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The thermal dependence of carbon stable isotope incorporation and trophic discrimination in the domestic cricket, *Acheta domesticus*

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ABSTRACT

Stable isotopes are valuable tools in physiological and ecological research, as they can be used to estimate diet, habitat use, and resource allocation. However, in most cases *a priori* knowledge of two key properties of stable isotopes is required, namely their rate of incorporation into the body (incorporation rate) and the change of isotope values between consumers and resources that arises during incorporation of the isotopes into the consumer's tissues (trophic discrimination). Previous studies have quantified these properties across species and tissue types, but little is known about how they vary with temperature, a key driver of many biological rates and times. Here, we explored for the first time how temperature affects both carbon incorporation rate and trophic discrimination via growth rates, using the domestic cricket, *Acheta domesticus*. We raised crickets at 16 °C, 21 °C, and 26 °C and showed that temperature increased carbon isotope incorporation rate, which was driven by both an increased growth rate and catabolism at higher temperatures. Trophic discrimination of carbon isotopes decreased at higher temperatures, which we attributed to either lower activation energies needed to synthesize non-essential amino acids at higher temperatures or the increased utilization of available resources of consumers at higher temperatures. Our results demonstrate that temperature is a key driver of both carbon isotope incorporation rate and trophic discrimination, via mechanisms that likely persist across all ectotherms. Experiments to determine incorporation rates and trophic discrimination factors in ectotherms must include temperature as a major factor, and natural variation in temperature might have significant effects on these isotopic properties that then can affect inferences made from isotope values.

1. Introduction

Stable isotopes are a standard tool in studies of insect physiology and ecology, used as natural tracers to quantify diets and habitat use, and to determine how resources consumed by an organism are allocated to maintenance, growth, and reproduction (Fry et al., 1978; McNabb et al., 2001; Yoshimura, 2013; Grant et al., 2014). However, the use of stable isotopes often requires *a priori* knowledge of two key properties (Vanderklift and Ponsard, 2003; Martínez del Rio et al., 2009a,b). First, isotopic incorporation rates must be known, which detail the rate at which isotopes are assimilated into an organism's body and provide a timeframe for when those isotopes were incorporated into the organism (Gratton and Forbes, 2006; Martínez del Rio et al., 2009a). Second, the difference in isotope values between a consumer and its resource - trophic discrimination factors (TDFs) – must be known in order to accurately quantify diet, habitat use, and resource allocation (Phillips et al., 2005; Moore and Semmens, 2008; Parnell et al., 2010). These TDF's arise when either heavy or light isotopes are selected for incorporation into a consumer's body (Fry 2006). Both incorporation rates and trophic discrimination vary considerably across taxa and tissue types (Tieszen et al., 1983; Vanderklift and Ponsard, 2003; Martínez del Rio et al., 2009a,b). Without accurate estimates of isotopic incorporation rates and TDFs, many inferences using stable isotopes will be erroneous (Phillips and Eldridge, 2006; Reich et al., 2008; Caut et al., 2009). For example, without knowledge of incorporation rates, researchers may make inferences regarding diet or habitat use from the wrong season, and without accurate TDFs researchers may incorrectly quantify diets or habitat use. Additionally, any fluctuations in these properties throughout an organism's life will adversely affect any inferences made. As such, knowledge of these isotopic properties and the factors that influence these properties is required for the correct use of stable isotope analyses in physiology and ecology.

Abbreviations: TDFs, Trophic discrimination factors

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Incorporation rates are affected by a number of properties, including taxon (Vanderklift and Ponsard, 2003; Martínez del Rio et al., 2009a), tissue type (Tieszen et al. 1983; Vanderklift and Ponsard 2003; Martínez del Rio et al., 2009a), body size (Schramski et al., 2015), and properties of the stable isotope itself (Martínez del Rio et al., 2009a; Caut et al., 2009). One driver that has not previously received much attention is temperature, which has profound influences on many aspects of biology, including growth and metabolic rate (Gillooly et al., 2001, 2002), both of which influence isotopic incorporation rate (MacAvoy et al., 2005; Reich et al., 2008; Murray and Wolf, 2012). Increased growth rates result in higher incorporation rates (Reich et al., 2008; Murray and Wolf, 2012), as growing organisms require a greater amount of resources in order to gain tissue mass. Higher environmental temperatures increase metabolic rates, which in turn increase growth rates (Gillooly et al., 2002), especially in ectotherms (Angilletta et al., 2004; Booth and Kiddell, 2007). Thus, in ectotherms increased environmental temperature, and thus body temperature, likely increases the rate of isotopic incorporation via increasing growth rates (Frazer et al., 1997). Despite the clear connections among temperature, growth, metabolism, and the rate of isotopic incorporation, no one has experimentally linked them. As such, physiologists and ecologists often assume that incorporation rates are temperature invariant, even though most organisms are subject to temperatures that fluctuate within and among seasons and habitats.

