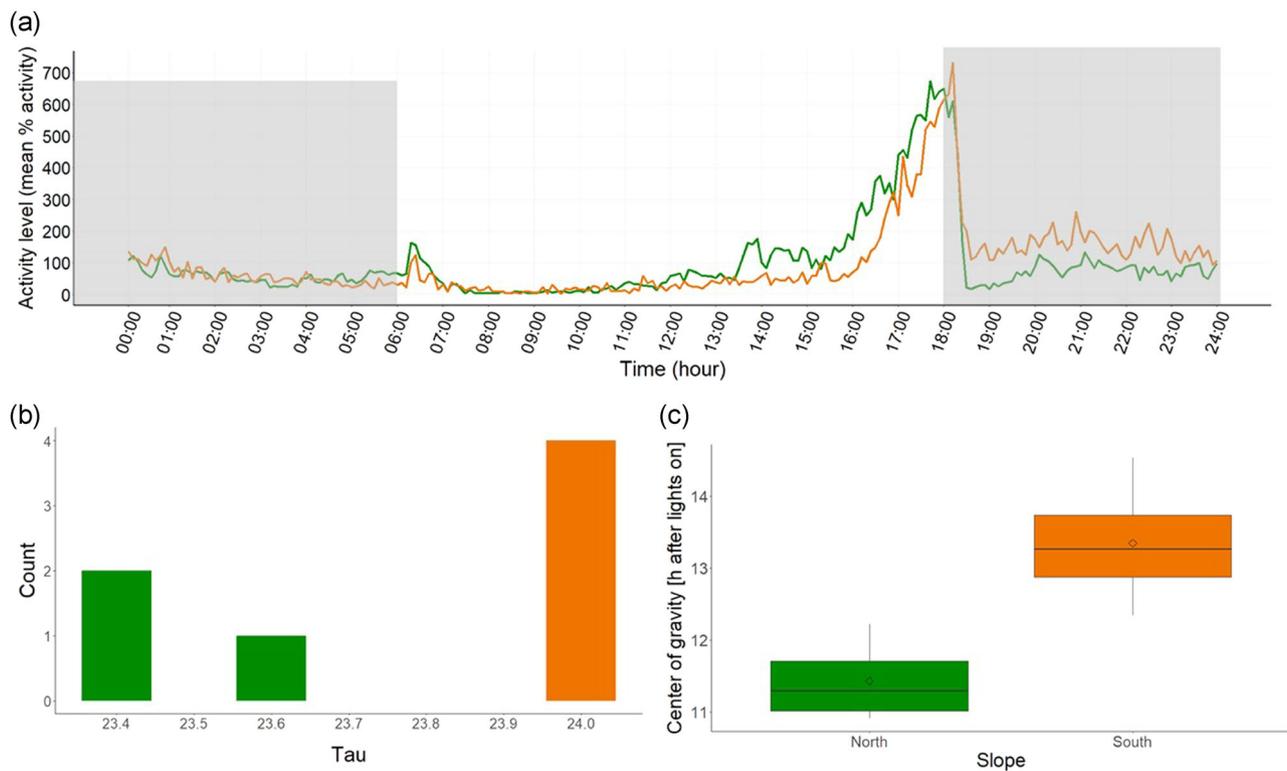
### 3.5 | Activity patterns

Individuals from both slopes had very similar activity patterns—a small peak in activity levels around the lights on (06:00–07:00), followed by lower activity levels for the rest of the first half of the light phase, and an ascent in activity levels from ~12:00 to ~18:00 (Figure 5a). At lights off (18:00) activity levels immediately dropped to an intermediate level and fluctuated until ~24:00. After midnight activity levels were minimal until lights on (Figure 5a). The activity level and pattern were not statistically significantly different between individuals from the north- and south-facing slopes, both in the light phase (south-facing slope:  $77 \pm 46$  moves·h<sup>-1</sup>; north-facing slope:  $118 \pm 67$  moves·h<sup>-1</sup>,  $n = 8$ ,  $t = -1.18$ ,  $p = 0.24$ ) and the dark phase (south-facing slope:  $124 \pm 66$  moves·h<sup>-1</sup>; north-facing slope:  $82 \pm 61$  moves·h<sup>-1</sup>,  $n = 8$ ,  $t = 1.40$ ,  $p = 0.16$ ). In constant darkness, individuals

