COLD ACCLIMATION RESPONSE OF NON-NATIVE ITALIAN WALL LIZARD

(*PODARCIS SICULUS*) POPULATIONS FROM NEW YORK AND CALIFORNIA

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ABSTRACT

Cold acclimation response of non-native Italian wall lizard (*Podarcis siculus*) populations from New York and California

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Understanding how organisms respond to climatic variability and novel conditions is becoming an increasingly important task for ecologists. For ectotherms in the northern hemisphere, the response to cold is of special interest, considering that poleward range expansion events and increasing variability of temperatures during winter are already being observed as consequences of a warming planet. Though direction of change in physiological variables in response to cold is well studied in ectotherms, the extent to which traits can change and the rate at which they can change is not.

We compared the extent and rate of change in cold tolerance (CT_{min}) between two long-term captive populations of the Italian wall lizard (*Podarcis siculus*) during a lab cold-acclimation treatment. Heat tolerance (CT_{max}), thermal preference (T_{pref}), temperature dependent rates of oxygen consumption (SMR_{O2}), and temperature dependent rates of water loss (EWL) were also compared between Italian wall lizards previously introduced to Long Island, NY and San Pedro, CA before and after the lab cold acclimation treatment. Because our study coincided with a cold snap during the spring 2018 season for the San Pedro, CA population, we also studied the effects of cold acclimatization on wild lizards from the CA population.

After initial lab acclimation of the lizards to laboratory conditions, SMR_{O2} at 15°C and EWL at 10°C were higher in NY lizards compared to CA lizards. Lizards from the two populations did not differ in any other variables measured before the cold acclimation treatment. We found that lizards from the NY population experienced an 80% decrease in CT_{min} following a switch from 20°C:18°C to 17.5°C:16°C (12h light:12h dark) acclimation treatment. Lizards from the CA population did not decrease CT_{min} in response to the same cold acclimation treatment. Overall, NY lizards decreased CT_{min}, CT_{max}, and T_{pref} following cold acclimation, whereas CA lizards decreased CT_{max} only. Wild CA lizards decreased CT_{max} following the cold spring 2018 season in a manner similar to that of lab acclimated NY and CA lizards, suggesting that these lizards do not maintain a high CT_{max} when the environment is unlikely to expose them to high temperatures. Thermal sensitivity (Q_{10}) of SMR_{O2} and EWL was lower in NY lizards, suggesting physiological adaptation to fluctuation in diurnal temperatures. The ratio of CO_2 produced to O_2 consumed (respiratory exchange ratio, RER) measured at 15°C increased in NY lizards following cold acclimation suggesting an increased use of carbohydrates and/or an increased production of lipids in the colder conditions.

These responses in combination with the higher observed plasticity in NY lizards are in accordance with the climatic variability hypothesis, which predicts that organisms from more variable climates will be better adapted to physiologically respond to variable conditions. The higher capacity for physiological plasticity may explain the relatively high success of *P. siculus* in NY and other northern U.S. states. By describing the rate of change of CT_{min} during cold acclimation we hope to better understand how these lizards minimize the risk of low temperature exposure during winter. We ultimately hope to

incorporate the rate at which cold tolerance can change into predictions of species distributions and hypothesis tests investigating the relationship between climatic variability and the rate at which animals can exhibit plasticity.

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1. GENERAL INTRODUCTION

In terrestrial ectotherms, which lack the physiological mechanisms necessary for maintenance of a high and stable internal body temperature (Porter and Gates 1969), few features of the abiotic environment are as fundamental in influencing physiology as temperature. At the sub-cellular level, temperature influences molecule kinematics and the rate of enzymatic processes (Hochachka & Somero 2002; Rogers, Seebacher & Thompson 2004). At stressful temperatures these effects can result in the denaturation of proteins, a reduced capacity for oxygen to power metabolic processes, and consequently a reduced capacity to repair any damage caused by stressful temperatures at either extreme (Hochachka & Somero 2002). At the organismal level, temperature strongly influences the performance of various processes including locomotion, cell growth, digestive efficiency, and gamete production (Huey *et al.* 1989; van Marken Lichtenbelt 1992; Angilletta, Steury & Sears 2004). At an ecological level, temperature is a consistently significant predictor in ectotherm species distribution models, especially in the case of reptiles (Kearney & Porter 2009; Aragon *et al.* 2010). For ectotherms in the northern hemisphere, cold climate boundaries can be extremely influential in determining a species' range (Thomas *et al.* 2001; Parmesan & Yohe 2003).

To characterize temperature sensitivity in ectotherms, researchers have typically generated thermal performance curves, with temperature on the x-axis and some measure of performance on the y-axis (Huey 1982). The temperatures at which performance is zero are defined as the critical thermal minimum at the cold end (CT_{min}) and critical thermal maximum at the hot end (CT_{max}). These are collectively known as measures of thermal tolerance in the field of eco-physiology. The study of thermal tolerance has been

an active area of research for ecologists since the 1940's, and researchers have since

developed a framework of methods and terminology that allow for broad comparisons

among taxa and for investigations of patterns across geographic gradients (Cowles &

Bogert 1944; Huey & Stevenson 1979; Lutterschmidt & Hutchison 1997; Angilletta,

Niewiarowski & Navas 2002). The most common way that thermal tolerances are

measured is by ramping the environmental temperature, and consequently the body

temperature, of an ectothermic organism and testing its righting reflex at various intervals

toward thermal extremes until the organism can no longer right itself when flipped onto

its back (Lutterschmidt & Hutchison 1997). The reasoning behind using such a simple

semi-behavioral assay in studies of eco-physiology is that loss of righting response is a

good indicator of where performance equals zero on the "performance curve," when we

define performance as mobility or related to mobility. Though performance can be

measured as gamete production, cell growth, digestive efficiency, or any other biological

measurement that can possibly vary with temperature, mobility is of interest to ecologists

because loss of mobility implies "ecological death" (Cowles & Bogert 1944). When

temperatures lead to immobility an organism is unlikely to defend itself, escape from

predators, forage, or find shelter from further climatic stress, likely leading to the death of

the organism before it reaches lethal temperatures associated strictly with the

physiological effects of temperature (e.g., freezing of tissue, denaturing of proteins, etc.).

Though there is currently no universally accepted description of the mechanisms

that determine thermal tolerance, recent studies have focused on the role of oxygen use in

determining both cold and heat tolerance of ectotherms (Smith *et al*. 2015; Verberk *et al*.

2016; Shea *et al*. 2016; Campbell-Staton *et al*. 2018). The oxygen and capacity limited

thermal tolerance hypothesis (OCLTT) states that at thermal extremes the demand for oxygen is outpaced by the organism's ability to supply oxygen (Pörtner 2001). In the case of cold tolerance, Campbell-Staton and others recently found that lactate concentration (a proxy for oxygen demand) and metabolic rate explained variation in observed CT_{min} across populations of green anole lizards (*Anolis carolinensis*) in Texas (Campbell-Staton *et al*. 2018). They identified transcriptional regulation of blood physiology, especially through upregulation of plasminogen, as a candidate for explaining how cold tolerance can change with acclimation within the lifetime of an organism (Campbell-Staton *et al*. 2018). In the case of heat tolerance, we know that in some organisms proteins can denature at ecologically relevant temperatures (Hochachka & Somero 2002), and that heat shock proteins (hsp's) can play a role in determining CT_{max} (Fangue, Hofmeister & Schulte 2006; Gao *et al*. 2014). In addition to the role hsp's themselves may have in determining CT_{max}, the synthesis of hsp's, the ubiquitination and degradation of denatured proteins, the replacement of damaged proteins, and the repairing of damaged structures such as cell membranes are all metabolically (O_2 and ATP) costly activities (Hochachka & Somero 2002), further complicating the role of oxygen transport and its interaction with hsp's in determining CT_{max}. As Hochachka and Somero point out in their book *Biochemical Adaptation* (2002), all biological systems are thermally sensitive and therefore all are likely to play a role in determining thermal tolerance. The goal of the researcher is then to determine which physiological mechanisms and systems play a larger role than the others in the determination of thermal tolerances.

Thermal tolerances, along with other organismal traits, are capable of changing within the lifetime of an organism. This is known as reversible plasticity (Fusco &

Minelli 2010). Plasticity is assumed to be beneficial to an organism if the resulting change in a trait caused by change in the environment results in a higher fitness in the acclimated organism relative to an un-acclimated organism (Fusco & Minelli 2010). In other words, the change in a trait does not need to completely void the negative effects of a change in the environment to be beneficial. The mechanisms of reversible plasticity involve environmentally stimulated adjustments to the regulation of the proteins that interact to determine trait values (Fusco & Minelli 2010). Modifications to the substrates of a system can occur through upregulation and down-regulation of key enzymes (such as plasminogens and hsp's), which in turn can occur through changes in the affinity of regulators and methylation of DNA itself (Zuckerkandl & Villet 1988).

One way to define thermal plasticity in an organism is to measure a trait at a given ambient temperature, and then measure the trait again after a given change in ambient temperature. Dividing the difference in trait values by the difference in ambient temperatures gives a value commonly referred to as the acclimation response ratio (ARR), which can be defined as change in a trait given a 1°C change in acclimation temperature (Claussen 1977). A meta-analysis involving amphibians, crustaceans, fish, insects and reptiles revealed that crustaceans and fish show a greater change in both CT_{min} and CT_{max} in response to a change in acclimation temperatures (Gunderson & Stillman 2015). The reason reptiles, amphibians, and insects exhibit lower overall plasticity compared to crustaceans and fish is not well known. Interestingly, the terrestrial taxa showed a negative correlation with CT_{min} plasticity and seasonality (Gunderson & Stillman 2015). This suggests that although overall plasticity in CT_{min} is weak in terrestrial ectotherm taxa compared to aquatic ectotherm taxa, CT_{min} is sensitive to

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environmental stimuli only in terrestrial ectotherm taxa. Clusella-Trullas and Chown (2014) found that ARR values for CT_{min} were higher than ARR values of both CT_{max} and preferred body temperatures in lizards. It should be noted that comparisons of thermal plasticity across studies are complicated by variation in methodologies. The ramping rate of temperatures during tolerance tests, the length between different acclimation treatments, the extent of the difference in acclimation temperatures, and the way that temperature itself is manipulated (i.e. constant, varying diurnally, or varying randomly) can all influence thermal tolerance values and the consequent ARR of ectotherms (Hutchison 1961; Claussen 1977; Terblanche *et al*. 2007; Allen *et al*. 2016; Kingsolver *et al*. 2016).

That some organisms have inherently higher capacities for plasticity in some traits has led to interesting hypotheses in ecological studies, especially relating to invasive species. For example, researchers hypothesized that plasticity may be important for invasion success. If so, invasive species should show more plasticity and this plasticity should result in a benefit for the invasive species. In an analysis involving 75 invasive/non-invasive plant species pairings, Davidson et al. (2011) found that although there was overwhelming support for higher plasticity in invasive plants, fitness advantages for invasive species were only detectable in resource limited environments. In a meta-analysis of mostly marine ectothermic animals, Kelley (2014) found that invasive species had higher levels of hsp expression compared to native species. ARR of upper thermal tolerance was also higher in invasive species compared to natives (Kelley 2014). Within closely related species of insects, several studies suggest that the more widespread species have higher capacities for plasticity in cold tolerance in the form of rapid cold

hardening (Chen *et al.* 1990; Kelty & Lee 2001; Bahrndorff *et al.* 2009; Overgaard *et al.* 2011). Within members of an invasive species, plasticity would be most beneficial to individuals more likely to experience novel climatic conditions (i.e., at the expanding range) and hence may be selected for in these individuals. Two studies on the highly successful invasive South American cane toads (*Rhinella marina*) in Australia provide some evidence for this idea (Kolbe, Kearney & Shine 2010; McCann *et al.* 2014). McCann et al. (2014) found that toads at the expanding southern edge were able to change their CT_{min} 12 h into a cold acclimation treatment. Kolbe et al. (2010) found that toads at a lower latitude (northward) were still decreasing CT_{min} 8 weeks into a cold acclimation treatment. Final CT_{min} values after cold acclimation were not reported in the Kolbe et al. (2010) study; although we do not know if the overall change in CT_{min} was the same in all populations, we do know that the population at the advancing edge changed its CT_{min} faster than the other two populations studied. Rapid decrease of CT_{min} in response to cold conditions may explain how toads at the southern edge are invading high elevation sites where ambient temperatures are likely colder than in any other native or non-native site inhabited by these toads (McCann *et al.* 2014).

Population level trends in thermal tolerances and plasticity also reveal geographic and climatic patterns. Daniel Janzen was one of the earliest ecologists to make predictions about how climate and climatic variability determine the distribution of organisms (Janzen 1967). Janzen argued that the reduced climatic variability in the tropics meant that mountains in the tropics were more effective at limiting dispersal compared to temperate zone mountains (Janzen 1967). The assumptions of Janzen's hypothesis have led to what is now known as the climatic variability hypothesis (CVH),

which states that organisms from more climatically variable environments are better adapted to physiologically deal with varying climatic conditions (Stevens 1989). If the CVH is true, and if plasticity of a trait is beneficial, then plasticity should increase with latitude (and therefore climatic variability). Indeed, a recent meta-analysis indicated that across taxa terrestrial ectotherms have higher thermal tolerance breadths (breadth = $CT_{max} - CT_{min}$) as latitude increases (Sunday, Bates & Dulvy 2010), the effect being greatly driven by lower CT_{min} as latitude increases. Additionally, Gunderson & Stillman (2015) found that across terrestrial ectotherms only CT_{min} plasticity was predicted by seasonality. The results of these meta-analyses suggest that terrestrial ectotherms from climatically variable environments do tolerate a wider range of temperatures, possibly due to plasticity in CT_{min}.

Mechanistic species distribution models apply these theories by incorporating thermal tolerance data to predict ectotherms' responses to changing climates (Kearney & Porter 2009; Buckley, Hurlbert & Jetz 2012). This is typically accomplished by assuming that activity time will be restricted by the amount of habitat suitable for thermoregulation throughout the day (Sinervo 2010; Buckley *et al.* 2012). Though informative, these studies often treat thermal tolerance values as fixed traits. Pintor *et al.* (2016a) recently found that most studies measuring cold tolerance of ectotherms may underestimate true values due to short experimental acclimation times of 0-2 weeks. By doing so, we overlook the impacts of reversible plasticity (i.e., the capacity for physiological traits to change within an individual's lifetime). Failure to take plasticity into account could overestimate the impacts of a changing climate and could underestimate true range expansion estimates in response to novel and changing climatic conditions. Indeed,

terrestrial organisms can be found outside of thermally suitable habitat as set by thermal

tolerance values, especially at the cold end of their habitat (Sunday, Bates & Dulvy

2010). Though this unpredictability may be due to behavioral buffering, physiological

plasticity is expected to play a role as well. For the most part, past literature investigating

plasticity has focused on the presence and direction of plasticity of informative traits,

without examining the extent to which a trait can change and the rate at which it changes.

In this study we investigate the capacity for plasticity of various traits, the extent to which

cold tolerance changes, and the rate at which cold tolerance changes in response to cold

acclimation in two non-native populations of a successfully introduced species of lizard:

the Italian wall lizard (*Podarcis siculus*).

P. siculus has been successfully introduced to various urban and suburban U.S.

cities in the last 100 years. In many cases, particularly in the northern U.S. where cold

winters prevent range expansion of native species, it is the only lizard species present.