Temperature likely also has strong effects on TDFs. Whether heavy or light isotopes are incorporated into an organisms' tissues (i.e., trophic discrimination) is largely determined by the synthesis of non-essential amino acids (Howland et al., 2003; McMahon et al., 2010; Newsome et al., 2014). The rates at which these amino acids are synthesized are metabolically constrained (MacAvoy et al., 2006; Tarboush et al., 2006). Therefore, due to the strong effect of temperature on metabolic rate (see above), temperature is likely to also affect trophic discrimination, although little work has tested this idea. Higher temperatures may possibly decrease trophic discrimination in two ways. First, at higher temperatures lower activation energies (i.e., the energy needed to initiate a chemical reaction) would be required to catalyze amino acid synthesis (Bloomfield et al., 2011; Gibert et al., 2016; Pawar et al., 2016). Second, warmer temperatures increase growth rates (Gillooly et al., 2002; Savage et al., 2004), which could decrease discrimination because fewer isotopes will be discriminated against as the organism rapidly uses available organic material to build tissues (Barnes et al., 2007; Sears et al., 2009).

Here, we used the domestic cricket, *Acheta domesticus,* to determine the effects of temperature on isotopic incorporation and discrimination. Specifically, we test two predictions: 1) incorporation rates will be highest in crickets grown at higher temperatures, where growth and metabolic rate are highest, and 2) TDFs will be smaller in crickets grown at higher temperatures. We used newly hatched crickets to perform a controlled feeding experiment, switching the crickets to an isotopically distinct diet. When the crickets were switched to the new diet, we then raised them at three different temperatures: 16 °C, 21 °C, and 26 °C.

2. Materials and methods

2.1. Experimental design

We used domestic crickets (*Acheta domesticus*) because they are easy to obtain and maintain in the laboratory, and their growth rates are strongly influenced by temperature (Booth and Kiddell, 2007). Five hundred pinhead crickets (~3–6 days old) were purchased from Carolina Crickets (Woodruff, South Carolina), where they and their parents were raised on a diet of chicken feed with a carbon isotopic ratio of −20.23‰ (SD = ± 0.19‰). Upon arrival at our laboratory, crickets were divided into three groups of approximately equal numbers and subsequently maintained at one of three environmental temperatures. The cold group was placed in a 10-gallon aquarium in an incubator (Precision Scientific; light:dark cycle 16:8) set to 16.0 °C (SD ± 0.01 measured with a built-in thermometer). The warm group was placed in the laboratory (light:dark cycle 16:8) in an empty 30-gallon trashcan with its lid removed, with the ambient temperature of the lab maintained at 21.0 °C (± 0.10 measured with a Taylor 6341 SAMA CP10 thermometer). The hot group was also placed in an empty 30-gallon trashcan, but with a heat lamp added that hung ~20 cm below the top of the can for the duration of the experiment (light:dark cycle 24:0), producing a mean temperature of 26.0 °C (± 0.10). Throughout the study, crickets were fed a diet of Nutro® catfood (containing chicken, salmon, and rice), chicken broth, powdered milk, and dried and powdered kale, providing a nutritionally balanced diet for crickets (Nakagaki and DeFoliart, 1991). Cricket food for our entire experiment was made in a single batch to minimize isotopic variation. Crickets at all temperatures were provided empty egg cartons and small boxes for refuges and wet sponges for water, which were cleaned and rewetted regularly. Crickets were maintained at these conditions for 32 days, throughout which they were sampled five times for stable isotope analysis. At each sampling point, we collected 5 to 6 crickets. The first sampling occurred on day 0, when the crickets had arrived at our laboratory and prior to being fed the new diet. All remaining crickets were fed the new diet immediately upon arrival to our laboratory. We sampled crickets on days 4, 8, 16, and 32 after they had been switched to the new diets.