from the south-facing slope had significantly longer  $\tau$  values (average  $24.0 \pm 0.0$  h,  $n = 4$ ), than those from the north-facing slope (average  $23.5 \pm 0.1$  h,  $n = 3$ ,  $t = 9.56$ ,  $p < 0.01$ ; Figure 5b). The average centre of gravity of individuals from the north-facing slope was significantly earlier than that of individuals from the south-facing slope (mean onset on the north-facing slope = 11.4 h,  $SD = 0.6$ ,  $n = 4$ ; mean onset on the south-facing slope = 13.3 h,  $SD = 0.9$ ,  $n = 4$ ;  $t = -3.52$ ,  $p = 0.01$ ; Figure 5c). Thus individuals were most active around 17:00 on the north-facing slope and around 19:00 on the south-facing slope (Figure 5a).

## 4 | DISCUSSION

Our hypothesis, that the ecophysiological traits of *P. guttatus* populations will differ between the slopes of Nahal Oren because of microclimatic differences in temperature, was overall refuted. Despite the 2°C and 10°C inter-slope difference between air and rock surface temperatures respectively (Pavlíček et al., 2003), there was no evidence of local adaptation to the slopes' microclimates. Thus, geckos do not seem to follow the predictions of the "labile" view of thermal physiology. Previous research in Nahal Oren on plants (Nevo et al., 2000; Raz et al., 2011), *Drosophila* (Debat et al., 2008; Lyman



**FIGURE 5** Activity patterns of *Ptyodactylus guttatus* from the north-facing (green) and south-facing (orange) slopes. (a) Mean activity level of geckos across slopes during the light and dark phases (indicated by a white or gray background). Mean activity level is presented as the mean percentage activity (calculated as the ratio between the average count of movements in each 6 min during the day and the average number of movements in 6 min across the entire day). (b) The distribution of circadian periods ( $\tau$ ) of free-running activity rhythms of *P. guttatus* individuals under constant darkness conditions. (c) Center of gravity (hours) after the beginning of the light phase at 6:00, averaged across individuals between slopes over 15 consecutive days

et al., 2002; Rashkovetsky et al., 2006; but see Gefen & Brendzel, 2011), Gastropoda (Raz et al., 2012), fungi (Miyazaki et al., 2003; Singaravelan et al., 2010), and bacteria (Sikorski & Nevo, 2007) did find differences in traits related to the microclimatic conditions in the opposing slopes. To our knowledge, however, no such differences were published for vertebrates. We suggest that *P. guttatus*, as opposed to the abovementioned mostly sessile taxa (apart from *Drosophila*), are more likely to cross between slopes, and thus gene-flow is more likely to occur and result in similar traits and exposure to the different microclimate regimes. Alternatively, behavioral effects may mask physiological adaptation according to the “Bogert effect,” thus minimizing physiological trait differences between slopes. A thorough investigation of the genetic diversity, movement ecology, and behavior of individuals from both slopes is needed to verify this hypothesis. Alternatively, the temperature-related traits we examined may be evolutionarily conservative, and thus insensitive to directional selection, as hypothesized by the “static” view (Hertz et al., 1983). The “static” hypothesis of thermal physiology has been supported for several other species, for instance, *Sceloporus undulatus*, in which cardiac performance, thermal tolerance, temperature preference, and sprint running, among others, were found to be conserved among populations occupying different microclimates throughout its range (Angilletta et al., 2013; Buckley et al., 2015; Crowley, 1985). Our results also stand in contrast to previous findings of differing temperature-dependent physiological traits between habitats (Angilletta, 2001; Gilbert & Miles, 2019; Rato & Carretero, 2015; Rodríguez-Serrano et al., 2009). These studies found interpopulation differences in thermal-sensitive traits among populations from different microclimates. Perhaps the discrepancy between previous studies and ours is the product of the small spatial scale over which we have tested trait differences. Perhaps a stronger contrast in climatic conditions, or a wider geographic gap, is needed to result in interpopulation divergence in temperature-dependent traits. Therefore, to know whether *P. guttatus* as a species have, at all, locally adapted their temperature-dependent physiological traits to different climates throughout their range and follow the “labile” hypothesis, one would need to test trait-variation between populations occupying habitats with a greater climatic contrast (e.g., between the Mediterranean and desert biomes).