One subspecies in particular, the northern Italian wall lizard (*P. siculus campestris*) is

currently found in six U.S. states (CA, CT, KS, MA, NJ, and NY), whereas the

Mediterranean Italian wall lizard (*P. siculus siculus*) is currently only found in southern

California within the U.S. (Kolbe *et al.* 2013; Donihue, Lambert & Watkins-Colwell

2015; Donihue 2017). To date, it is not known how much of this species' remarkable

success in these novel climates can be attributed to pre-existing adaptation, rapid

adaptation, or phenotypic plasticity. To chip away at this is gap in knowledge, I

investigated the capacity for plasticity in commonly measured physiological variables

using two populations of this species: one population from San Pedro, CA (*P. siculus*

siculus) and one from Long Island, NY (*P. siculus campestris*). Lizards were lab

acclimated to similar conditions for eight months prior to any measurements. After initial measurement of variables, lizards were exposed to a cold acclimation treatment designed to mimic New York surface air temperatures during the onset of winter. The full cold acclimation treatment lasted 13 weeks. Thermal tolerance values, thermal preference, metabolic rate, and evaporative water loss were measured before and after 5 weeks of cold acclimation. Cold tolerance was measured once weekly during the cold acclimation treatment for 5 weeks, and once more at 12 weeks to determine if cold tolerance continuously decreased with cold acclimation. After the 13-week cold acclimation treatment lizards were returned to their original laboratory housing conditions.

The accumulation of information on plasticity will improve the predictability of empirical and theoretical models aiming to investigate the response of ectotherms to novel and changing climates, including but not limited to a warming planet. With increasing global connectivity, we are likely to see an increase in the introduction of species to novel conditions. With climate change, we are likely to see range expansions as species seek to behaviorally buffer the effects of stressful conditions. Although there are many biotic interactions that determine a species' success in the face of novel and changing conditions, the first hurdle in survival is an adequate individual response to the abiotic environment. Understanding the capacity for change, the extent of change, and the speed of change is necessary for an understanding of how and why some species are more successful than others. Future work must also determine the role of pre-existing genetic differences, as well as the mechanistic processes that allow for plasticity, for us to better understand the role of plasticity in successful species.

2. COLD ACCLIMATION RESPONSE OF NON-NATIVE ITALIAN WALL LIZARD (*PODARCIS SICULUS*) POPULATIONS FROM NEW YORK AND CALIFORNIA

2.1 Introduction

Temperature has a profound impact on terrestrial ectotherms at multiple biological scales; consequently, temperature preferences and tolerances, along with temperature sensitivity of biological rates, are commonly measured when investigating an ectothermic organism's adaptation to its thermal environment (Mautz 1982a; Al-Sadoon & Spellerberg 1985; Kearney & Porter 2004; Huey *et al*. 2009; Clusella-Trullas, Blackburn & Chown 2011). These physiological estimates can be used to empirically and theoretically investigate ectotherms' responses to novel stressful conditions such as a warming climate (Kearney & Porter 2004; Deutsch *et al*. 2008; Sinervo *et al*. 2010; Levy *et al*. 2016; Carlo *et al*. 2018). Predictive models typically assume that potential activity time is restricted to times when ambient temperatures fall within the thermal tolerance range, to allow for proper thermoregulation (Sinervo *et al*. 2010; Buckley, Hurlbert & Jetz 2012). For ectotherms the results of these studies suggest decreased fitness at the equator as global temperatures increase (Deutsch *et al*. 2008; Huey *et al*. 2009). Indeed, poleward range expansion and equatorial range contraction have been observed across many ectotherm taxa (Sunday, Bates & Dulvy 2012). In addition to fixed trait values for animals measured in wild or lab acclimated conditions, the capacity for change in physiological traits within an individual's lifetime (i.e., reversible plasticity) can also alter an organism's fitness (Fusco & Minelli 2010).

Studies in eco-physiology have often focused on determining whether a trait

changes in response to different ambient temperatures and the direction of that change

(Clusella-Trullas & Chown 2014; Gunderson & Stillman 2015). To further understand

how traits change in response to different temperatures, we also need to know the extent

to which a trait can change, the rate at which it changes, and whether the response is

linear (Pintor, Schwarzkopf & Krockenberger 2016a). Of special interest is the

acclimation ability of cold tolerance in terrestrial ectotherms due to the relatively high

capacity for change in CT_{min} (Clusella-Trullas & Chown 2014; Gunderson & Stillman

2015) and due to its correlation with ambient temperatures (Araujo *et al*. 2013). In the

northern U.S., an increase of extreme winter weather events is predicted as a consequence

of a warming Arctic (Cohen, Pfeiffer & Francis 2018). Severe winter weather events

have been shown to have profound impacts on cold tolerance of ectotherm populations

(Campbell-Staton *et al*. 2017). Pintor *et al* (2016a) recently found that most studies

measuring cold tolerance of ectotherms may underestimate true values due to short

experimental acclimation times of 0-2 weeks. Incorporation of the extent and rate of

plastic responses in models and meta-analyses is made difficult by the lack of published

work on the topic, although some ways around this are to include values for traits

measured across time and space for a species (e.g., Sinervo *et al*. 2010), or to use the

most extreme values observed in the literature (e.g., Sunday, Bates & Dulvy 2010). In

addition to improving model predictability, accumulation of information on the rate at

which cold tolerance can change within an individual's lifetime will allow us to

investigate the relationship between climatic variability and capacity for plasticity in cold

tolerance (e.g., Gunderson & Stillman 2015), thus improving our ability to predict the responses of ectotherms to climates experiencing winter extremes.

In this study we report the plasticity of multiple physiological traits and the time-course of change in cold tolerance for two non-native populations of the Italian wall lizard, *Podarcis siculus* (Rafinesque 1810), following cold acclimation. Lizards from a Long Island, New York population and a San Pedro, California population were used to investigate the capacity for cold acclimation in this species. *Podarcis siculus* has been successfully introduced from Italy to various U.S. urban and suburban sites in the last 100 years (Kolbe *et al.* 2013). The Long Island, New York population has been geographically separated from its source population in Tuscany, Italy for 50 years and has been identified as belonging to the *Podarcis siculus campestris* subspecies complex (Kolbe *et al.* 2013). The San Pedro, California population has been geographically separated from its source population in Sicily, Italy for over 23 years and has been identified as belonging to the *Podarcis siculus siculus* subspecies complex (Kolbe *et al.* 2013). The NY population is thought to have originated from a pet shop after storm damage led to their escape in 1967 (Gossweiler 1975). The CA population was introduced in 1994 by a local resident who wanted to keep a small population (seven adults) as pets in his backyard (Deichsel *et al.* 2010), and the population has since been spreading at a rate of about 100m per year (G.B. Pauly, pers. comm.).

Magnitude of seasonal change is high in the Long Island, New York habitat, with a summer to winter difference of 20.4°C and a standard deviation of 8.5°C in average low monthly surface air temperatures (Arguez *et al.* 2012). Consequently, cold snaps tend to be more extreme in the NY habitat, with surface temperatures as low as -20°C being

reported during winter in Long Island (Arguez *et al.* 2012). NY lizards are less active

than Italian populations during their respective winter months (Burke & Ner 2005),

suggesting selection pressures from the winter environment on NY lizards in the short

time since these lizards arrived (Burke *et al.* 2002). San Pedro, CA experiences a summer

to winter difference of 9°C and a standard deviation of 3.9°C in average low monthly

surface air temperatures (Arguez *et al.* 2012). In general, it seems that the introductions

to these respective U.S. locations have extended pre-existing differences in low

temperature patterns that *P. siculus campestris* and *P. siculus siculus* were experiencing

in Italy (Table A1). Precipitation and annual rainfall in the NY and CA habitats have also

diverged in a way that extends pre-existing differences from their Italian habitats (Table

A2).

We hypothesized that due to the NY lizard's historical Italian climate and due to

their recent success in NY, lizards from this population would have a relatively high

capacity for plasticity in cold tolerance compared to lizards from the CA population.

Specifically, we aimed to determine: (1) if after initial lab acclimation there were

persistent differences between these two non-native populations of *P. siculus*, (2) if

lizards from these two populations would differ in their response to cold acclimation, and

(3) the extent to which cold tolerance would change and the rate at which it changed for

both populations.

To do this, we measured changes in traits in response to a cold acclimation

treatment. Specifically, we measured thermal tolerance limits (CT_{min} and CT_{max}) and

thermal preference (T_{pref}) to examine physiological and behavioral traits associated with

thermoregulation. We measured thermal tolerance limits as critical thermal maxima

(CT_{max}) and critical thermal minima (CT_{min}) because these values are frequently used to define limits to activity periods of ectotherms in simulation models (Sinervo *et al.* 2010; Buckley *et al.* 2012), and they are measured in similar ways across taxa, allowing for data to be used in future meta-analyses and hypothesis testing (Huey 1982; Lutterschmidt & Hutchison 1997; Angilletta, Niewiarowski & Navas 2002; Araujo *et al*. 2013). We measured preferred temperatures (T_{pref}) to investigate if any changes in thermal tolerance would be accompanied by changes in behavioral thermoregulation. The T_{pref} range also tends to encompass the optimal temperature for various physiological processes and performance of whole organism traits (Dawson 1975; but see Huey & Bennett 1987 for exceptions; Martin & Huey 2008). Although acute temperature stressors can inform us about responses to temperature extremes, consistent temperature stressors inform us about long term costs associated with stressful ambient temperatures (Bennett 1982; Mautz 1982b). Thus, we also measured temperature dependent rates of evaporative water loss (EWL) and standard metabolic rate (SMR_{O2}). To determine if overall hydration state of lizards changed before and after cold acclimation, we measured serum osmolality in a subset of lizards used. To determine if a change in SMR_{O2} was correlated with hemoglobin concentration, we measured hemoglobin concentration in a subset of lizards. Through comparing the effects of cold acclimation on the overall thermal physiology of lizards from these two non-native populations of *P. siculus,* we hope to gain a better understanding of the extent to which acclimation ability can differ between individuals of the same species.

2.2 MATERIALS AND METHODS

2.2.1 Study organism and cold acclimation

We collected 36 *P. siculus* in July 2016 from the two previously mentioned U.S. populations: 24 *P. siculus campestris* (12 males, 12 females) from Hempstead, New York (33°44′10″N, 118°17′32″W, elevation 36 m) and 12 *P. siculus siculus* (six males, six females) from San Pedro, California (33°44′10″N, 118°17′32″W, elevation 36 m). Lizards were housed individually in 10 L plastic terraria (27 cm x 16 cm x 16 cm, Lee's Aquarium & Pet Products, San Marcos, CA, USA) with approximately 1.5 cm of shredded bark as substrate (Zilla bark blend, Franklin, WI, USA). Each terrarium was placed halfway atop a heating pad (Sunbeam, Boca Raton, FL, USA) on the "low" setting, creating a 27°C-32°C gradient across the terrarium and relative humidity of 30 % within the terrarium. A water bowl/hide was placed on the cooler side of the terrarium to reduce evaporation. All terraria were situated under full spectrum lighting set for a 12 h light: 12 h dark cycle. Lizards were fed 4-5 crickets bi-weekly and given water *ad libitum*. Crickets were dusted with vitamins (calcium and vitamin D) every third feeding. Lizards were housed under identical laboratory conditions for at least 8 months before any testing, to acclimate to common laboratory conditions and therefore test for fixed differences between populations, and to ensure that responses to cold acclimation were due to the acclimation treatment and not recent thermal history prior to the treatment. All animal use protocols were evaluated and approved by the Institutional Animal Care and Use Committee at California Polytechnic State University (Cal Poly; protocol #1514).

Prior to cold-acclimation, subsets of captured lizards were tested for thermal tolerance, thermal preference, metabolic rate, evaporative water loss, hemoglobin concentration, hematocrit, and serum osmolality. We performed studies on subsets of lizards for each variable due to material and time constraints. We chose lizards at random while balancing for equal sex ratios between populations. Once lizards were chosen for pre-acclimation measurements, these same lizards were used for post-acclimation measurements. Missing values occasionally occurred due to equipment difficulties or death of animals, resulting in different pre vs. post acclimation sample sizes. We report the specific sample sizes for all variables measured under their respective subsections.

Our acclimation treatment was a temperature regime designed to mimic average surface temperatures in the New York population's location at a time when activity is severely decreased (Burke & Ner 2005; Arguez *et al.* 2012). This temperature acclimation treatment began mid-November 2017 with one week of 20°C: 18°C (Light: Dark), followed by four weeks of 17 °C: 16.5 °C. Photoperiod remained the same as before acclimation: 12 hours light and 12 hours dark. To achieve these conditions, lizards were moved within their original cages into large refrigerated incubators (Model 818, Thermo Scientific, Waltham, MA, USA). During cold acclimation, lizards were offered 1-2 crickets weekly and given water *ad libitum*. CT_{min} was tested weekly during the acclimation period for 5 weeks on all lizards with the exception of four NY lizards only tested at the 5-week mark to determine if repeated testing had an effect on CT_{min}. After 5 weeks of cold acclimation, all other variables were re-measured. Lizards were maintained at 17°C: 16.5°C for an additional seven weeks, and cold tolerance was tested once more. The full temperature acclimation treatment lasted 12 weeks. After the treatment, lizards

were returned to room temperature for two weeks, then provided heat mats to achieve pre-acclimation temperatures.

2.2.2 Thermal tolerances

We measured CT_{min} before cold acclimation in 16 lizards (n=eight NY, n=eight CA). A week into the cold acclimation treatment, we measured CT_{min} in 29 lizards (n=17 NY, n=12 CA) weekly until the 5th week, when an additional four NY lizards were tested to see if repeated testing had an effect on final cold tolerance value. We measured CT_{min} as the lowest temperature at which a lizard was still mobile (Cowles & Bogert 1944; Huey & Stevenson 1979; Angilletta, Niewiarowski & Navas 2002). We recorded CT_{min} using a custom designed device similar to that described by Shea et al. (2016), built by engineers Rob and Doug Brewster at Cal Poly, called the Gas Analysis, Temperature, and Oxygen Regulation System (GATORS).

The GATORS device consists of five temperature-controlled acrylic tubes, allowing for simultaneous testing of five lizards per trial (Fig. A1). Each tube consists of three chambers: primary, secondary, and an insulating air gap in the outermost chamber (Fig. A2). Each lizard was placed inside a primary (innermost) chamber measuring 23 cm in length and 4 cm in diameter. These tubes could be flipped independently of each other to test a lizard's loss of righting response (see below). Air flow during trials circulated between the primary and secondary chambers. Air temperature within the primary and secondary chamber loop was controlled through a TEC1-12710 Peltier thermoelectric module attached to a LPF70A50-5-B pinned heat sync and a 12V PC fan. A more detailed explanation of how air temperature is manipulated by the device is given in the figure caption for Fig. A2. Custom Arduino software (Penscil, Inc., Los Osos, CA, USA)

allowed for individual temperature ramping behavior within each tube and recording of lizard body temperatures (T_b) at times of interest, including loss of righting response. A resistance temperature detector probe (HEL-705-U-0-12-00, Honeywell, Morris Plains, NJ, USA) was inserted ~3mm into each lizard's cloaca and secured with medical tape around the lizard's tail. Lizard T_b was reported every 6 seconds. Once lizard T_b reached room temperature, surrounding air temperature was cooled 1 °C·min^{-1}. When lizard T_b reached 15 °C, the primary chamber was quickly turned to flip the lizard onto its back and the lizard was given 5 s to right itself. If the lizard righted itself within 5 s, cooling continued at 1 °C·min^{-1}. Lizards were re-tested every 1.5 min two more times. After these initial 3 minutes, loss of righting response was then tested every minute for the remainder of the trial. Loss of righting response was typically coupled with a posture in which lizards could no longer support themselves and front limbs stiffened backward toward the body. Some lizards that had lost righting response continued walking within their temperature-controlled tube in an apparent attempt to escape the stressful conditions. Due to mobility's relevance for predicting survival at sub-lethal temperatures (Cowles & Bogert 1944), when voluntary walking was observed we continued the trial until limb stiffening was also observed and defined the lizard's T_b at this point as its CT$_{min}$.

We assessed CT$_{max}$ using the same equipment and in a manner similar to that described for CT$_{min}$, with a heating rate of 1 °C·min^{-1}. We measured CT$_{max}$ before cold acclimation in 19 lizards (nine NY, 10 CA), and after acclimation in 17 lizards (eight NY, nine CA). The difference in sample size reflects attrition of two lizards at week 5 of cold acclimation. Once T_b reached 36°C lizards were tested for righting response every 1.5 min until they reached 40°C. Lizards were then tested every 30 s because lethal

temperatures are relatively close to loss of righting response at warmer temperatures in most lizard species (Lutterschmidt & Hutchison 1997). Because we did not observe the onset of muscle spasms for any individuals in this study, T_b at loss of righting response was the only variable measured during heating and was used to define CT_{max} (Lutterschmidt & Hutchison 1997).