2.2. Stable isotope analysis

Following collection, crickets were frozen, weighed, and then dried in an oven at 55 °C for 48 h. Each cricket was then homogenized with a mortar and pestle, and we performed isotopic analysis on the whole body. About 0.5 – 0.6 mg of this whole-body material was placed in a 3 mm x 5 mm tin capsule and sent by mail to the UC Davis Stable Isotope Facility for analysis of $\delta^{13}C$ isotope values. This involved combusting samples in a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotope values (δ) were expressed in parts per thousand (‰), where $\delta^3C = ({}^{13}C_{sample}/{}^{13}C_{standard} - 1) \times 1000$. ${}^{13}C_{sample}$ and ${}^{13}C_{standard}$ represent molar ratios of C^{13}/C^{12} of the sample and a standard reference material (Vienna-Pee Dee belemnite).

2.3. Analyses

To determine the temperature dependence of cricket growth rate, we fitted the following exponential equation to the data on cricket weights,

$$W = Pe^{rt} \quad (1)$$

where W is wet weight (g), P is a coefficient, r is growth rate (g/day), and t is time in days. Curves were fitted to this data using iterative, non-linear fitting functions to determine P and r. The value of r represents the fractional rate of growth (k_g; see below) in isotopic incorporation (Reich et al., 2008; Carleton and Martínez del Rio, 2010).

Initial analysis using the *outliers* package (Komsta, 2011) in the R statistical program (R Core Development Team 2016) revealed that four of our 91 data points were marginally significant outliers (21°: day 0: $\chi^2 = 2.766$, $p = .096$; day 4: $\chi^2 = 2.83$, $p = .092$; 26°: day 4: $\chi^2 = 3.534$, $p = .098$; day 32: $\chi^2 = 3.534$, $p = .06$). Due to low sample sizes these outliers had a strong effect on the analysis, as the iterative, non-linear fitting routines (see below) failed to converge on an estimate of k, and so these four data points were removed before further analysis.

We modeled the isotopic incorporation of carbon as

$$\delta C_t = \delta C_\infty - (\delta C_\infty - \delta C_0)e^{-kt} \quad (2)$$

where C_t is the isotopic composition at time t, C_∞ is the isotopic composition at equilibrium on the new diet, C_0 is the initial stable isotope

composition before switching to the new diet, and k is the fractional rate of isotopic incorporation estimated using iterative, non-linear fitting routines. To partition the effects of growth and catabolism on isotopic incorporation, k was divided into k_g (the fractional rate of growth) and k_c (the fractional rate of incorporation driven by catabolism), where $k = k_g + k_c$ (Reich et al., 2008; Carleton and Martínez del Rio, 2010). Thus, the proportion of isotopic incorporation driven by growth is defined as k_g/k. As $k_g \rightarrow k$ the isotopic incorporation is dominated by growth, and as $k_g \rightarrow 0$ isotopic incorporation is dominated by catabolism (Reich et al., 2008; Carleton and Martínez del Rio, 2010).

Trophic discrimination factors (TDFs) were calculated as the difference between the $\delta^{13}C$ isotope value of the consumer and its resource (e.g., $\delta^{13}C_{sample} - \delta^{13}C_{resource}$), commonly denoted as $\Delta^{13}C$. We pooled isotope data from days 32 and 16 in our analysis of TDFs, since carbon isotopes had reached equilibrium by day 16 for crickets grown at 21 °C and 26 °C. Since crickets grown at 16 °C did not survive past day 16 we could not accurately estimate their TDFs, and so data from crickets at 16 °C were not included in our analysis of TDFs.

We used an ANCOVA to determine whether crickets raised at different temperatures grew at different rates, where cricket weight was the response variable and both day number and temperature were explanatory variables. Likewise, ANCOVA was used to determine differences in stable isotope incorporation rates across temperatures, where the stable isotope value was the response variable and day number and temperature were explanatory variables. To determine whether temperature affected TDF, we performed a Welch's t-test on the TDFs of carbon from crickets raised at 21 °C and 26 °C.