While we found no difference between the metabolic rates of geckos across slopes, Plasman et al. (2020) found that high altitude *Sceloporus grammicus* lizards had higher metabolic rates than low altitude individuals at all measured temperatures (ranging between 15°C and 35°C). The study sites investigated by Plasman et al. (2020) were located 5–11 km apart, and mean annual temperatures differed by ~5°C between sites and by ~10°C between the highest and lowest sites. This is a much larger contrast than the 2°C difference in annual air temperature between slopes in Nahal Oren. The difference in rock surface temperature we measured between the slopes, however, was 10°C. Based on the positive correlation between rock and body temperatures, rock temperature may be more important for *P. guttatus* thermoregulation than the air temperature. Rato and Carretero (2015) found variation in water loss rates among populations and

among the European and Iberian clades of *Tarentola mauritanica* geckos, contrary to our results, in which no significant difference was found between individuals across slopes. The populations Rato and Carretero (2015) examined, however, were situated at least 150 km apart, and belonged to two distinct clades, thus the genetic variation was likely much greater than in our study. Because of the greater distance, the difference in humidity may also have been greater than in our study site, and therefore promoted more pronounced differences in water loss rates. These examples raise the possibility that physical distance, rather than microclimate differences alone, is needed for local adaptation of some physiological traits to evolve, as gene-flow may prevent the manifestation of trait differences. Populations occupying different microclimates in close proximity may therefore have the phenotypic plasticity in thermal-sensitive traits that are needed to withstand the conditions in those microhabitats. A thorough investigation of the way individuals across slopes respond to extreme temperatures and the dependence of metabolic and water loss rates on temperature across slopes is needed to gain more insight on the matter.

Although the preferred temperatures of individuals from the opposing slopes did not differ in the laboratory, they were higher than the body temperatures measured in the field across slopes, and similar to preferred temperatures previously found for this species (30.9 ± 2.4°C; Arad et al., 1989). The higher body temperatures in the laboratory may result from the fact that we caught all individuals during the morning, when they were still either basking outside their shelters, or waiting to reach higher temperatures by means of conductance from the rocks. It is thus possible that they only reach their preferred body temperatures in the field later during the day, before retreating to their shelters and commencing their activity. The fact that the field body and substrate temperatures, but not air temperatures, were positively correlated, suggests that *P. guttatus* may be thigmothermic rather than heliothermic, meaning they absorb heat through the substrate rather than through the air. This matches our field observations, where geckos are very seldom observed basking. But, despite the 10°C difference in substrate temperatures measured between slopes, individuals from the opposing slopes do not differ in their preferred temperatures. Because Nahal Oren is close to the species' northernmost distribution, and the genus *Ptyodactylus* has a Saharo-Arabian distribution (Metallinou et al., 2015; Roll et al., 2017), perhaps the Nahal Oren population experiences lower ambient temperatures than more southern populations, and seldom reaches their preferred temperatures in nature. This possibility supports the “static” hypothesis of physiological traits because it would explain why even populations that experience different conditions will possess similar temperature-dependent traits. If the “static” hypothesis is correct, this species, and possibly other species, may have managed to expand their range to habitats with differing microclimates (Crowley, 1985). Following the “static” hypothesis, however, can also mean that those populations that occupy habitats at the edge of their range may be more prone to local extinction if the conditions become more extreme due to global warming, as they may already be at the edge of their thermal tolerance (Buckley et al., 2015).