2.2.3 Thermal preference

We measured T_{pref} before cold acclimation in 16 lizards (n=eight NY, n=eight CA) and post-acclimation in 15 lizards (n=six NY, n=nine CA). Difference in sample size reflects the attrition of two NY lizards and one CA lizard following cold acclimation. One of the NY lizards died during an evaporative water loss trial. Two additional CA lizards were included in post-acclimation trials to increase sample size and to balance sex ratios.

We measured T_{pref} using a linear temperature gradient (163 cm L x 46 cm W) divided into four lanes running the length of the gradient, with a temperature range from 10°C to 40°C along its length. White dividers were placed between lanes so that lizards could not see each other, to reduce the potential effects of visual cues on spacing. A 1 cm thick plastic sheet was placed above the gradient to restrict air flow between lanes and to reduce potential effects of neighboring scents on spacing. The cold end of the gradient was maintained with cold water circulated through copper piping beneath the metal floor of the gradient, using a circulating water bath (SD07R-20, PolyScience, Niles, IL, USA) set to 10°C. The warm end of the gradient was maintained by electric heat strips beneath the metal floor of the gradient. The floor of the gradient was covered with reptile sand (Zoo Med, San Luis Obispo, CA, USA) so that the lizards were not in direct contact with the metal floor. The gradient was encased in a Plexiglas chamber lined with Drierite

(W.A. Hammond, Xenia, OH, USA) to reduce condensation. Each lizard was fitted with a flexible, 40-gauge type K thermocouple (Omega Engineering, Stamford, CT, USA) inserted into the lizard's cloaca and securely taped with medical tape. Four lizards were tested simultaneously, with a single lizard in each lane of the gradient, and lizard T_b was monitored continuously with a four-channel thermocouple reader (Model RDXL4SD, Omega Engineering, Stamford, CT, USA). We recorded T_b every 10 min for 4 h and determined T_{pref} as the average T_b within the last hour.

2.2.4 Metabolic rate and hemoglobin concentration

We measured standard rates of oxygen consumption ($SMR_{O2,}$ mL $O_2 \cdot h^{-1}$) and carbon dioxide expiration ($SMR_{CO2,}$ mL $O_2 \cdot h^{-1}$) using manual bolus integration at two different temperatures both before and after the cold acclimation treatment (Lighton 2008). We measured SMR_{O2} before cold acclimation in 24 lizards (n=12 NY, n=12 CA), and after cold acclimation in 23 lizards (n=11 NY, n=12 CA). One NY lizard was not used in SMR_{O2} measurements post-cold acclimation because we observed muscle stiffening at the end of the cold acclimation treatment. Lizards were fasted 5-7 days prior to any testing. Before each trial, fasted lizards were weighed to the nearest 0.001g with a digital balance (Practicum 213, Sartorius, Bohemia, NY, USA) then individually placed into 150 mL plastic syringes with a hole drilled into the 150 mL mark for ventilation (Fig. A4). Syringes with lizards were then placed into an environmental chamber set to either 15°C or 35°C and allowed to rest for 45 min with access to ambient air through the drilled hole. After this time each syringe was flushed with ambient air for 5 min, then sealed at the 140 mL mark and returned to the environmental chamber for an additional 45 min. We then injected a 20 mL subsample of air from the syringe into a flow of baseline air

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that was being analyzed for O_2 and CO_2 concentrations (Fig. A5). O_2 concentration was measured using a Sable Systems FC-10 Oxygen Analyzer (Sable Systems International, Las Vegas, NV, USA). CO_2 concentration was measured using a Sable Systems CA-10 Carbon Dioxide analyzer. Using an air pump and flowmeter located at the end of our system, baseline air was pulled through the analyzers at a rate of 100 mL·min^{-1} from outside of the building through tubing connected to a carboy (Fig. A5). This air was scrubbed of water prior to entering the CO_2 analyzer using a magnesium perchlorate $(Mg(ClO_4)_2)$ column, then scrubbed of CO_2 and water again using a DAD (Drierite/Ascarite/Drierite) column prior to entering the O_2 analyzer (Fig. A5).

Fractional concentration of O_2 in dry and CO_2-free outside air was assumed to be 20.94%. Following the conventions of Lighton (2008), the rate of O_2 consumption for each 20 mL sample of injected air was calculated using the ExpeData data analysis software (Sable Systems International, Las Vegas, NV, USA) as follows:

$$M_s O_2 = \frac{\left[100\text{mL·min}^{-1}(0.2094\text{-}F'_e O_2)\right]}{(1\text{-}0.2094)}$$

where $F'_e O_2$ is the fractional concentration of O_2 in the sample. This rate was then plotted as a function of time, and the volume of O_2 for each sample was determined by integrating under the curve of $M_s O_2$ with respect to time. We estimated volume of gas consumed in the chamber with the following equation:

$$\text{VolO}_{2\,\text{final}} = \text{VolO}_{2\,\text{sample}} \cdot \left(\frac{\text{Vol}_{\text{syringe}}\text{-Vol}_{\text{animal}}}{\text{Vol}_{\text{sample}}} \right)$$

where VolO$_2$ final is the final volume of gas consumed by the lizard, VolO$_2$ sample is the

volume consumed in the sample, Vol$_{syringe}$ is the syringe volume of 140 mL, Vol$_{animal}$ is

the volume of the animal estimated using the animal's mass and assuming a density of

0.98 g · cm^{-3}, and Vol$_{sample}$ is the sample volume of 20 mL. We then divided this volume

of gas by total time sealed to determine the rate of O$_2$ consumption for each lizard in its

container. Carbon dioxide concentration of each sample was used to calculate CO$_2$

production rate in a similar manner. The following equation was used to determine the

uncorrected sample rate of CO$_2$ production:

$$M_s CO_2 = \left[100\text{mL·min}^{-1}(F'_i CO_2 - F'_e CO_2) \right]$$

Because the CO$_2$ analyzer was the first analyzer in the series, this calculation could not

take the effects of CO$_2$ enrichment by O$_2$ depletion into account. Uncorrected volume of

CO$_2$ produced (Vol$_u$CO$_2$) was calculated using integration as described for O$_2$ and then

corrected for CO$_2$ enrichment using the following equation:

$$\text{VolCO}_2 = \frac{\left[\text{Vol}_u CO_2 - (F'_i CO_2 \cdot \text{VolO}_2) \right]}{(1 - F'_i CO_2)}$$

where VolCO$_2$ is the corrected volume of CO$_2$ produced in the sample, Vol$_u$CO$_2$ is the

uncorrected volume of CO$_2$ produced in the sample, $F'_i CO_2$ is the fractional

concentration of CO$_2$ background levels measured for each trial, and VolO$_2$ is the volume

of O$_2$ consumed in the sample. Rate of CO$_2$ production by each lizard was then calculated

as described for O$_2$ using the corrected volume of CO$_2$. CO$_2$ volume produced was used

to define a respiratory exchange ratio (RER) for each lizard as CO_2 volume produced to O_2 volume consumed.

To take into account the allometric relationship between mass and metabolism, we log transformed SMR_{O2} and included log transformed mass as a covariate in our statistical models (Hayes & Shonkwiler 1996; Packard & Boardman 1998; Lighton 2008). An interaction between log-transformed mass and population was included in the model to test for different scaling coefficients of mass and metabolic rate between populations. We calculated a simple metric of temperature sensitivity (MR Q_{10}, the ratio of metabolic rate at a given temperature to metabolic rate at 10°C lower) by using the following formula:

$$MR\ Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10\ ^{\circ}C}{T_2 - T_1}}$$

where MR Q_{10} is the factor by which standard metabolic rate increases given a 10°C increase in temperature, R_2 is the rate of oxygen consumption at 35 °C, R_1 is the rate of oxygen consumption at 15°C, and $T_2 - T_1$ is the difference in temperatures at which the rates were measured (35°C – 15°C in our case).

We were interested in seeing if changes in SMR_{O2} (and presumably in ATP production) would correlate with changes in free hemoglobin, and if this relationship would differ between populations. Thus, we investigated the effects of cold acclimation on hemoglobin concentration, and the effect of hemoglobin concentration on metabolic rate, for a small subset of lizards (n=five NY, n=six CA). Approximately 70 μL of whole

blood were collected from lizards via the infra-orbital sinus using self-sealing heparinized micro-capillary tubes. 10 μL of this whole blood was immediately analyzed for hemoglobin concentration (g · dL^{-1}) using a HemoCue hemoglobin analyzer (HemoCue, model 121721, Brea, CA, USA). This was done on the same lizards once before cold acclimation and once again after cold acclimation. The respective hemoglobin concentrations were regressed against SMR$_{O2}$ at 15°C and 35°C for pre- and post-acclimation measurements.

2.2.5 Evaporative water loss and hydration state

We estimated rates of evaporative water loss (EWL, mg H$_2$O · h^{-1}) by observing mass lost over time at each of four experimental temperatures (10 °C, 20 °C, 30 °C, and 40 °C). We measured EWL before cold acclimation in 24 lizards (n=12 NY, n=12 CA), and after cold acclimation in 19 lizards (n=seven NY, n=12 CA). Following 5 weeks of the cold acclimation treatment, the first EWL trial was randomly selected to be 40 °C. This proved to be too hot for lizards following cold acclimation, and death of four lizards from the NY population occurred within 1.5 hrs. We consequently terminated the 40°C post-acclimation trial to prevent further animal loss and excluded the 40°C level within the temperature factor from statistical comparisons of pre- and post-acclimation values. An additional lizard died of unknown causes in August 2017 before the cold acclimation treatment.

Prior to each trial lizards were fasted for 5-7 days, then placed inside individual fiberglass mesh pouches (18 x 6 mesh count, 0.11ga diameter) and weighed to the nearest 0.001g using a digital balance (Practicum 213, Sartorius, Bohemia, NY). The lizards in

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pouches were placed atop individual petri dishes on three separate shelves inside an environmental chamber (Precision model 818 Plant Growth Chamber, Thermo Scientific, Somerset, NJ, USA) set to either 10°C, 20°C, 30°C, or 40°C for 7.5 h. Experimental temperatures were tested in random order. Relative humidity within the chamber scaled linearly with temperature (RH = 34.3 – 0.63T, R^2 = 0.87), measuring 28%, 21.7%, 15.4%, and 9.1% at 10°C, 20°C, 30°C, and 40°C respectively. Speed of air flow was measured to be 1.4 km · h^{-1} at the vent within the chamber, but was undetectable at shelf height. Lizards were re-weighed every 1.5 h and their positions were rotated among the shelves between weightings to control for any potential inconsistency of temperature, humidity, and air flow within the chamber. Urination or defecation was never detected on petri dishes and urination was never detected in pouches; on the rare occasion when feces was observed in a pouch between weightings its mass was undetectable, so the trials were continued with the small amount of feces removed.

Rate of evaporative water loss was calculated as the net loss in body mass over the duration of a trial (Withers, Aplin & Werner 2000; Pintor, Schwarzkopf & Krockenberger 2016b). We analyzed log transformed EWL with log transformed mass as a covariate to investigate and take into account the allometric effects of size on rates of physiological processes (Schmidt-Nielsen 1984; Hayes & Shonkwiler 1996; Packard & Boardman 1999). We calculated a simple metric of temperature sensitivity (EWL Q_{10}, the ratio of EWL at a given temperature to EWL at 10°C lower) in the same manner as described for MR Q_{10} using the following equation:

$$EWL\ Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10\ \text{°C}}{T_2 - T_1}}$$

where EWL Q_{10} is the factor by which evaporative water loss rate increases given a 10°C increase in temperature, R_2 is the rate of water loss at 30 °C, R_1 is the rate of water loss at 10 °C, and $T_2 - T_1$ is the difference in temperatures at which the rates were measured (30°C – 10°C in our case).

We investigated the effects of cold acclimation on hydration in a subset of lizards (n=five NY, n=six CA) by measuring serum osmolality before and after cold acclimation. This was done using a separate subgroup of lizards than the group used for hemoglobin analysis. To investigate hydration state, approximately 70 µL of whole blood were collected from lizards via the infra-orbital sinus using glass micro-capillary tubes coated with a minimum of 2 USP units ammonium heparin per tube. Blood was transferred into 0.5 mL micro-centrifuge tubes and allowed to form a clot for 15 minutes at room temperature. Blood was then centrifuged (13,000 rpm, 2 min) (Eppendorf, model 22620207, Hauppauge, NY, USA) and 10 µL of the resulting supernatant was measured for osmolality (mOsm · kg^{-1}) using a vapor pressure osmometer (Wescor model 5600, Logan, Utah, USA).

2.2.6 Field comparison in CA

To investigate whether changes in physiological properties of lab acclimated lizards were representative of the physiology of lizards in the field, we performed a series of measurements on wild-caught, non-acclimated lizards before and after the natural winter

season in California. We measured CT_{max}, hemoglobin concentration, and serum

osmolality of wild lizards from San Pedro, CA on August 23rd, 2017 and again on April

10th, 2018. Data reported by Torrance Airport (www.ncdc.noaa.gov/cdo-web/datasets,

accessed June 6, 2018), which is approximately 5 miles from our collection site, showed

that daily average surface air temperatures for August 2017 ranged from 18.33°C to

25.56 °C, with no precipitation observed. Daily average surface air temperatures for

March 1st-April 8th, 2018 ranged from 10.87°C to 19.72°C, with a total 14.48 mm of rain

observed. On April 9th, surface air temperatures increased to an observed maximum of

31.67°C and stayed relatively warm for the rest of the month. This increase in ambient

temperatures allowed us to collect lizards for studies on April 10th following the cold

snap, as lizards were less active and thus difficult to find in the colder weather. A total of

14 lizards (nine males, five females) were caught for CT_{max} and seven additional lizards

(four males, three females) were caught for analysis of blood properties in August 2017.

Ten lizards (five males, five females) were caught for CT_{max} and six lizards (four males,

two females) were caught for analysis of blood properties in April 2018. Lizards were

tested within 24 h of capture and euthanized post-testing by intracardiac injection of

sodium pentobarbital.

2.2.7 Data analysis

We analyzed our data with linear-mixed effects models (LMMs) using the 'lme4'

package in the R statistical environment (Bates *et al.* 2017), with lizard ID treated as a

random effect when repeated measures were performed. We accounted for sex by

including sex as a fixed effect in all models. For all measured variables, we investigated

an interaction between cold acclimation status (pre vs. post) and population (NY vs. CA)

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to determine if populations differed in their capacity for plasticity. When significant

interactions were present we compared independent means by pairwise comparisons

(PWC) using the 'multcomp' package in the R environment (Hothorn *et al.* 2017). *P*-

values were corrected for multiple tests using the Holm method (Holm 1979). Model

residuals were visually inspected to ensure that data met the assumptions of parametric

analysis and data were transformed if necessary to meet these assumptions (i.e.,

transformed so that model residuals did not significantly deviate from a normal

distribution through use of Shapiro-Wilks tests). Summary statistics presented in the text

are mean ± SEM.

2.3 Results

2.3.1 Thermal tolerances

We found that after the initial lab acclimation period, lizards from both populations did

not differ in CT_{min} (PWC, $z = 0.28$, corrected $P = 1.00$; Tables 1 and 2). The effect of cold

acclimation on CT_{min} did significantly differ between populations, however (LMM,

$F_{1,147.6} = 5.92$, $P < 0.001$; Tables 1 and 2; Fig. 1). Lizards from the California population

did not exhibit a significant decrease in CT_{min} after 12 weeks of cold acclimation (PWC, z

$= 0.79$, corrected $P = 1.00$), whereas New York lizards did (PWC, $z = 3.61$, corrected $P =$

0.005; Fig. 1). Overall, CT_{min} of NY lizards decreased from $10.16 \pm 0.72°C$ to $7.14 \pm$

0.52°C over the 12-week acclimation period. The only significant change on a week-to-

week basis for either population occurred during the shift from 20°C: 18°C to 17.5°C:

16°C for NY lizards, when CT_{min} changed from $9.99 \pm 0.56°C$ to $8.00 \pm 0.42°C$ (week 1

to week 2; PWC, $z = 3.39$, corrected $P = 0.011$). The four lizards that had not been tested

for CT_{min} weekly but were tested at week 5 did not significantly differ from lizards that had been repeatedly tested (PWC, $z = 0.46$, corrected $P = 1.00$), suggesting no effect of repeated testing on CT_{min} in this group.