3. Results

Crickets grew faster and had higher variation in growth rates at higher temperatures (Table 1, Fig. 1; $t_{3,80} = 11.53$, $p < .001$), with growth rates (r) increasing by about 0.03 (g/day) every 5 °C (Table 1). Crickets grown at 16 °C did not survive past day 16.

Crickets grown at higher temperatures had higher rates of isotopic incorporation (Table 1, Fig. 2; $t = -2.64$, $df = 3, 78$, $p = .01$), and isotope half-lives decreased at higher temperatures (Table 1). The increase in isotopic incorporation rates at higher temperatures was due to an increase in both growth and catabolism (Table 2). The values of k_g and k_c were highest at 26 °C, intermediate at 21 °C, and lowest at 16 °C (Table 2).

Temperature had a significant effect on TDFs ($t = 2.467$, $df = 21.81$, $p = 0.022$), being lowest at 26 °C (Fig. 3).

4. Discussion

To the best of our knowledge, ours is the first study to explicitly link the effects of temperature on growth rates and isotopic incorporation and trophic discrimination (TDF), and our results clearly show that temperature has a strong influence on the rate of incorporation and TDF of carbon isotopes. Our results also supported our two predictions. First, cricket growth rates were higher and isotopic incorporation was fastest at warmer temperatures. Second, carbon TDFs were reduced at warmer temperatures.

Table 1
Growth equations, growth rates (r), and R^2 values of the growth equations for crickets grown at 16°, 21°, and 26 °C. Compared to the growth rate at 16 °C, growth rate approximately doubled at 21 °C and tripled at 26 °C.

Temperature	Growth Equation	r	R^2
16 °C	$W = 0.0075e^{0.0318t}$	0.0318	0.62
21 °C	$W = 0.0009e^{0.0626t}$	0.0626	0.99
26 °C	$W = 0.0034e^{0.097t}$	0.0970	0.99

For ectotherms, temperature affects carbon isotopic incorporation by increasing growth rates via the effects of temperature on metabolism. While researchers have demonstrated that growth rate influences the rate of isotopic incorporation (Logan et al., 2006; Reich et al., 2008; Carleton and Martínez del Rio, 2010; Heady and Moore, 2013), researchers have not previously shown that different temperatures change growth rates and thereby alter isotopic incorporation rates. Our finding has important implications for studies and experiments performed in the field, where fluctuating temperatures can alter growth rates in ectotherms (Angilletta et al., 2004; Booth and Kiddell, 2007) and thus are likely to influence isotopic incorporation. Similarly, latitudinal, altitudinal, and seasonal differences in temperature result in differences in growth rates, which should be considered in future isotopic studies, as well as the elemental flow through food webs (Schramski et al., 2015). Additionally, many insect species are heterothermic and behaviorally and physiologically thermoregulate to above or below ambient temperatures (Stevenson, 1985). Some heterotherms regulate different parts of their bodies to different temperatures, e.g. warming the thorax for flight while maintaining a cool abdomen, a trait likely to result in complex patterns of isotopic incorporation across tissues and body regions. A greater knowledge of the degree to which temperature affects incorporation across a range of ectotherms is needed to improve the accuracy of stable isotopes as a tool for ecologists.

Our results also show that despite a significant increase at higher temperatures in the amount of isotopic incorporation due to growth, across the range of temperatures in this study catabolism accounted for much more of the incorporation than did growth. Few studies exist whereby authors partition the contributions of catabolism and growth to isotopic incorporation, but the results from those studies do suggest there may be interspecific variation in the degree to which growth affects incorporation (Logan et al., 2006; Reich et al., 2008; Carleton and Martínez del Rio, 2010). In addition, the age of the focal animal is likely to be a primary determinant of the relative importance of growth versus catabolism in many species because growth rates will vary through an individual's life. In our study, isotopic incorporation of mature crickets was driven by catabolism, as growth becomes reduced. In hatchling and juvenile sea turtles, *Caretta caretta*, growth accounted for all the incorporation in tissues such as skin, scute, and red blood cells (Reich et al., 2008). In other tissues, such as whole blood, plasma, and scute (in juveniles), catabolism also made contributions to incorporation, ranging from 0.64 to 3.5 times those of growth (Reich et al., 2008). In juvenile Nile tilapia, *Oreochromis niloticus*, catabolism contributed 2.6–5 times more to incorporation than did growth (Carleton and Martínez del Rio, 2010). In our study, we found that catabolism contributed 6.5–12.2 times more to incorporation than growth, and that the fractional contribution of catabolism increased with temperature. Thus, the effects of temperature on isotopic incorporation goes beyond increased growth rates, and temperature may directly affect incorporation rates, possibly by increasing the rate at which elements are overturned at higher temperature. This increase in the contribution of catabolism with temperature is a result that we would expect with an increase in metabolic rate as higher metabolic rates are associated with faster tissue turnover rates (Welle and Nair, 1990).