Somewhat counterintuitively, gut passage times were shortest at the medium temperature than at the highest temperature. Many functional traits are maximized at some optimal temperature range at the middle of the species' thermal performance breadth, and drop as temperature rises, according to the thermal dependence curve (Huey & Stevenson, 1979). If the mean preferred temperature, in this case, 32–33°C, is an indicator of this species' optimal temperature, then because 35°C exceeds this optimum, an increase in gut passage time at this temperature is logical, as there is a need to compensate for the decreased absorption ability at higher temperatures, (Beaupre et al., 1993; Angilletta, 2001). Moreover, different traits may have different optimal temperatures in the same species, a phenomenon that has become known as the "multiple optima" hypothesis (Huey, 1982). Van Damme et al. (1991) examined the gut passage time of *Zootoca vivipara* at temperatures ranging between 20°C and 35°C and detected a decline at 35°C, while the shortest time was reached at 30°C, slightly lower than the optimal temperatures for other functions (32–34°C). The fact that the warm-adapted *P. guttatus* has a similar performance breadth in gut passage time as does the cold-adapted *Z. vivipara* despite being phylogenetically distant, gives further support for the "static" view of physiological traits. A study of the trade-off between gut passage time and absorption efficiency in *P. guttatus* under different temperature regimes is however required to better understand the thermal performance breadth of this species.

While the activity pattern of individuals from both slopes was similar, individuals from the north-facing slope were generally more active around 17:00 while those from the south-facing slope were more active around 19:00. Individuals from the north-facing slope also had a shorter circadian cycle compared to individuals from the south-facing slope. In the more shaded, cooler north-facing slope, activity may already be possible at late afternoon (around 17:00 in the season we sampled in), while in the more open south-facing slope, temperatures may remain inhospitably hot until later in the evening (around 19:00). Our finding, that body temperatures measured in the field were higher in individuals from the north-facing slope compared to individuals from the south-facing slope despite most being caught earlier in the morning (6 out of 10 compared to 1 out of 9 caught before 09:00 on the north and south-facing slopes, respectively; Table S1), seems counterintuitive. The north-facing slope probably heats up later during the day, due to lower solar radiation and fuller canopy cover, which would lead to lower body temperatures of individuals from the north-facing slope earlier in the morning. Strong intraspecific competition for favorable basking spots may lead to earlier emergence from the shelter in north-facing slope individuals. Alternatively, individuals from warmer microclimates, unlike those from cooler ones, can restrict their activity times to when the ambient temperatures enable the achievement of preferable temperatures, as observed in a high-altitude population of *Podarcis tiliguerta* (Van Damme et al., 1989). A thorough day-round field study of the activity of the geckos across slopes, the thermal quality of microhabitats across slopes and gecko thermoregulation behavior in natural conditions of light and temperature is nonetheless needed to test this hypothesis.

We conclude that *P. guttatus* geckos probably did not locally adapt their temperature-dependent physiological traits to the differing microclimate conditions occurring across the opposing slopes of the same canyon. This species therefore seems to either conform to the "static view of thermal physiology, at least in the microscale context of Nahal Oren, or did not evolve inter-slope differences as a consequence of dispersal and gene-flow, or as a result of behavioral adjustments. The contrast in microclimatic conditions between slopes and the close geographic proximity between slopes probably result in dispersal that overcomes local adaptation to the different climatic and microhabitat conditions across them. Our study highlights the importance of studying intraspecific trait variation at small scales and contributes to our understanding of the evolution of temperature-related traits in relation to microclimates.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## ETHICS STATEMENT

The study has been conducted under permit no. 2017/41741 from the Israeli Nature and Parks Authority, and under permits 034\_b11850 and 034\_b15191\_26 from the Tel Aviv University ethics in animal experimentation committee.

## DATA AVAILABILITY STATEMENT

All data are included in the Supporting Information. The data set is available as Supporting Information.

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