Using backward stepwise selection we chose parameters used to describe the relationship between time (week) and CT_{min} in NY lizards and found time to be the only significant predictor. We fit a non-linear model using the 'nlme' package in R (Pinheiro *et al.* 2016) to describe the relationship between time and CT_{min} for NY lizards during weeks 1-12 of cold acclimation at 17.5 °C: 16 °C. The exponential decay function took the following form, where w = week:

$$CT_{min} = 6.90 + 3.07 \cdot e^{-1.11 \cdot w}$$

As with CT_{min}, the two populations showed no significant difference in CT_{max} after the initial lab acclimation period (PWC, $z = 3.01$, corrected $P = 0.71$, Tables 1 and 2). Unlike with CT_{min}, both populations showed a significant decrease in CT_{max} after 5 weeks of cold acclimation (LMM, $F_{1,19.4} = 22.40$, $P < 0.001$, Tables 1 and 2; Fig. 2). The decrease in CT_{max} following cold acclimation was not significantly different between populations (PWC, $z = 1.83$, corrected $P = 0.22$).

2.3.2 Thermal preference

We found that T_{pref} did not differ between populations after initial lab acclimation, but there was a significant interaction between cold acclimation and population (LMM, $F_{1,10.3} = 6.94$, $P = 0.024$, Tables 1 and 2). After 5 weeks of cold acclimation, T_{pref} significantly decreased from 36.11 ± 0.48°C to 30.58 ± 2.53°C in NY lizards (PWC, $z = 4.02$,

corrected $P < 0.001$; Fig. 2) but did not significantly decrease in CA lizards (PWC, $z =$ 0.92, corrected $P = 0.74$; Fig. 2).

2.3.3 Metabolic rate and hemoglobin concentration

As expected, mass was a significant predictor of metabolic rate in both populations (LMM, $F_{1,\,23.3} = 9.78$, $P = 0.005$, Table 3). The effect of mass on SMR_{O2} did not differ between populations. After the initial lab acclimation period, we found that NY lizards had a significantly higher SMR_{O2} at 15°C compared to CA lizards at 15°C (PWC, $z = $ -4.31, corrected $P < 0.001$; Fig. 3). We found a significant interaction between temperature and cold acclimation (LMM, $F_{1,63.2} = 17.08$, $P < 0.001$, Table 3), such that SMR_{O2} at 15°C decreased after cold acclimation in CA lizards (PWC, $z = 3.09$, corrected $P = 0.016$; Fig. 3) but did not significantly change with cold acclimation in NY lizards at either temperature (15°C PWC, $z = 1.84$, corrected $P = 0.40$; 35°C PWC, $z = -1.48$, corrected $P = 0.67$; Table 3, Fig. 3). We also found a significant interaction between temperature and population (LMM, $F_{1,63.2} = 13.16$, $P < 0.001$; Table 3, Fig. 3). SMR_{O2} significantly increased with temperature for both populations before and after cold acclimation (NY PWC, $z = -20.62$, corrected $P < 0.001$; CA PWC, $z = -26.51$, corrected $P < 0.001$). The factor by which SMR_{O2} changed with a 10°C increase in temperature (MR Q_{10}) significantly increased after cold acclimation in lizards from both populations (LMM, $F_{1,21} = 45.64$, $P < 0.001$), from 2.15 ± 0.15 to 2.88 ± 0.13 in NY lizards and from 2.54 ± 0.12 to 3.86 ± 0.22 in CA lizards. Overall, CA lizards had significantly higher MR Q_{10} values compared to NY lizards (LMM, $F_{1,21} = 16.47$, $P < 0.001$).

We saw a trend toward an increasing respiratory exchange ratio (RER) at 15°C

for both populations, so we followed this up with an additional measurement of standard

metabolic rate at 15°C, 11 weeks into cold acclimation. Post-acclimation RER was

significantly higher than pre-acclimation RER only for CA lizards (NY PWC, z = -1.71,

corrected P = 0.45; CA PWC, z = -3.09, corrected P = 0.015; Table 4). Follow-up RER

was significantly higher than pre-acclimation RER for both populations (NY PWC, z = -

3.02, corrected P = 0.019; CA PWC, z = -2.94, corrected P = 0.024; Table 4). Following

the initial lab acclimation period, populations did not significantly differ from each other

in RER (15°C PWC, z = -0.71, corrected P = 0.98; 35°C PWC, z = 1.01, corrected P =

0.90). Hemoglobin concentration was significantly higher in NY lizards (10.51 ± 0.49 g

dL^{-1}) than in CA lizards (8.32 ± 0.39 g dL^{-1}) (LMM, $F_{1,9}$ = 7.49, P = 0.023; Table 2), and

did not change with cold acclimation for either population. Hemoglobin was not

significantly related to SMR$_{O2}$ at either temperature, though the trend was positive at

35°C before (R^2 = 0.22, P = 0.20) and after cold acclimation (R^2 = 0.14, P = 0.32).

2.3.4 Evaporative water loss and hydration state

We found that after the initial lab acclimation period, NY lizards exhibited higher EWL

at 10°C compared to CA lizards (PWC, z = 4.14, corrected P < 0.001). There was a

significant interaction between cold acclimation and temperature (LMM, $F_{2,96.2}$ = 6.89,

P = 0.002; Table 3, Fig. 4), such that the only significant increase in EWL after cold

acclimation occurred at 10°C for both NY lizards (PWC, z = -3.14, corrected P = 0.021;

Fig. 4) and CA lizards (PWC, z = -5.70, corrected P < 0.001; Fig. 4). The factor by which

EWL changed with a 10°C increase in temperature (EWL Q$_{10}$) significantly decreased

after cold acclimation in lizards from both populations (LMM, $F_{1,20.4} = 20.76$, $P < 0.001$), from 3.21 ± 0.12 to 2.32 ± 0.37 in NY lizards and from 4.44 ± 0.26 to 3.22 ± 0.23 in CA lizards. CA lizards had an overall higher EWL Q_{10} compared to NY lizards (LMM, $F_{1,21.6} = 18.29$, $P < 0.001$).

Following the initial lab acclimation period, serum osmolality was not significantly different between populations (PWC, $z = 0.94$, corrected $P = 0.74$). Overall serum osmolality was significantly different between populations (LMM, $F_{1,9} = 5.63$, $P = 0.042$, Table 2), though this significance was driven by two outliers post-cold acclimation in the NY population. Removal of the paired (pre and post) observations of the outliers resulted in a non-significant difference between populations (LMM, $F_{1,7} = 2.42$, $P = 0.16$). We used $Q_1 - 1.5 \times IQR$ to define lower outliers and $Q_3 - 1.5 \times IQR$ to define upper outliers. Serum osmolality was not different after cold acclimation either (Table 2). Mass was not a significant predictor of EWL in our model (Table 3).

2.3.5 Field comparison in CA

When we combined CT_{max} of wild lizards and CT_{max} of lab acclimated lizards into a single mixed model, we found that time (month and acclimation period) was still a significant predictor of CT_{max} (LMM, $F_{3,32.7} = 4.62$, $P = 0.008$). CT_{max} of wild CA lizards was significantly lower during April compared to August (PWC, $z = 2.48$, corrected $P = 0.047$; Fig. 5A). CT_{max} of wild lizards collected during August was not significantly different from CT_{max} of lab acclimated lizards measured after the initial lab acclimation period (PWC, $z = 0.66$, corrected $P = 0.89$). CT_{max} of wild lizards collected during April

was not significantly different from CT_{max} of lab acclimated lizards following the cold acclimation treatment (PWC, $z = 0.61$, corrected $P = 0.91$).

As was the case with cold acclimation in the lab, hemoglobin concentration did not significantly differ in wild CA lizards between August and April (PWC, $z = -1.01$, corrected $P = 0.53$). We also found that wild CA lizards had consistently higher hemoglobin concentrations when compared to lab acclimated lizards (PWC, $z = 2.47$, corrected $P = 0.027$; Fig. 5B). Similarly, wild CA lizards had consistently higher serum osmolalities when compared to lab acclimated lizards (PWC, $z = 4.13$, corrected $P < 0.001$; Fig. 5C), with no differences between the months investigated (PWC, $z = 0.80$, corrected $P = 0.67$).

2.3.6 Tables

Table 1. Summary statistics (mean ± SE) of thermal physiology variables for two non-native populations of *Podarcis siculus* before (pre-acclimation) and after (post-acclimation) 5 weeks of a cold acclimation treatment. NY = values for *Podarcis siculus* from Long Island, NY, CA = values for *Podarcis siculus* from San Pedro, CA. CT_{min} = critical thermal minimum (°C), CT_{max} = critical thermal maximum (°C), T_{pref} = preferred body temperature (°C), $N_{lizards}$ = sample size.

Variable	Population	Time	$N_{lizards}$	Mean values (± SE)
CT_{min}	NY	pre-acclimation	8	10.16 (0.72)
		post-acclimation	21	6.48 (0.42)
	CA	pre-acclimation	8	9.71 (0.90)
		post-acclimation	12	10.41 (0.70)
CT_{max}	NY	pre-acclimation	9	42.99 (0.44)
		post-acclimation	8	41.11 (0.47)
	CA	pre-acclimation	10	43.58 (0.27)
		post-acclimation	9	42.29 (0.51)
T_{pref}	NY	pre-acclimation	8	36.11 (0.48)
		post-acclimation	6	30.58 (2.53)
	CA	pre-acclimation	8	34.50 (0.35)
		post-acclimation	9	31.64 (1.53)

Table 2. Linear mixed model results for critical thermal minimum (CT_{min}, °C), critical thermal maximum (CT_{max}, °C), thermal preference (T_{pref}, °C), serum osmolality (Osmolality, mOsm · kg^{-1}), and blood hemoglobin concentration (Hemoglobin, g · dL^{-1}) for two non-native populations of *Podarcis siculus* before (pre-acclimation) and after (post-acclimation) 5 weeks of a cold acclimation treatment. The two populations compared were lizards from Long Island, NY and San Pedro, CA. Subject ID was included as a random effect for all models. In all models an interaction between time (pre vs. post acclimation) and population (NY vs. CA) was included. Levels of time for each analysis are given in the first column. Significant terms not complicated by a significant higher order interaction are emboldened. Post-hoc analyses of these terms are presented in the text.

Time levels	Dependent Variable	Effect	Num df, Den df	*F*, *P*-value
pre-acc., week 1, 2, 3, 4, 5, & 12	CT_{min}	Mass	1, 103.69	0.29, 0.590
		Sex	1, 35.92	3.22, 0.081
		Time	6, 147.78	4.46, <0.001
		Population	1, 42.91	7.07, 0.011
		Time × Population	**6, 147.57**	**5.92, <0.001**
pre-acc., post-acclimation	CT_{max}	Mass	1, 23.76	0.32, 0.578
		Sex	1, 18.36	0.18. 0.678
		Time	**1, 19.42**	**22.40, <0.001**
		Population	1, 20.24	0.57, 0.458
		Time × Population	1, 20.41	0.21, 0.653
pre-acc., post-acclimation	Exp (T_{pref})	Mass	1, 11.55	1.56, 0.236
		Sex	1, 15.90	0.69, 0.417
		Time	1, 10.66	13.29, 0.004
		Population	1, 16.62	0.93, 0.349
		Time × Population	**1, 10.33**	**6.94, 0.024**
pre-acc., post-acc.	Osmolality	Time	1, 9	3.22, 0.106
		Population	**1, 9**	**5.63, 0.042**
		Time × Population	1, 9	1.10, 0.321
pre-acc., post-acclimation	Hemoglobin	Time	1, 9	0.66, 0.137
		Population	**1, 9**	**7.49, 0.023**
		Time × Population	1, 9	0.330, 0.580

Table 3. Linear mixed model results for standard metabolic rate (SMR$_{O2}$, mL O$_2 \cdot$ h^{-1}) and evaporative water loss rate (EWL, mg H$_2$O $\cdot$ h^{-1}) for two non-native populations of *Podarcis siculus* before and after 5 weeks of a cold acclimation treatment. The two populations compared were lizards from Long Island, NY and San Pedro, CA. Subject ID was included as a random effect for all models. All models were fit with an interaction between temperature (levels given in the first column), time (pre vs. post acclimation), and population (NY vs. CA). Significant terms not complicated by a significant higher order interaction are emboldened. Post-hoc analyses of these terms are presented in the text.

Temperature	Dependent Variable	Effect	Num df, Den df	*F*, *P*-value
15 °C, 35 °C	log$_{10}$ (SMR$_{O2}$)	**Log$_{10}$ (Mass)**	**1, 23.28**	**9.78, 0.005**
		Log$_{10}$ (Mass) × Population	1, 22.53	0.05, 0.829
		Sex	1, 19.62	1.37, 0.256
		Temperature	1, 63.24	1001, <0.001
		Time	1, 71.47	0.20, 0.655
		Population	1, 22.60	0.05, 0.824
		Temperature × Time	**1, 63.24**	**17.08, <0.001**
		Temperature × Population	**1, 63.24**	**13.16, <0.001**
		Time × Population	1, 72.48	0.28, 0.596
		Temperature × Time × Population	1, 63.24	0.78, 0.380
10 °C, 20 °C, 30 °C	log$_{10}$ (EWL)	Log$_{10}$ (Mass)	1, 29.37	1.94, 0.174
		Log$_{10}$ (Mass) × Population	1, 26.57	1.07, 0.310
		Sex	1, 20.67	0.17, 0.682
		Temperature	2, 94.22	194.7, <0.001
		Time	1, 108.52	19.55, <0.001
		Population	1, 27.25	1.48, 0.234
		Temperature × Time	**2, 96.25**	**6.89, 0.002**
		Temperature × Population	**2, 94.13**	**5.91, 0.004**
		Time × Population	1, 108.62	1.95, 0.165
		Temperature × Time × Population	2, 95.74	0.136, 0.873

Table 4. Summary statistics of respiratory exchange ratios (RER = $SMR_{CO2} \div SMR_{O2}$) for two non-native populations of *Podarcis siculus* before (pre-acclimation), 5 weeks into cold acclimation (post-acclimation), and 11 weeks into cold acclimation (follow-up). The two populations compared were lizards from Long Island, NY and San Pedro, CA.