Several lines of evidence suggest that the effects of temperature on isotopic incorporation are caused by increased metabolism at higher temperatures. The rate of isotopic incorporation is negatively correlated with body size (MacAvoy et al., 2006; Schramski et al., 2015), as is metabolic rate (Gillooly et al., 2001; Brown et al., 2004). Isotopic incorporation is also slower in ectotherms compared to similarly sized endotherms (Reich et al., 2008; Murray and Wolf, 2012; Rosenblatt and Heithaus, 2013; Cloyed et al., 2015), and ectotherms have much lower metabolic rates than do endotherms. Finally, metabolic rate drives ontogenetic development (Gillooly et al., 2002), and in ectotherms, the higher rates of ontogenetic development at higher temperatures (e.g. increased growth rates observed in this study, Fig. 1) are driven by

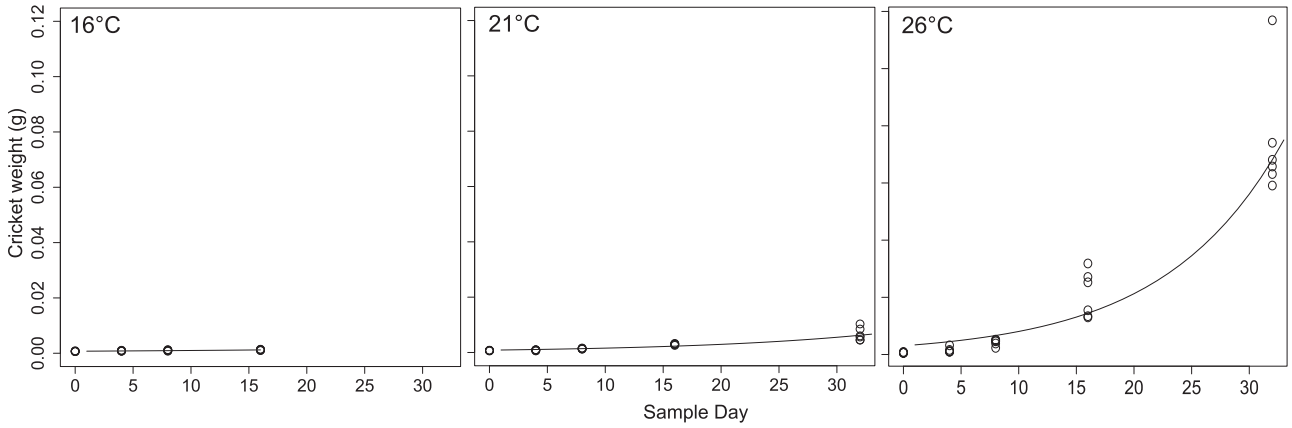

Fig. 1. Growth curves of domestic crickets grown at 16 °C, 21 °C, and 26 °C. Equations of the growth curves are provided in Table 1.

increased metabolism (Gillooly et al., 2002). Despite this evidence, several researchers argue that incorporation is not driven by metabolic rate (Carleton and Martínez del Rio, 2005), but instead more narrowly by the rate of protein synthesis (Carleton and Martínez del Rio, 2005; Newsome et al., 2014). However, the rate of protein synthesis is itself metabolically constrained (Robertson et al., 2001), and it is therefore possible that metabolism drives isotopic incorporation via its effects on protein synthesis.

Less is known about how temperature may influence TDFs in carbon. Temperature may affect TDFs via the lowering of activation energies at warmer temperatures, or because at warmer temperatures organisms grow faster and have a higher metabolic rate, resulting in individuals using more of the available molecules to build/replace tissues. Although we cannot distinguish between the two mechanisms, there is evidence that this pattern is at least partially driven by the lowering of activation energies. Given that isotopic discrimination occurs when different stable isotopes have different activation energies (Fry 2006), at warmer temperatures the lower activation energy should be more easily reached than the higher activation energy required at cooler temperatures (Gibert et al., 2016; Pawar et al., 2016), and isotopic discrimination should thus be lower. Although our results supported this prediction, results from published studies vary, possibly due to dietary differences. Carbon fractionation is sensitive to diet, and differences in diet, particularly lipid content, can obscure how temperature affects discrimination (Barnes et al., 2007; Bloomfield et al., 2011). In the European sea bass, *Dicentrarchus labrax*, carbon discrimination was greater at higher than at lower temperatures (Barnes et al., 2007), which the authors attributed to the greater proportion of lipids found in bass raised at lower temperatures. On the other hand, black bream, *Acanthopagrus butcheri*, had less carbon discrimination at higher temperatures when fed a vegetable diet but not when fed an animal diet (Bloomfield et al., 2011).

Like incorporation rates, the effects of temperature on TDFs are likely driven by metabolic rate, as increasing temperature decreases activation energies required for the physiological processes that drive metabolic rate (Gibert et al., 2016; Pawar et al., 2016). Research on nitrogen discrimination provides some support for the idea that metabolic rate drives trophic discrimination. While diet does affect trophic discrimination of nitrogen, diet quality affects nitrogen discrimination less than it affects carbon discrimination (Caut et al., 2009). Studies have found that nitrogen discrimination is lower at warmer temperatures than at cooler temperatures (Barnes et al., 2007), even when diets are different (Bloomfield et al., 2011). This suggests that when diet quality is accounted for, especially for carbon, metabolic rate may limit how much the lighter and/or heavier isotopes are discriminated against. In order to determine definitively whether metabolic rate drives trophic discrimination, future research should investigate how temperature affects discrimination independent of the protein and fat content of the food.

These temperature effects on TDFs and isotopic incorporation can be significant, but authors can take steps to account for them. For example, we found that the difference in carbon TDFs between the 21 °C and 26 °C treatments was 0.24, which is approximately the average carbon TDF value for invertebrates (~0.25; Caut et al., 2009). Thus, using TDFs measured at an incorrect temperature can result in either under- or over-estimating food sources and habitat usage. To improve

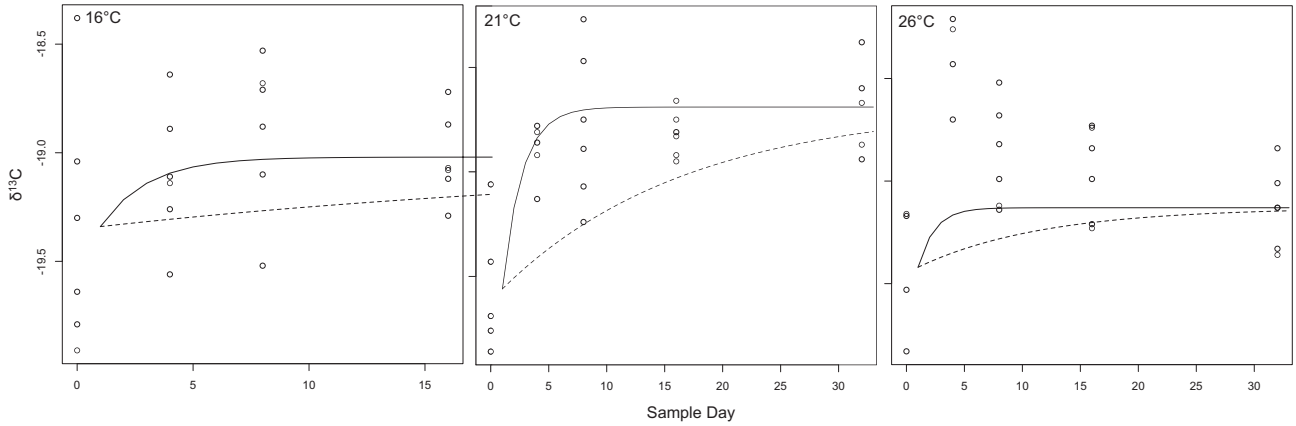

Fig. 2. $\delta^{13}C$ incorporation rates in domestic crickets raised at 16 °C, 21 °C, and 26 °C. Solid lines depict a fractional rate of incorporation that includes both growth and catabolism components. Dashed lines depict a fractional rate of incorporation that includes only growth component. Isotopic incorporation proceeds faster at warmer temperatures than at cooler temperatures.