Temperature	Population	Time	$N_{lizards}$	Mean ($\pm$ SE)
15 °C	NY	pre-acclimation	12	0.83 (0.04)
		post-acclimation	11	0.94 (0.07)
		follow-up	13	1.01 (0.03)
	CA	pre-acclimation	12	0.73 (0.02)
		post-acclimation	9	0.90 (0.04)
35 °C	NY	pre-acclimation	12	0.85 (0.02)
		post-acclimation	11	0.87 (0.03)
	CA	pre-acclimation	12	0.85 (0.01)
		post-acclimation	12	0.84 (0.02)

2.3.7 Figures
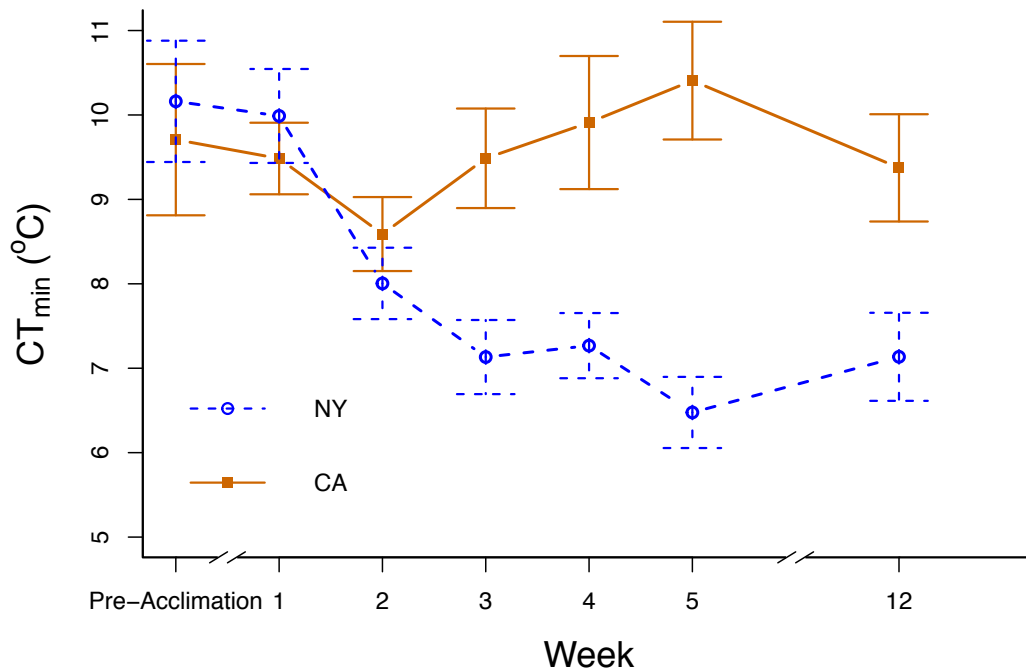
Fig. 1. Cold tolerance (CT_{min}, °C) before and during cold acclimation for two non-native populations of *Podarcis siculus*: from Long Island, NY and San Pedro, CA. CT_{min} decreased over time during cold acclimation in NY lizards (blue circles with dotted lines) but not in CA lizards (orange squares with solid lines). Shapes with bars denote means ± SEM. Pre-acclimation lizards were housed in an environment spanning 27 °C-32 °C. At week 1, lizards had been in an environment set to 20°C: 18°C (Light:Dark) for 1 week. Between weeks 1-12 lizards were housed in an environment set to 17.5°C: 16°C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this document.)

Fig. 2. Thermal tolerances and thermal preferences of two non-native populations of *Podarcis siculus* before and after 5 weeks of cold acclimation. The two populations compared were lizards from Long Island, NY and San Pedro, CA. A) Thermal tolerance ranges for NY lizards are given by the blue bars, where the lighter (top) bar denotes pre-acclimation values and the darker (bottom) bar denotes post-acclimation values. B) Thermal tolerance ranges for CA lizards are given by the orange bars, where the lighter (top) bar denotes pre-acclimation values and the darker (bottom) bar denotes post-acclimation values. Critical thermal minimum (CT_{min}, mean ± SEM) is plotted in blue on the left edge of each bar and critical thermal maximum (CT_{max}, mean ± SEM) is plotted in red on the right edge of each bar. Thermal preference (T_{pref}) box and whisker plots are plotted in orange toward the middle of each bar. We used $Q_1 - 1.5 \times IQR$ to define lower outliers and $Q_3 - 1.5 \times IQR$ to define upper outliers. Levels of significance for thermal tolerance differences between pre- and post-acclimation values within each population are marked as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this document.)
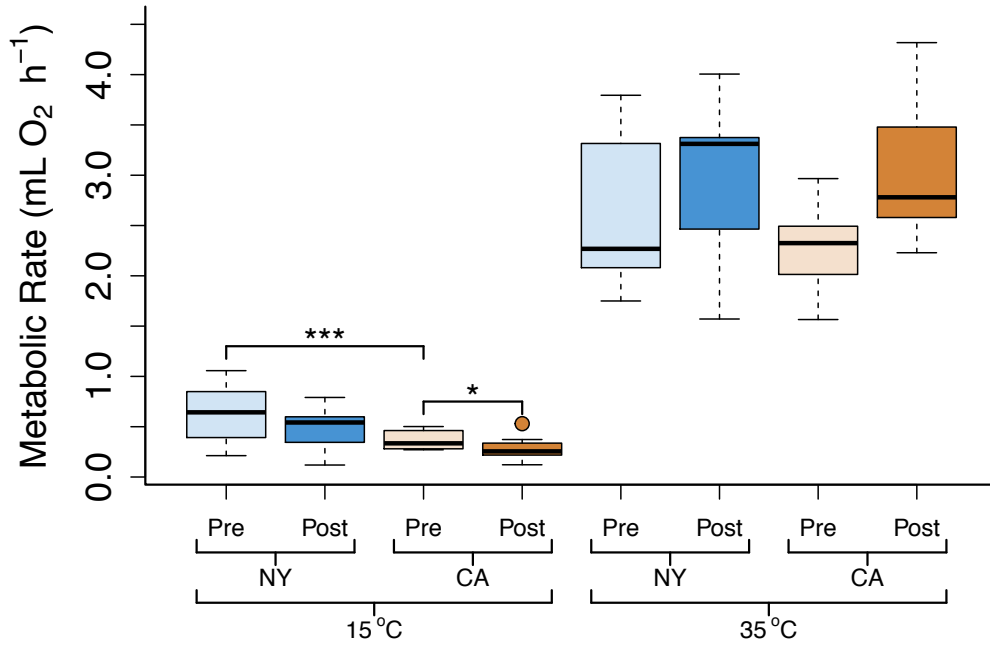
Fig. 3. Box and whisker plots of standard metabolic rate (mL $O_2 \cdot h^{-1}$) for two non-native populations of *Podarcis siculus* at 15°C (left) and 35°C (right), before (Pre, light colored boxes) and after (Post, dark colored boxes) 5 weeks of cold acclimation. The two populations compared were lizards from Long Island, NY (shown in blue) and San Pedro, CA (shown in orange). Levels of significance for differences are marked as follows: * P <0.05, ** P <0.01, *** P <0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this document.)
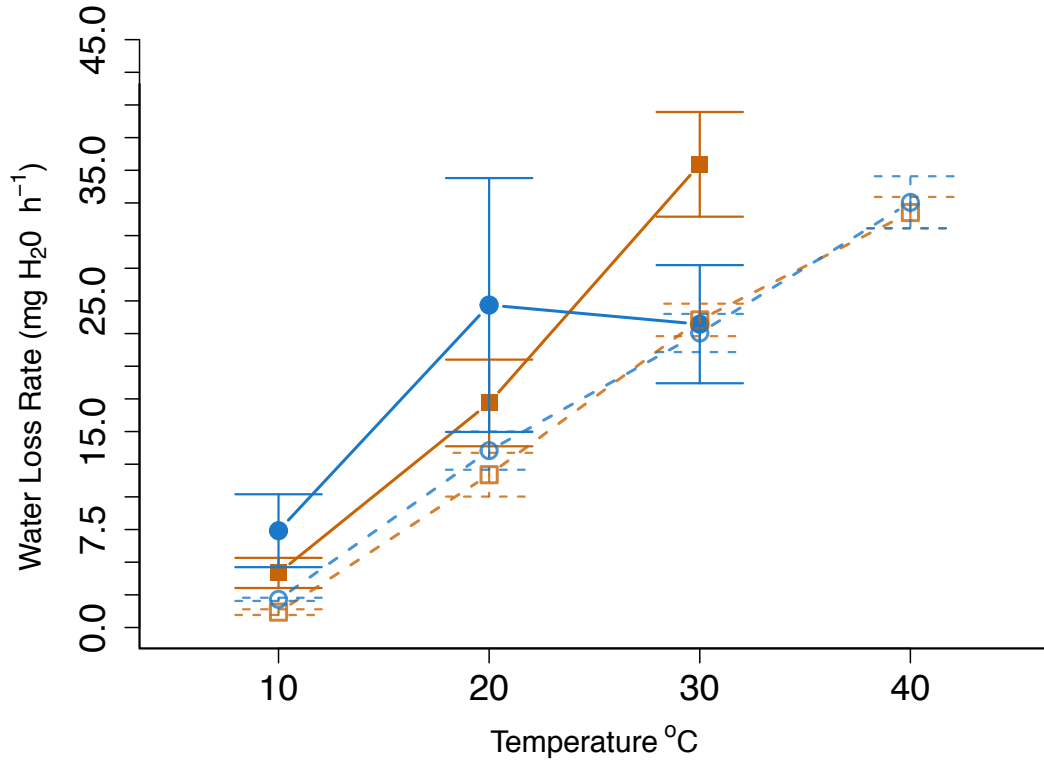
Fig. 4. Rates of evaporative water loss (mg $H_2O \cdot h^{-1}$) for two non-native populations of *Podarcis siculus*: from Long Island, NY (blue circles) and San Pedro, CA (orange squares) at 10°C, 20°C, 30°C, and 40°C (X-axis) before (dotted lines, open shapes) and after (solid lines, filled shapes) 5 weeks of cold acclimation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this document.)
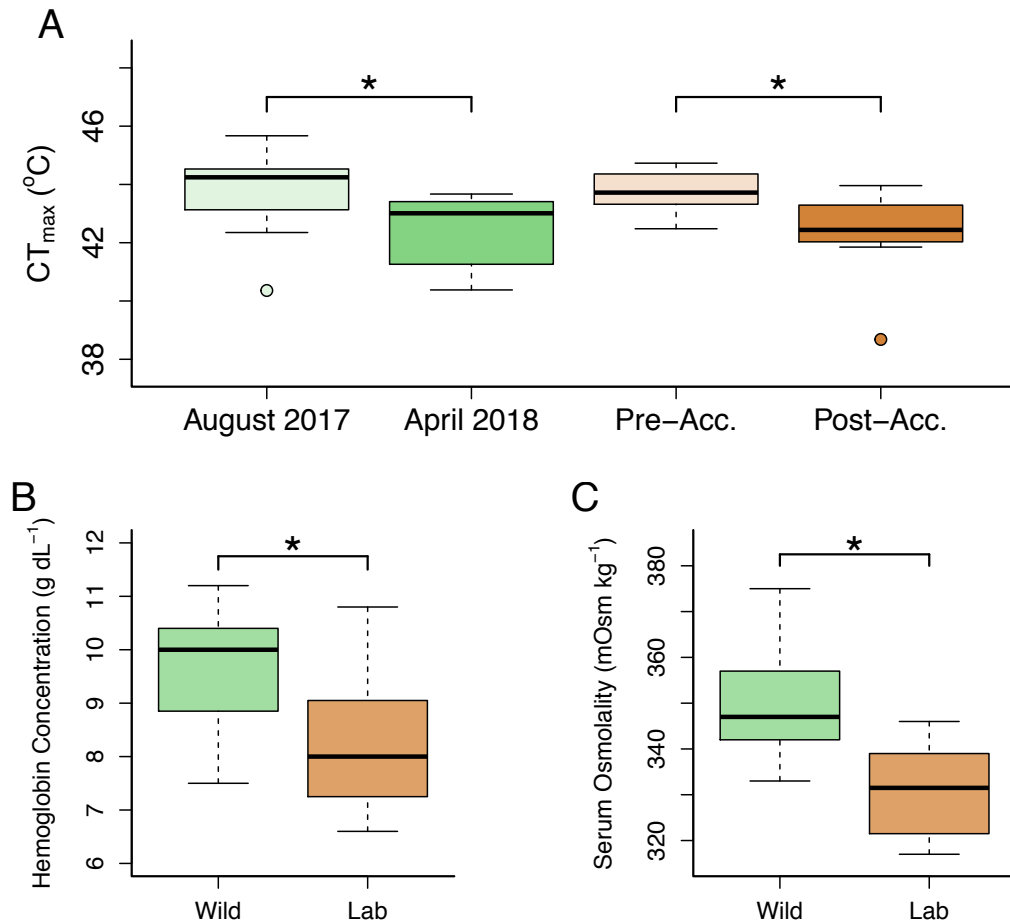
Fig. 5. Comparison of field active *Podarcis siculus* (measured same day as capture) to lab acclimated lizards from the same non-native population (San Pedro, CA). A) Box and whisker plot of heat tolerance (CT_{max}, °C) across seasons for wild CA lizards (green plots on the left), and before (Pre-Acc.) and after (Post-Acc.) cold acclimation for lab acclimated CA lizards (orange plots on the right). B) Box and whisker plot of hemoglobin concentration for wild CA lizards (green on the left) and lab acclimated CA lizards (orange on the right) grouped across seasons and time respectively. C) Box and whisker plot of serum osmolality for wild CA lizards (green on the left) and lab acclimated CA lizards (orange on the right) grouped across seasons and time respectively. Levels of significance for differences are marked as follows: * $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this document.)

2.4 Discussion

A capacity for plasticity in adaptive traits allows organisms to alter their fitness within their lifetime (Fusco & Minelli 2010). Consequently, the extent to which a trait can change and the rate at which it can change should be studied when examining an organism's adaptation to its environment. In this study we show that traits such as cold tolerance can vary in their capacity to change in response to changing climatic conditions within the lifetime 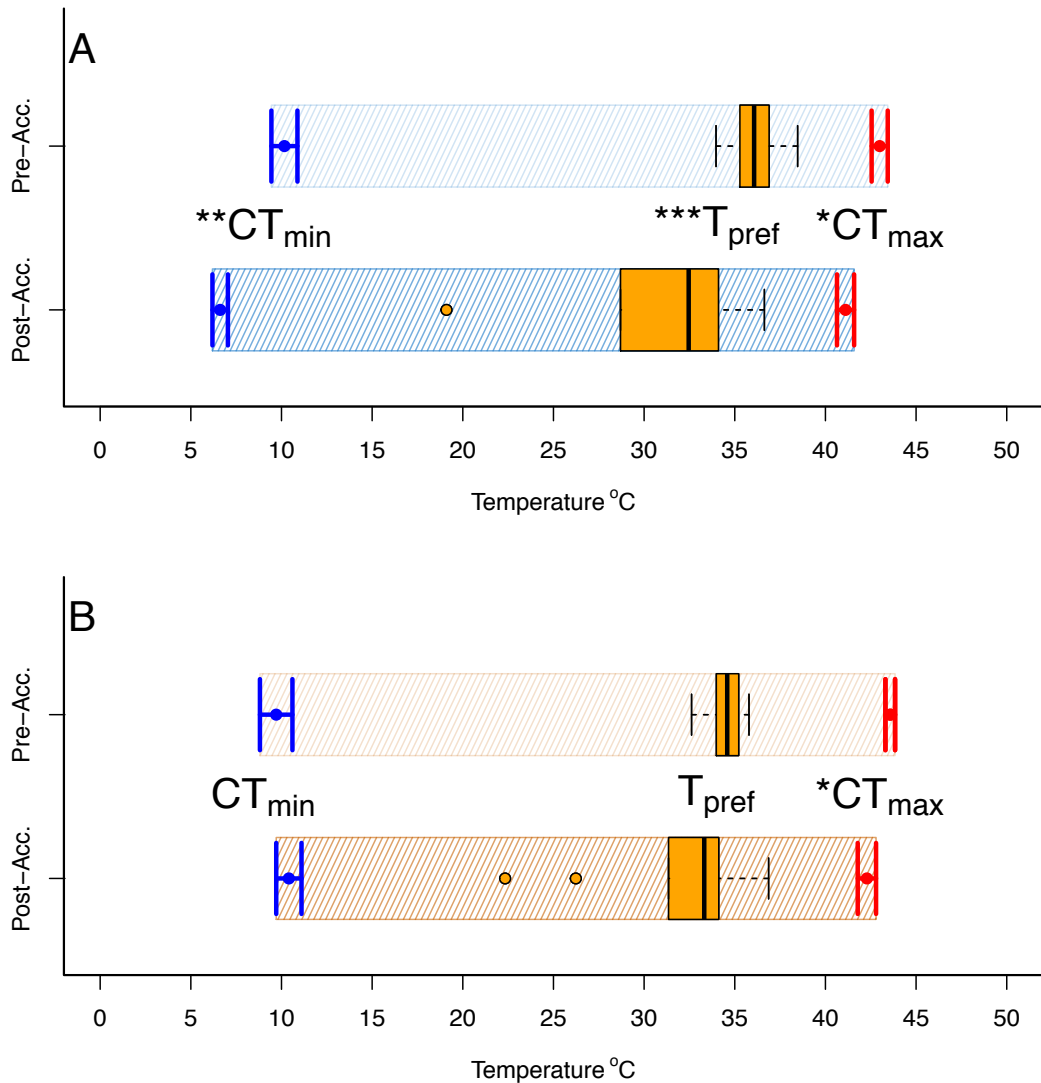of an individual, even between different populations of the same species. We found that *P. siculus* from a non-native Long Island, New York population showed plasticity in CT_{min} in response to cold acclimation, whereas *P. siculus* from a San Pedro, California population did not (Fig. 1). Differing capacities for plasticity of CT_{min} within a species have also been found in non-native Australian cane toads (*Rhinella marina*), with toads from the cooler southern edge of the expanding range showing the most rapid change in CT_{min} in response to cold climates (Kolbe, Kearney & Shine 2010; McCann *et al.* 2014). Similarly, the non-native subspecies (*P. siculus campestris*) of Italian wall lizard that showed a capacity for plasticity in CT_{min} appears to be more successful at spreading in the U.S., especially at cold range boundaries, compared to the Mediterranean subspecies (*P. siculus siculus*). To date, there have been no reports of the subspecies inhabiting San Pedro, CA (*P. siculus siculus*) spreading beyond this southern California neighborhood. The subspecies inhabiting Long Island, NY (*P. siculus campestris*), however, has been recently described as established and mating in Boston, MA (Donihue 2017), which is now its northernmost habitat in the U.S. and likely the coldest habitat across the globe for this species. When comparing closely related species of insects, several studies suggest that the more widespread species have higher

capacities for plasticity in cold tolerance in the form of rapid cold hardening (Chen *et al.* 1990; Kelty & Lee 2001; Bahrndorff *et al.* 2009; Overgaard *et al.* 2011). Whether these findings are similar for plasticity *within* species successfully expanding at their cold boundaries is currently not well known, but it does seem likely that a higher capacity for plasticity in CT_{min} would be beneficial in this regard.