Table 2
Carbon isotopic incorporation equations, total fractional rate of isotopic incorporation (k_t), fractional rate associated with growth (k_g), fractional rate associated with catabolism (k_c), half lives ($t_{1/2}$), and trophic discrimination factors (± 95% CI). Incorporation proceeds faster at warmer temperatures, as is indicted by the higher values of k_t and the lower half-lives. Growth rates double between 16° and 21 °C and triple between 16° and 26 °C. The TDFs are significantly lower at warmer temperatures.

Temperature	Isotopic Incorporation Equation	k_t	k_g	k_c	$t_{1/2}$ (d)	TDF
16 °C	$\delta^{13}C = -19.02 - 0.32e^{-0.489t}$	0.489	0.0318	0.461	1.417	NA
21 °C	$\delta^{13}C = -18.69 - 0.87e^{-0.591t}$	0.591	0.0626	0.528	1.173	0.79 (± 0.24)
26 °C	$\delta^{13}C = -19.13 - 0.29e^{-0.7t}$	0.700	0.0970	0.607	0.990	0.55 (± 0.22)

the accuracy of stable isotope analyses in future studies in which temperature varies, we propose using temperature-specific measures of discrimination by calculating temperature-specific isotopic incorporation and discrimination. We can model the half-life of isotopes across temperatures by determining the proportion of time that organisms spend at different temperatures (or temperature ranges, depending on the level of accuracy that is feasible or desired) and then summing the incorporation for each temperature,

$$t_{1/2} = \sum_{i=1}^{n} \alpha_i \tau_i \quad (3)$$

where $t_{1/2}$ is the overall half-life, n is the number of different temperatures observed, α_i is the proportion of time spent at temperature i, and τ_i is the half-life at temperature i. Additionally, using the Arrhenius equation (Brown et al., 2004), we can normalize trophic incorporation rates to specific temperatures and compare rates across species. Likewise, we can model TDF in a similar way,

$$TDF_{total} = \sum_{i=1}^{n} \alpha_i TDF_i \quad (4)$$

where TDF_{total} represents the overall trophic discrimination factor and TDF_i is trophic discrimination factor at temperature i.

There are two caveats in our experimental design, although we are confident they do not substantively affect the overall results. First, differences in cricket housing and how temperature was controlled across treatments could potentially have had some effect on our results. Crickets in the 16 °C treatment were held in an aquarium in an incubator while crickets in the 21 °C and 26 °C treatments were housed in large plastic bins in the laboratory. The differences in container type likely had little effect on cricket growth and isotopic incorporation, as all cricket were provided with the same amount of food, water, and shelter. Temperature was tightly controlled in the incubator in which the 16 °C group was held and light:dark cycles were 16:8 h. The 21 °C group was held at room temperature, which varied more than in the incubator but overall varied only slightly (see methods), and light:dark cycles were similarly set to 16:8 h. The temperature of the 26 °C group was elevated with the 24-h use of a heat lamp, a method commonly used to elevate temperatures for invertebrates and reptiles. Effects from the differences in light cycles between the 16 °C/21 °C groups and the 26 °C were likely overridden by the temperature effects for two reasons. First, the crickets were provided ample shelter that they could find many dark places to hide for extended periods of time (8–12 h). Second, the effects of temperature on physiological processes are so substantial