A high degree of plasticity in cold tolerance for NY lizards may have come at the cost of a higher likelihood of death during prolonged exposure to high temperatures. The cold acclimation-induced decrease in T_{pref} was highly significant for NY lizards but not for CA lizards (Fig. 2). It appears that NY lizards chose to avoid higher temperatures following cold acclimation. The death of four NY lizards 1.5 h into our first EWL trial (at 40 °C) following cold acclimation suggests that increased stress, possibly due to water loss, may be the reason for the avoidance of high temperatures by NY lizards following cold acclimation. Whether wild NY lizards will ever experience rapid warming toward the end of winter depends on their winter refugia, though it has been hypothesized that in Long Island, NY they must burrow at least 24 cm deep into soil to avoid lethal conditions (Burke *et al.* 2002). They have also been observed around plumbing of urban residences during winter (R.L. Burke, pers. comm.), suggesting they may use the warmth of water running through pipes to thermoregulate. Studies on Mexican *Sceloporus* species have found positive correlations between the rate of temperature change from winter to spring and local extinction events, with rate of warming being most intense at the northern range (Sinervo *et al.* 2010), suggesting that rapid warming after winter can have negative effects on fitness. Future use of operative temperature models through winter and across various refugia type and depths would allow us to quantify where NY lizards would be

vulnerable to rapid post-winter warming. Additionally, further work is required to determine the extent to which NY lizards behaviorally buffer themselves from stressful winter conditions and stressful post-winter conditions.

The increase in metabolism at a given temperature typically observed in ectotherms after cold acclimation is thought to occur to combat the passive effects of cold temperatures on ATP production (Clarke 1991). Additionally, cold acclimation may elicit a metabolic depression to conserve energy stores (Christian, Bedford & Schultz 1999). Though CA lizards did not show plasticity in CT_{min}, they did show evidence for a metabolic depression at lower temperatures (Fig. 3), which may help retain energy stores during periods antagonistic to energy assimilation in ectotherms (Christian, Bedford & Schultz 1999). However, thermal sensitivity of metabolism (MR Q_{10}) increased by 34% for NY lizards and by 52% for CA lizards following cold acclimation and was overall higher in CA lizards, suggesting that NY lizards would be better equipped than CA lizards to conserve energy during cold hibernation should occasional warm snaps happen (Ruel & Ayres 1999; Williams *et al*. 2012). RER also increased and was higher 11 weeks into cold acclimation for lizards from both populations (Table 4), possibly due to a difference in metabolic substrates used after cold acclimation. High RER values (RER $\geq$ 1) could reflect an increased use of carbohydrates as a fuel, an increase in lipid assimilation, or both. Williams *et al.* (2016) showed that cold hardy *Drosophila* lines increase accumulation of labeled carbon into lipids and increase CO_2 production during cold recovery relative to cold susceptible lines. Whether these mechanisms reflect a response to damaged storage lipids or cell membrane lipids is not known (Williams *et al*. 2016).

In contrast to SMR_{O2}, EWL increased at lower temperatures in both NY and CA lizards following cold acclimation (Fig. 4). The increase in EWL is most likely due to an increase in cutaneous EWL as opposed to respiratory EWL, because respiratory EWL would be associated with an increase in SMR_{O2} (Tattersall, Cadena & Skinner 2006). Additionally, we did not observe any gaping or panting during EWL trials. Though serum osmolality can also change in response to cold acclimation as a freeze avoidance strategy in some taxa (Costanzo & Lee 2013), it appears this is not the strategy used by overwintering lacertids (Voituron *et al*. 2002). Based on our measurements of serum osmolality, the increase in EWL at low temperatures did not seem to be caused by an increase in hydration state (and therefore an increase in stored water) during cold acclimation, suggesting a physiological change in cutaneous lipid composition instead (Hadley 1991; Muñoz-Garcia, Cox & Williams 2008). Thermal sensitivity of EWL decreased by 28% in NY lizards and by 27% in CA lizards, and was generally higher in CA lizards. Overall, the consistent finding of higher thermal sensitivity of physiological rates in CA lizards suggests that NY lizards are better able to physiologically deal with variable diurnal temperatures by buffering the impact that acute exposures have on important physiological rates.

When we compared our CT_{max} findings in response to cold acclimation for lab acclimated CA lizards to the CT_{max} response after a cold season in wild CA lizards, we found similar patterns of decrease in response to cold (Fig. 5A). San Pedro, CA is a coastal population that likely experiences considerably low temperatures at night relative to other southern California locations. It is possible that our lab cold acclimation treatment reflected the conditions experienced by CA lizards through the spring 2018

season and elicited a similar physiological response as that elicited by natural acclimatization. Current findings across the literature support a role for heat shock proteins and a capacity for oxygen delivery in shaping thermal tolerance of ectotherms (Fangue, Hofmeister & Schulte 2006; Gao *et al.* 2014; Smith *et al.* 2015; Verberk *et al.* 2016; Shea *et al.* 2016). Additionally, it is possible that there is a minimum reachable CT_{max} in CA lizards; both the cold acclimation treatment and the CA winter may have stimulated this decrease in CT_{max}. An investigation into the presence of a "triggering" temperature and time of exposure would be a promising area of research for future studies. With the response of NY lizards in mind, our findings also suggest that a high CT_{max} is not maintained during conditions that are unlikely to expose lizards to extreme heat. This may be because maintaining a high CT_{max} is costly, or the mechanisms involved in cold acclimation are linked to mechanisms decreasing heat tolerance, or both. Wild lizards also differed from lab acclimated lizards in hemoglobin concentration and serum osmolality across seasons (Fig. 5B,C). This likely reflects an artifact of long-term lab acclimation that we were not able to take into account. Whether this was caused by a difference in diet, lighting, water availability, or temperature is unfortunately not known and should be investigated in future experiments.

Though we were able to compare some of our findings with wild lizards, it is important to keep in mind the ecological implications of the variables we measured. Thermal tolerance represents values that reflect a response to acute temperature stress, but the likelihood of organisms ever experiencing temperatures that ramp in a controlled manner is low. A more practical interpretation would be to consider thermal tolerance values as bounds that restrict activity time, as previous authors have done with modelling

approaches (Sinervo *et al.* 2010; Buckley, Hurlbert & Jetz 2012). Additionally, we have

shown that although CT_{max} can decrease in a similar manner for two groups, one group

may still be more susceptible to heat stress than the other when the heat is lower in

intensity but longer in exposure (e.g., during an EWL trial). Furthermore, the phenotypic

plasticity of traits can mask our understanding of adaptation and the ecological

consequences of novel climatic conditions if not taken into account (Pintor *et al.* 2016).

In the case of ectotherms, the response to novel temperatures experienced due to

expansion into novel habitats or due to a changing climate are of particular relevance to

predicting range expansion and survival. Studying how quickly a trait can change during

acclimation, as we have done for CT_{min}, allows us to understand how quickly organisms

can physiologically buffer themselves from stressful conditions. A better understanding

of plasticity will not only improve our understanding of individual species' responses to

novel climates, it will also allow us to test broad ecological theories of interest related to

climatic variability. For example, in a highly seasonal environment where there is a large

decrease in temperature from summer to fall, we expect the temperatures that an

organism experiences to decrease rapidly as well. If this is the case, it may be beneficial

if that organism could rapidly decrease its thermal tolerance to maximize the time it can

be actively foraging, digesting, defending territory, or doing some other activity essential

to the maintenance of its life. Assuming this additional activity time is beneficial enough

to affect fitness, we would then expect temperate ectotherms to have faster rates of

acclimation in addition to a larger effect of acclimation compared to tropical ectotherms.

With the accumulation of data on the rate of acclimation of various traits, it will be

possible to conduct meta-analyses to test hypotheses that explain observed patterns in

plasticity. An understanding of the broad patterns of plasticity and geographical trends will further improve our understanding of how organisms may respond to novel climatic conditions within their lifetime, and of which organisms will be better able to physiologically respond to novel climates within their lifetime.

Of interest for future work is a robust test of the climatic variability hypothesis (CVH), which states that as a consequence of larger variability in experienced climatic conditions, organisms from more variable environments should have a higher capacity for change in physiological traits (Janzen 1967; Stevens 1989; Gaston & Chown 1999). Though we can interpret our results in the context of the CVH, we cannot explicitly test this hypothesis due to a lack of replicates across different climates. Incorporation of *P. siculus* populations from different U.S. states will undoubtedly help answer whether it is those populations from more variable environments that show a higher degree of plasticity. We must also acknowledge the limitations in interpretation of our findings caused by the testing of two recognized subspecies. The differences we observed may be due to inherent differences present in source populations, rapid adaptation caused by introduction to the novel U.S. climates, or both, and so an investigation involving lizards collected from source populations would be beneficial. This would ideally involve a common garden experiment where the effects of developmental plasticity could also be accounted for. We do find it interesting that it is the subspecies which is most successful in the U.S., and the one present in the coldest regions of the U.S., that shows a higher degree of plasticity. Perhaps the most intriguing direction for future work would be to determine if successful non-natives in cold climates show higher degrees of plasticity in

cold tolerance across taxa, and determining if plasticity allows for success of non-native or if novel and variable climates select for plasticity.

2.5 Conclusions

Podarcis siculus campestris from a Long Island, NY population had a higher capacity for plasticity of physiological traits in response to cold acclimation compared to *P. siculus siculus* from San Pedro, CA, consistent with predictions of the climatic variability hypothesis. Both CT_{min} and T_{pref} decreased in NY lizards following cold acclimation, whereas CA lizards showed no significant change in these traits. CT_{min} decreased relatively fast following cold acclimation, with the most significant decrease occurring within the first week at the final cold acclimation treatment temperature regime. CT_{max} decreased equally in lizards from both populations, and was shown to decrease equally in wild CA lizards following an unusually cold spring season. SMR_{O2} and EWL were only different following cold acclimation when measured at low temperatures (15°C and 10°C respectively). Four of the NY lizards were unresponsive 1.5 h into a EWL trial at 40°C, suggesting that though heat tolerance measured as acute heat stress (CT_{max}) decreased similarly in all groups tested, prolonged heat stress at 40°C may be more lethal to NY lizards following cold acclimation. RER at 15°C was significantly changed in NY lizards 11 weeks into the cold acclimation treatment; this reflects a heavier dependence on carbohydrates as a food source and the synthesis of lipids upon reintroduction to cold temperatures. Overall thermal sensitivity of physiological rates was higher in CA lizards, and so acute differences likely to be diurnal in nature may have a higher impact on the physiology of CA lizards. The findings of this study suggest that *P. siculus campestris* from Long Island, NY are highly plastic. This plasticity, especially in cold tolerance, may

contribute to the success of this subspecies throughout the northern U.S. Increased

capacity for plasticity in cold tolerance may come at the price of increased heat stress,

however.

REFERENCES

Al-Sadoon, M. K. & Spellerberg, I. F. (1985) Effect of temperature on the oxygen consumption of lizards from different climatic regions. *Amphibia-Reptilia*, **6**, 241-258.

Allen, J. L., Chown, S. L., Janion-Scheepers, C. & Clusella-Trullas, S. (2016) Interactions between rates of temperature change and acclimation affect latitudinal patterns of warming tolerance. *Conservation Physiology*, **4**.

Angilletta, M. J., Niewiarowski, P. H. & Navas, C. A. (2002) The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology*, **27**, 249-268.

Angilletta, M. J., Steury, T. D. & Sears, M. W. (2004) Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integrative and Comparative Biology*, **44**, 498-509.

Arguez, A., Durre, I., Applequist, S., Vose, R.S., Squires, M.F., Yin, X., Heim Jr, R.R. & Owen, T.W. (2012) NOAA's 1981–2010 US climate normals: An overview. *Bulletin of the American Meteorological Society*, **93**, 1687-1697.

Aragón, P., Lobo, J. M., Olalla-Tárraga, M. Á. & Rodríguez, M. Á. (2010) The contribution of contemporary climate to ectothermic and endothermic vertebrate distributions in a glacial refuge. *Global Ecology and Biogeography*, **19**, 40-49.

Bahrndorff, S., Loeschcke, V., Pertoldi, C., Beier, C. & Holmstrup, M. (2009) The rapid cold hardening response of Collembola is influenced by thermal variability of the habitat. *Functional Ecology*, **23**, 340-347.

Bates, D., Mächler, M., Bolker, B., Walker, S., Rune, H.B.C., Henrik, S., Bin, D., Fabian, S., Gabor, G. & Peter, G. (2017) Linear mixed-effects models using 'Eigen' and S4. R package version 1.1–14.

Bennett, A. F. (1982) The energetics of reptilian activity. *Biology of the Reptilia*, **13**, 155-199. Academic Press.

Buckley, L. B., Hurlbert, A. H. & Jetz, W. (2012) Broad-scale ecological implications of ectothermy and endothermy in changing environments. *Global Ecology and Biogeography*, **21**, 873-885.

Burke, R. L., Hussain, A. A., Storey, J. M. & Storey, K. B. (2002) Freeze tolerance and supercooling ability in the Italian wall lizard, *Podarcis sicula*, introduced to Long Island, New York. *Copeia*, **2002**, 836-842.

Burke, R. L. & Ner, S. E. (2005) Seasonal and Diel Activity Patterns of Italian Wall Lizards, *Podarcis sicula campestris*, in New York. *Northeastern Naturalist*, **12**, 349-360.

Campbell-Staton, S. C., Cheviron, Z. A., Rochette, N., Catchen, J., Losos, J. B. & Edwards, S. V. (2017) Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science*, **357**, 495-498.

Campbell-Staton, S. C., Bare, A., Losos, J. B., Edwards, S. V., & Cheviron, Z. A. (2018). Physiological and regulatory underpinnings of geographic variation in reptilian cold tolerance across a latitudinal cline. *Molecular ecology*, *27*(9), 2243-2255.