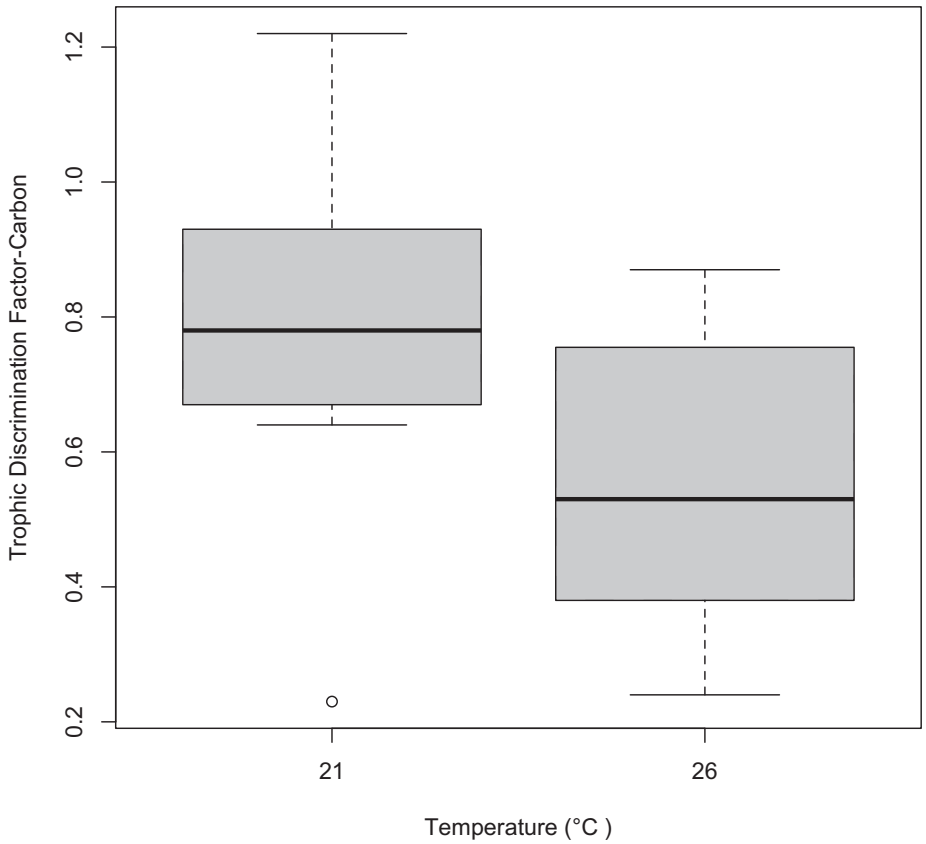

Fig. 3. Boxplot depicting trophic discrimination factors (TDF) of carbon in the domestic cricket. Crickets raised at 26° had lower trophic discrimination factors for carbon than did crickets raised at 21°. Crickets from 16° were not included in this analysis as none of them lived past day 16.

(Gillooly et al., 2001, 2002) that they likely overwhelmed any effects from differences in either light cycles or container type and our results are consistent with our expectations of how biological rates vary with temperature.

The second caveat is that crickets from the 16 °C group all died shortly after day 16, and 16 °C is at the lower end of the operational temperature range for domestic crickets (Booth and Kiddell, 2007). The other temperature treatments are well within the range of operational temperatures for the domestic cricket. Having a temperature treatment at the lower end of the temperature range highlights the effects of temperature on growth rates and isotopic incorporation rates. Thus, we believe that the results of our experiment are robust enough to overcome these potential shortcomings in our experimental design.

Our results highlight the complicated ways that isotopes, and other elements, move through organisms and food webs. The isotopic incorporation values and TDFs of individual species obtained in the lab are likely overly simplistic. Natural variation in temperature can affect growth rates (Gillooly et al., 2002), which in turn will affect isotopic incorporation rates (Frazer et al., 1997). Likewise, temperature can influence trophic discrimination factors. In order to improve the accuracy of stable isotope analysis, a better understanding of how temperature affects fundamental properties of isotope ecology is needed for a broad collection of species. Furthermore, researchers cannot assume that the incorporation rates and TDFs obtained from thermally constant laboratory experiments can be directly applied to studies performed in natural environments, where ecological inferences are made from stable isotope ratios. We suggest adding temperature treatments to controlled feeding experiments that determine isotopic incorporation and discrimination. Temperatures in these treatments should span an ecologically relevant range for the species in question. In this way, researchers performing field studies with the isotopic properties obtained from these experiments may estimate the times that the organism spent at various temperatures and adjust the rate of incorporation and the amount of discrimination to suit the temperatures experienced.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2018.02.003.

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