Carlo, M. A., Riddell, E. A., Levy, O. & Sears, M. W. (2018) Recurrent sublethal warming reduces embryonic survival, inhibits juvenile growth, and alters species distribution projections under climate change. *Ecology Letters*, **21**, 104-116.

Chen, C. P., Lee, R. E. & Denlinger, D. L. (1990) A comparison of the responses of tropical and temperate flies (Diptera: Sarcophagidae) to cold and heat stress. *Journal of Comparative Physiology B*, **160**, 543-547.

Christian, K. A., Bedford, G. S. & Schultz, T. J. (1999) Energetic consequences of metabolic depression in tropical and temperate-zone lizards. *Australian Journal of Zoology*, **47**, 133-141.\

Clarke, A. (1991) What is cold adaptation and how should we measure it? *American Zoologist*, **31**, 81-92.

Claussen, D.L. (1977) Thermal acclimation in ambystomatid salamanders. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **58**, 333-340.

Clusella-Trullas, S., Blackburn, T. M. & Chown, S. L. (2011) Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *The American Naturalist*, **177**, 738-751.

Clusella-Trullas, S. & Chown, S. L. (2014) Lizard thermal trait variation at multiple scales: a review. *Journal of Comparative Physiology B*, **184**, 5-21.

Cohen, J., Pfeiffer, K. & Francis, J. A. (2018) Warm Arctic episodes linked with increased frequency of extreme winter weather in the United States. *Nature Communications*, **9**, 869.

Costanzo, J. P. & Lee, R. E. (2013) Avoidance and tolerance of freezing in ectothermic vertebrates. *Journal of Experimental Biology*, **216**, 1961-1967.

Cowles, R.B. & Bogert, C.M. (1944) A preliminary study of the thermal requirements of desert reptiles. *Bulletin of the American Museum of Natural History*, **83**, 261-296.

Davidson, A. M., Jennions, M. & Nicotra, A. B. (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters*, **14**, 419-431.

Dawson, W.R. (1975) On the Physiological Significance of the Preferred Body Temperatures of Reptiles. *Perspectives of Biophysical Ecology*, **12**, 443. Springer, Berlin, Heidelberg.

Deichsel, G., Nafis, G. & Hakim, J. (2010) Geographic distribution: *Podarcis siculus*. *Herpetological Review*, **41**, 513-514.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. & Martin, P. R. (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, **105**, 6668-6672.

Donihue, C.M., Lambert, M.R. & Watkins-Colwell, G.J. (2015) *Podarcis sicula* (Italian

wall lizard) habitat invasion of suburban New England. *Herpetological Review*,

46, 260-261.

Donihue, C.M. (2017) *Podarcis siculus:* A breeding population in Boston's Fenway

Victory Gardens. *Herpetological Review*, **48**, 126.

Fangue, N. A., Hofmeister, M. & Schulte, P. M. (2006) Intraspecific variation in thermal

tolerance and heat shock protein gene expression in common killifish, *Fundulus*

heteroclitus. *Journal of Experimental Biology*, **209**, 2859-2872.

Fusco, G. & Minelli, A. (2010) Phenotypic plasticity in development and evolution: facts

and concepts. *Philosophical transactions of the Royal Society B*, **365**, 547-556.

Gao, J., Zhang, W., Dang, W., Mou, Y., Gao, Y., Sun, B. J. & Du, W. G. (2014) Heat

shock protein expression enhances heat tolerance of reptile embryos. *Proceedings*

of the Royal Society B, Biological Sciences, **281**, 20141135.

Gaston, K. J., & Chown, S. L. (1999) Why Rapoport's rule does not generalise. *Oikos*, **84**,

309-312.

Gossweiler, W. A. (1975) European lizards established on Long Island. *Copeia*, **1975**,

584-585.

Gunderson, A. R. & Stillman, J. H. (2015) Plasticity in thermal tolerance has limited

potential to buffer ectotherms from global warming. *Proceedings of the Royal*

Society B, Biological Sciences, **282**, 20150401.

Hadley, N. F. (1991) Integumental lipids of plants and animals: comparative function and biochemistry. *Advances in Lipid Research*, **24**, 303-320.

Hayes, J. P. & Shonkwiler, J. S. (1996) Analyzing mass-independent data. *Physiological Zoology*, **69**, 974-980.

Hochochka, P. W. & Somero, G. N. (2002) Biochemical adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press.

Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 65-70.

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A. & Scheibe, S. (2017) Simultaneous inference in general parametric models. R package version 1.1–7.

Huey, R. B. (1982) Temperature, physiology, and the ecology of reptiles. *Biology of the Reptili*a*, **12**, 25-91. Academic Press.

Huey, R. B. & Bennett, A. F. (1987) Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution*, **41**, 1098-1115.

Huey, R. B. & Kingsolver, J. G. (1989) Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, **4**, 131-135.

Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Pérez, H. J. Á. & Garland, T. (2009) Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B, Biological Sciences*, **276**, 1939-1948.

Hutchison, V. H. (1961) Critical thermal maxima in salamanders. *Physiological Zoology*, **34**, 92-125.

Janzen, D. H. (1967) Why mountain passes are higher in the tropics. *The American Naturalist*, **101**, 233-249.

Kearney, M. & Porter, W. P. (2004) Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology*, **85**, 3119-3131.

Kearney, M. & Porter, W. (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology letters*, **12**, 334-350.

Kelley, A. L. (2014) The role thermal physiology plays in species invasion. *Conservation Physiology*, **2**.

Kelty, J. D. & Lee, R. E. (2001) Rapid cold-hardening of Drosophila melanogaster (Diptera: Drosophiladae) during ecologically based thermoperiodic cycles. *Journal of Experimental Biology*, **204**, 1659-1666.

Kingsolver, J. G., MacLean, H. J., Goddin, S. B. & Augustine, K. E. (2016) Plasticity of upper thermal limits to acute and chronic temperature variation in *Manduca sexta* larvae. *Journal of Experimental Biology*, **219**, 1290-1294.

Kolbe, J. J., Kearney, M. & Shine, R. (2010) Modeling the consequences of thermal trait variation for the cane toad invasion of Australia. *Ecological Applications*, **20**, 2273-2285.

Kolbe, J. J., Lavin, B. R., Burke, R. L., Rugiero, L., Capula, M. & Luiselli, L. (2013) The desire for variety: Italian wall lizard (*Podarcis siculus*) populations introduced to

the United States via the pet trade are derived from multiple native-range sources. *Biological Invasions*, **15**, 775-783.

Levy, O., Buckley, L. B., Keitt, T. H. & Angilletta, M. J. (2016) Ontogeny constrains phenology: opportunities for activity and reproduction interact to dictate potential phenologies in a changing climate. *Ecology Letters*, **19**, 620-628.

Lighton, J. R. (2008) *Measuring Metabolic Rates: A Manual for Scientists*. Oxford University Press.

Lutterschmidt, W. I. & Hutchison, V. H. (1997) The critical thermal maximum: history and critique. *Canadian Journal of Zoology*, **75**, 1561-1574.

Martin, T. L. & Huey, R. B. (2008) Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *The American Naturalist*, **171**, 102-118.

Mautz, W. J. (1982a) Correlation of both respiratory and cutaneous water losses of lizards with habitat aridity. *Journal of Comparative Physiology*, **149**, 25-30.

Mautz, W. J. (1982b). Patterns of evaporative water loss. *Biology of the Reptilia*, **12**, 443-481. Academic Press.

McCann, S., Greenlees, M. J., Newell, D. & Shine, R. (2014) Rapid acclimation to cold allows the cane toad to invade montane areas within its Australian range. *Functional Ecology*, **28**, 1166-1174.

Muñoz-Garcia, A., Cox, R. M. & Williams, J. B. (2007) Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum in house sparrows (*Passer*

domesticus) following acclimation to high and low humidity. *Physiological and Biochemical Zoology*, **81**, 87-96.

Overgaard, J., Kristensen, T. N., Mitchell, K. A. & Hoffmann, A. A. (2011) Thermal tolerance in widespread and tropical Drosophila species: does phenotypic plasticity increase with latitude?. *The American Naturalist*, **178**, S80-S96.

Packard, G. C. & Boardman, T. J. (1999) The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **122**, 37-44.

Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2016a) Linear and nonlinear mixed effects models. R package version 3.1-128.

Pintor, A. F., Schwarzkopf, L. & Krockenberger, A. K. (2016b) Extensive acclimation in ectotherms conceals interspecific variation in thermal tolerance limits. *PloS ONE*, **11**, e0150408.

Pintor, A. F., Schwarzkopf, L. & Krockenberger, A. K. (2016b) Hydroregulation in a tropical dry-skinned ectotherm. *Oecologia*, **182**, 925-931.

Porter, W. P. & Gates, D. M. (1969) Thermodynamic equilibria of animals with environment. *Ecological Monographs*, **39**, 227-244.

Pörtner, H. (2001). Climate change and temperature-dependent biogeography: oxygen

limitation of thermal tolerance in animals. *Naturwissenschaften*, *88*(4), 137-146.

Rafinesque-Schmaltz, C. S. (1810*) Caratteri di alcuni nuovi generi e nuove specie di*

animali e plante della Sicilia. Sanfilippo, Palermo, 105-109.

Rogers, K. D., Seebacher, F. & Thompson, M. B. (2004) Biochemical acclimation of

metabolic enzymes in response to lowered temperature in tadpoles of

Limnodynastes peronii. Comparative Biochemistry and Physiology Part A:

Molecular & Integrative Physiology, **137**, 731-738.

Ruel, J. J. & Ayres, M. P. (1999) Jensen's inequality predicts effects of environmental

variation. *Trends in Ecology & Evolution*, **14**, 361-366.

Schmidt-Nielsen, K. (1984) *Scaling: why is animal size so important*? Cambridge

University Press.

Shea, T. K., DuBois, P. M., Claunch, N. M., Murphey, N. E., Rucker, K. A., Brewster, R.

A. & Taylor, E. N. (2016) Oxygen concentration affects upper thermal tolerance

in a terrestrial vertebrate. *Comparative Biochemistry and Physiology Part A:*

Molecular & Integrative Physiology, **199**, 87-94.

Sinervo, B., Mendez-De-La-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagrán-

Santa Cruz, M., Lara-Resendiz, R., Martínez-Méndez, N., Calderón-Espinosa,

M.L., Meza-Lázaro, R.N. & Gadsden, H. (2010) Erosion of lizard diversity by

climate change and altered thermal niches. *Science*, **328**, 894-899.

Smith, C., Telemeco, R. S., Angilletta, M. J. & VandenBrooks, J. M. (2015) Oxygen

   supply limits the heat tolerance of lizard embryos. *Biology Letters*, **11**, 20150113.

Stevens, G. C. (1989) The latitudinal gradient in geographical range: how so many

   species coexist in the tropics. *The American Naturalist*, **133**, 240-256.

Sunday, J. M., Bates, A. E. & Dulvy, N. K. (2010) Global analysis of thermal tolerance

   and latitude in ectotherms. *Proceedings of the Royal Society B, Biological

   Sciences*, **278**, 1823-1830.

Sunday, J. M., Bates, A. E. & Dulvy, N. K. (2012) Thermal tolerance and the global

   redistribution of animals. *Nature Climate Change*, **2**, 686.

Tattersall, G. J., Cadena, V. & Skinner, M. C. (2006) Respiratory cooling and

   thermoregulatory coupling in reptiles. *Respiratory Physiology &

   Neurobiology*, **154**, 302-318.

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C. & Chown, S. L. (2007)

   Critical thermal limits depend on methodological context. *Proceedings of the

   Royal Society of London B: Biological Sciences*, **274**, 2935-2943.

Thomas, C. D., Bodsworth, E. J., Wilson, R. J., Simmons, A. D., Davies, Z. G., Musche,

   M. & Conradt, L. (2001) Ecological and evolutionary processes at expanding

   range margins. *Nature*, **411**, 577.

Verberk, W. C., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L. &

   Terblanche, J. S. (2016) Does oxygen limit thermal tolerance in arthropods? A

critical review of current evidence. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **192**, 64-78.

van Marken Lichtenbelt, W. D. (1992) Digestion in an ectothermic herbivore, the green iguana (*Iguana iguana*): effect of food composition and body temperature. *Physiological Zoology*, **65**, 649-673.

Voituron, Y., Storey, J., Grenot, C. & Storey, K. (2002) Freezing survival, body ice content and blood composition of the freeze-tolerant European common lizard, *Lacerta vivipara*. *Journal of Comparative Physiology B*, **172**, 71-76.

Williams, C. M., Marshall, K. E., MacMillan, H. A., Dzurisin, J. D., Hellmann, J. J. & Sinclair, B. J. (2012) Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. *PLoS One*, **7**, e34470.

Williams, C. M., McCue, M. D., Sunny, N. E., Szejner-Sigal, A., Morgan, T. J., Allison, D. B. & Hahn, D. A. (2016) Cold adaptation increases rates of nutrient flow and metabolic plasticity during cold exposure in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, **283**, 20161317.

Withers, P. C., Aplin, K. P. & Werner, Y. L. (2000) Metabolism and evaporative water loss of Western Australian geckos (Reptilia: Sauria: Gekkonomorpha). *Australian Journal of Zoology*, **48**, 111-126.

Zuckerkandl, E. & Villet, R. (1988) Concentration-affinity equivalence in gene

regulation: convergence of genetic and environmental effects. *Proceedings of the*

National Academy of Sciences, **85**, 4784-4788.

A. Supplementary Tables

Table A1. Monthly low temperatures grouped across seasons for different *Podarcis siculus* habitat locations. Climatic data for Hempstead, New York were averaged using data from NOAA climate normals from the JFK Airport weather station. Climatic data for Long Beach, California were also averaged using NOAA climate normals (NOAA 1981-2010; Arguez *et al*. 2012). Climatic data for Italian locations were averaged across seasons from monthly temperatures presented by www.currentresults.com for 1971-2000 (Nazionale di Meterologia e Climatologia Aeronautica).

P. siculus campestris	Spring	Summer	Fall	Winter	Summer — Winter Difference	Standard Deviation
Hempstead, New York	6.3°C	19°C	10.3°C	-1.7°C	20.7°C	8.5°C
Firenze, Florence	8.3°C	17°C	9.7°C	2.7°C	14.3°C	5.9°C
P. siculus siculus						
Long Beach, California	12°C	17.3°C	14°C	8.3°C	9°C	3.9°C
Catania, Sicily	8.7°C	18.3°C	14°C	5.7°C	12.7°C	5.6°C

Table A2. Monthly average temperatures grouped across seasons for different *Podarcis siculus* habitat locations. Climatic data for Hempstead, New York were averaged using data from NOAA climate normals from the JFK Airport weather station. Climatic data for Long Beach, California were also averaged using NOAA climate normals (NOAA 1981-2010; Arguez *et al*. 2012). Climatic data for Italian locations were averaged across seasons from monthly temperatures presented by www.currentresults.com for 1971-2000 (Nazionale di Meterologia e Climatologia Aeronautica).

P. siculus campestris	Spring	Summer	Fall	Winter	Summer — Winter Difference	Standard Deviation
Hempstead, New York	10.5°C	23.2°C	14.2°C	1.8°C	21.3°C	8.7°C
Firenze, Florence	14°C	23.7°C	15.3°C	7.2°C	16.5°C	6.8°C
P. siculus siculus						
Long Beach, California	16.8°C	22.3°C	19.5°C	14°C	8.3°C	3.7°C
Catania, Sicily	15°C	25.2°C	19.5°C	11°C	14.2°C	6.1°C

Table A3. Monthly high temperatures grouped across seasons for different *Podarcis siculus* habitat locations. Climatic data for Hempstead, New York were averaged using data from NOAA climate normals from the JFK Airport weather station. Climatic data for Long Beach, California were also averaged using NOAA climate normals (NOAA 1981-2010; Arguez *et al*. 2012). Climatic data for Italian locations were averaged across seasons from monthly temperatures presented by www.currentresults.com for 1971-2000 (Nazionale di Meterologia e Climatologia Aeronautica).

P. siculus campestris	Spring	Summer	Fall	Winter	Summer — Winter Difference	Standard Deviation
Hempstead, New York	14.7°C	27.3°C	18°C	5.3°C	22°C	9°C
Firenze, Florence	19.7°C	30.3 °C	21°C	11.7°C	18.7°C	7.6°C
P. siculus siculus						
Long Beach, California	21.7°C	27.3°C	25°C	19.7°C	7.7°C	3.5°C
Catania, Sicily	21.3°C	32°C	25°C	16.3°C	15.7°C	6.6°C

Table A4. Average total days of rain grouped across seasons for different *Podarcis siculus* habitat locations. Climatic data for Hempstead, New York were averaged using data from NOAA climate normals from the JFK Airport weather station. Climatic data for Long Beach, California were also averaged using NOAA climate normals (NOAA 1981-2010; Arguez *et al*. 2012). Climatic data for Italian locations were averaged across seasons from averages presented by www.currentresults.com for 1971-2000 (Nazionale di Meterologia e Climatologia Aeronautica).

P. siculus campestris	Spring	Summer	Fall	Winter	Annual Total
Hempstead, New York	34	29	26	32	121
Firenze, Florence	26	15	24	23	88
P. siculus siculus					
Long Beach, California	9	2	7	18	36
Catania, Sicily	13	4	15	19	51

Table A5. Average amount of monthly rain grouped across seasons for different *Podarcis siculus* habitat locations. Climatic data for Hempstead, New York were averaged using data from NOAA climate normals from the JFK Airport weather station. Climatic data for Long Beach, California were also averaged using NOAA climate normals (NOAA 1981-2010; Arguez et al. 2012). Climatic data for Italian locations were averaged across seasons from averages presented by www.currentresults.com for 1971-2000 (Nazionale di Meterologia e Climatologia Aeronautica).

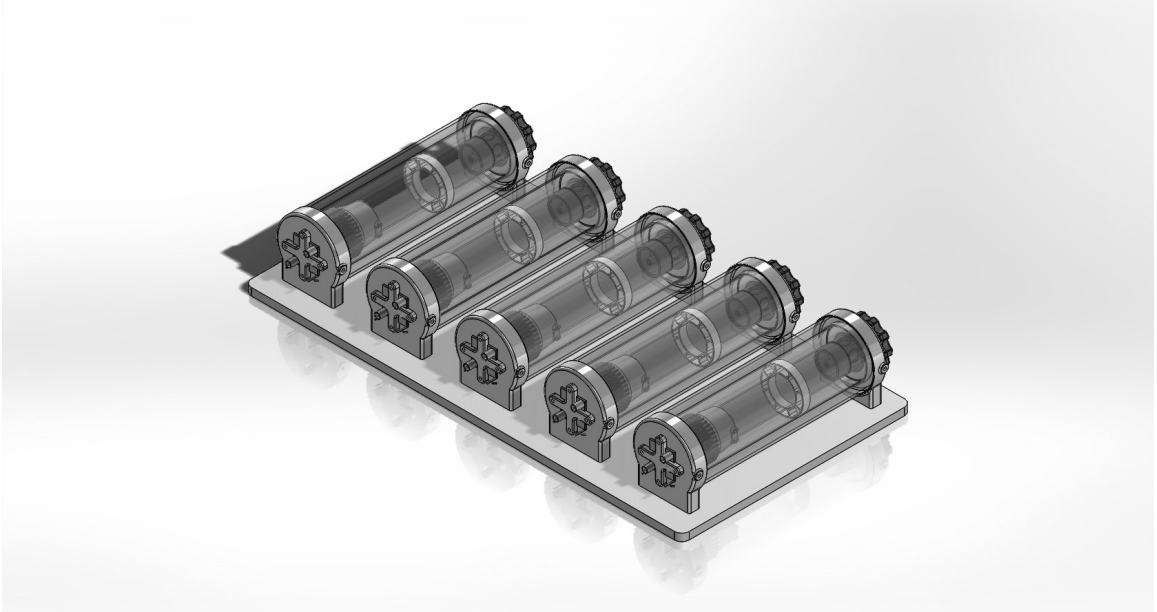| *P. siculus campestris*	Spring	Summer	Fall	Winter	Annual Total
Hempstead, New York	294 mm	295 mm	265 mm	232 mm	1086 mm
Firenze, Florence	220 mm	150 mm	298 mm	206 mm	874 mm
P. siculus siculus					
Long Beach, California	67 mm	4 mm	46 mm	194 mm	311 mm
Catania, Sicily	79 mm	28 mm	160 mm	181 mm	448 mm

Fig. A1. A simple schematic of the Gas Analysis, Temperature, and Oxygen Regulation System (GATORS) when viewed from behind and not plugged into a power source and running water. The device is composed of electrical components housed in the rear side of each chamber, acrylic tubes, and polylactic acid (PLA) 3D printing filament. A detailed description of the components of each chamber is shown in Fig. A2.
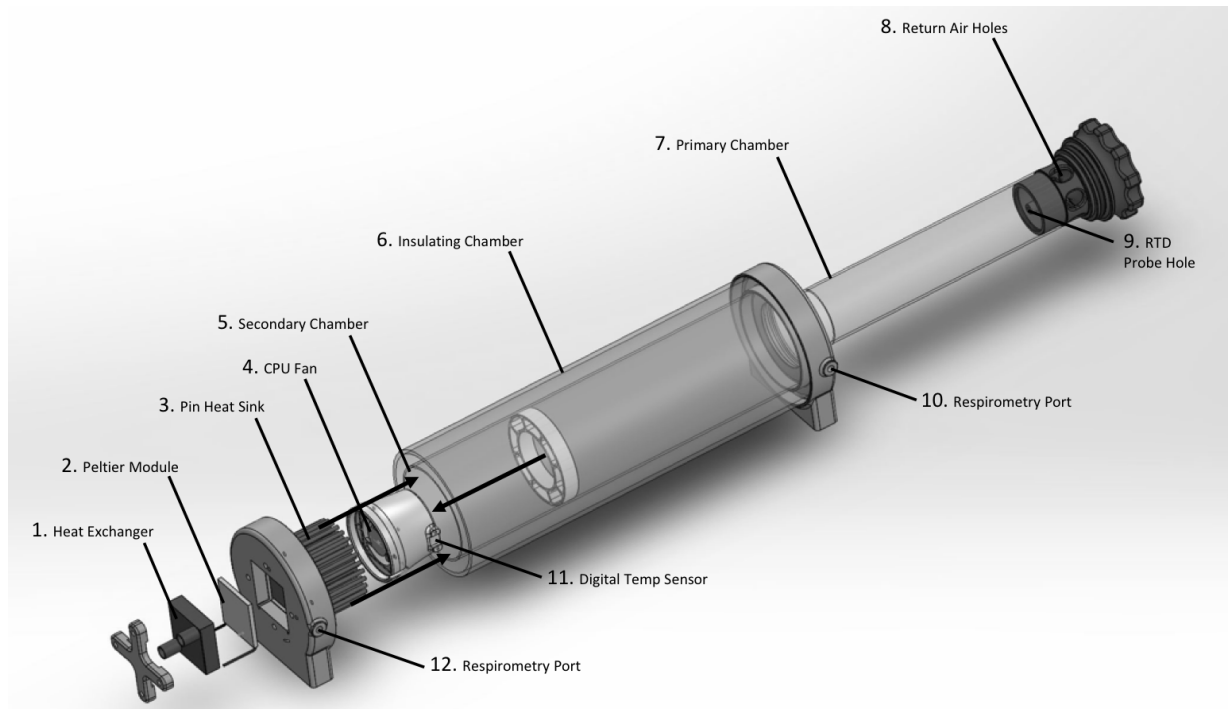
Fig. A2. A schematic of the components making each individual chamber of the Gas Analysis, Temperature, and Oxygen Regulation System (GATORS). The primary component that controls temperature is the Peltier thermoelectric cooler module (2), which creates a temperature differential (i.e., one side warms while the other side cools) according to power supply input. If the current running through the module is reversed, the side which was previously warming will begin to cool and vice versa. The ability of one side of the Peltier module to cool or heat is dependent on the ability of the other side to dissipate heat or to gain heat respectively. A circulating water bath with temperature-controlled water is pumped through an aluminum heat exchanger (1) in contact with the Peltier module to aid in the module's function. A pin heat sink (3) allows the local air to cool or warm and a 12V CPU fan (4) circulates this air by pulling from the innermost primary chamber (7) and pushing it through the secondary chamber (5). The air returns to the innermost chamber through holes made for this purpose (8). The arrows show the direction in which air circulates between the primary and secondary chambers. Lizards are kept in the primary chamber (7) during testing and their body temperatures are measured with resistance temperature detector (RTD) probes passed through the primary chamber (9). The rate of cooling or heating is set manually using custom Arduino software that uses a digital temperature sensor (11) as its input. There are optional ports (10 and 12) that aid in sampling and manipulation of air composition for metabolism studies, but this feature was not used in the experiments described in this study.
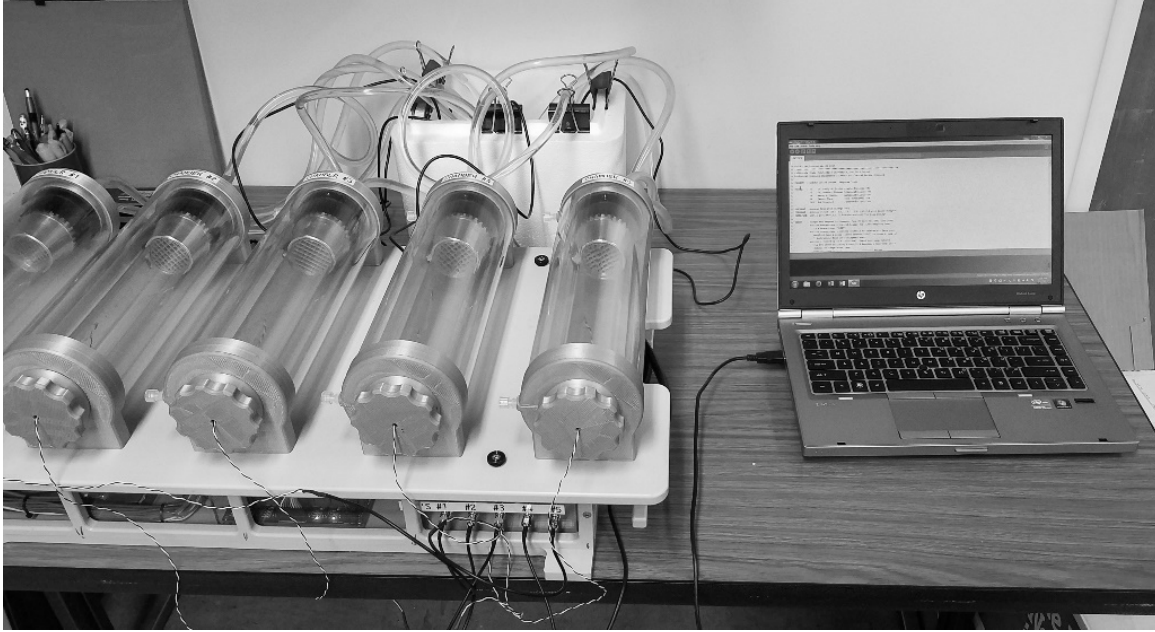
Fig. A3. A front view of the Gas Analysis, Temperature, and Oxygen Regulation System (GATORS) while powered on and running. The braided wires entering each chamber are the resistance temperature detector (RTD) probes described in the text and in the caption for Fig. A2. Plastic tubing toward the back circulates water from a water bath through the aluminum heat exchanger described in the caption for Fig. A2. Air temperature and lizard body temperature are reported on the computer using custom Arduino software. Using commands in this software, the direction of temperature ramping (i.e., cooling or heating) and the rate of air temperature ramping ($.5°C \cdot min^{-1}$ or $1°C \cdot min^{-1}$) can be manipulated. For the experiments described in this study only $1°C \cdot min^{-1}$ was used. The entire device is powered via 120 V AC power.
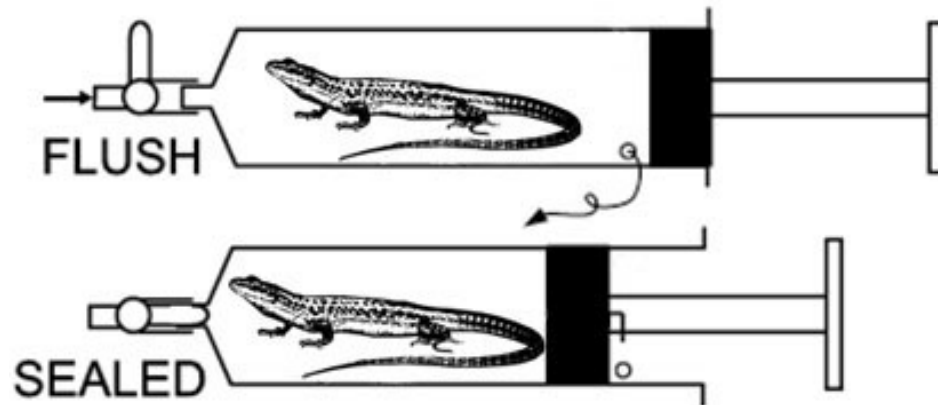
Fig. A4. Diagram of a modified syringe enclosure being flushed with ambient air to give animals a stable and repeatable initial gas source for metabolic rate measurements. Air is pumped through the syringe, then sealed using a three-way stopcock at the injection site and by pushing the plunger past the drilled hole on the other end. Figure modified after Lighton (2008).
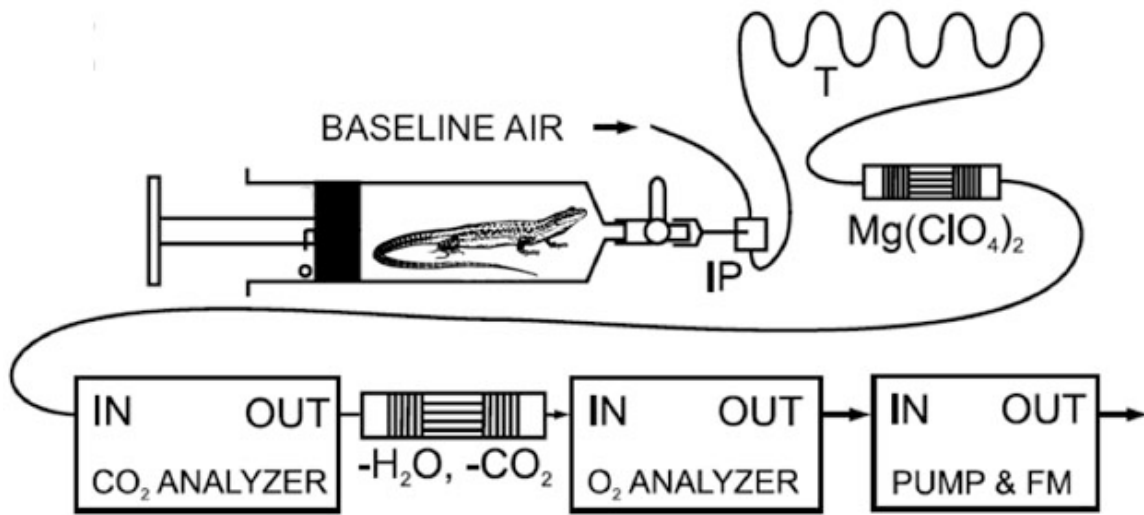
Fig. A5. Diagram of manual bolus integration for metabolic rate measurements. A pump and flow meter (PUMP and FM) pulled air through the system at 100 mL · min^{-1}. Air from each syringe was fed into the system by opening the three-way stopcock and connecting it to an injection port (IP), then pushing 20 mL of air into tubing (T) long enough to hold the injected volume of air. This air was then pulled through an H_2O scrubbing column ($Mg(ClO_4)_2$) and into a CO_2 analyzer, then through an H_2O and CO_2 scrubbing column and into an O_2 analyzer. Figure modified after Lighton (2008).