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LETTER

U-shaped response Unifies views on temperature dependency of stoichiometric requirements

Thomas Ruiz,^{1*}  Apostolos-Manuel Koussoroplis,¹  Michael Danger,²  Jean-Pierre Aguer,¹ Nicole Morel-Desrosiers¹ and Alexandre Bec¹ 

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Abstract

Temperature and nutrient availability, which are major drivers of consumer performance, are dramatically affected by global change. To date, there is no consensus on whether warming increases or decreases consumer needs for dietary carbon (C) relatively to phosphorus (P), thus hindering predictions of secondary production responses to global change. Here, we investigate how the dietary C:P ratio optimising consumer growth ($TER_{C:P}$: Threshold Elemental Ratio) changes along temperature gradients by combining a temperature-dependent $TER_{C:P}$ model with growth experiments on *Daphnia magna*. Both lines of evidence show that the $TER_{C:P}$ response to temperature is U-shaped. This shape indicates that consumer nutrient requirements can both increase or decrease with increasing temperature, thus reconciling previous contradictory observations into a common framework. This unified framework improves our capacity to forecast the combined effects of nutrient cycle and climatic alterations on invertebrate production.

Keywords

Daphnia, ectotherm, global change, nutrient, stoichiometry, temperature, TER.

Ecology Letters (2020)

INTRODUCTION

In the last decades, temperature of lake surface water increased on average by $0.34\text{ }^{\circ}\text{C decade}^{-1}$ (O'Reilly *et al.*, 2015), a trend which may worsen regarding the speed at which global change occurs (Rogelj *et al.*, 2012). In the same time, urbanisation and agricultural practices lead to significant alterations of elemental cycles (especially C, N and P) and nutrient availability in the biosphere (Falkowski *et al.*, 2000). These two factors are important drivers of ectotherm herbivore physiology, temperature regulating the rate of all biological process occurring in the organism (Angilletta, 2006) and nutrient uptake fuelling physiological needs for growth or reproduction (Elser *et al.*, 2003). The independent effects of temperature and nutrient availability on organisms has long been studied separately, however, in natural environments submitted to global change, these two factors change in parallel, affecting consumer performance interactively (Kingsolver *et al.*, 2006). Hence, it is essential to consider any temperature change within its nutritional context to understand on-going and future changes in invertebrates' populations and their interactions with other trophic levels.

Several studies have explored the thermal sensitivity of consumer nutritional requirements, particularly focusing on C:N:P stoichiometry (Cross *et al.*, 2015) and two competing predictions emerge from this literature. The first emphasises the temperature sensitivity of individual metabolic rate (Brown *et al.*, 2004) arguing that a higher metabolic activity resulting from higher temperature should increase the energetic requirements of the organism. As energy results from the oxidation of C-rich compounds, an increased temperature should therefore increase consumer C needs. This hypothesis is supported

by experimental evidence both for terrestrial (Lee *et al.*, 2015) or aquatic (Boersma *et al.*, 2016; Malzahn *et al.*, 2016) consumers which tend to select for C-rich diets at higher temperatures. However, other authors claim that higher temperature also accelerates growth (Frazier *et al.*, 2006). According to the growth rate hypothesis (Elser *et al.*, 2003), an increased growth rate should raise nutrient demand (N and P) to sustain increasing protein synthesis and DNA replication. This hypothesis is also supported by empirical evidence showing an increasing nutrient demand at higher temperatures both in terrestrial (Lemoine *et al.*, 2013) or aquatic (Persson *et al.*, 2011; Wojewodzic *et al.*, 2011) consumers. To date, these apparently contradictory results remain a conundrum and the evolution of consumer stoichiometric requirements under warming scenarios a subject of vivid debate (Boersma *et al.*, 2016; Winder *et al.*, 2016). A recent opinion paper by Schmitz & Rosenblatt (2017), however, contains an appealing solution to the above controversy. The authors propose four theoretical models for the interactive effects between temperature and predation stress on consumer stoichiometric requirements. One of their models predicts that both C and nutrient demand can increase with warming and that previous contradictory results could simply reflect different regions of the same underlying U-shaped response.

The models in Schmitz & Rosenblatt (2017) are based on the threshold elemental ratio (TER), a well-established framework defining how consumer biomass production responds to the elemental composition of food (Frost *et al.*, 2006). The TER is the ratio at which elements should be present in diet to maximise consumer growth and where the limitation of growth switches from one element to another. The most commonly studied TER are those relating carbon (C) to

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phosphorus (P) or nitrogen (N) and they reflect the switching point from an energy-limited (i.e. C-limited) growth to a nutrient-limited growth. Deviations of food stoichiometry below or above the *TER* induce a dietary (C- or nutrient-) limitation for consumers (Frost *et al.*, 2006) and lead to a unimodal response of growth to food stoichiometry also known as the “stoichiometric knife-edge” (Elser *et al.*, 2016; Zhou & Declerck, 2019). Besides the effects on consumer performance, the *TER* can explain consumer-driven P and N recycling in ecosystems (Cherif & Loreau, 2013), population dynamics (Elser *et al.*, 2000; Frost *et al.*, 2006) and species interactions (Boersma *et al.*, 2016). The main innovation in Schmitz & Rosenblatt (2017) is that certain physiological processes underlying the *TER* such as mass-specific ingestion and respiration rate do not simply rise exponentially as usually assumed (Anderson *et al.*, 2017) but rather exhibit a unimodal response to temperature (McMahon, 1969; Kibby, 1971; Yurista, 1999). Under this assumption, the *TER* of a consumer can exhibit a U-shaped response to temperature, an exciting prediction that remains to be tested experimentally.

The aim of our study is twofold. First, we extend the theoretical work of Schmitz & Rosenblatt (2017) and thoroughly explore the conditions under which the U-shaped response of the *TER* emerges. Departing from a model parametrisation based on the freshwater cladoceran *Daphnia magna*, we analyse the robustness of the U-shape to variations in the thermal reaction norms of the physiological traits underlying the *TER*. Second, we experimentally determine how the *TER* of a *Daphnia magna* clone changes with temperature by measuring its somatic growth responses under a factorial combination of temperatures and dietary stoichiometry.

MATERIAL AND METHOD

Model equations and analysis

The $TER_{C:nutrient}$ can be expressed as the product of nutrient use efficiency and the consumer’s elemental composition:

$$TER_{C:nutrient} = \frac{AE_{nutrient}}{GGE_C} * Q_{C:nutrient} \quad (1)$$

where $Q_{C:nutrient}$ is the body composition of organisms, $AE_{nutrient}$ is the assimilation efficiency of a specific nutrient (N or P) and GGE_C the gross growth efficiency of C. GGE_C refers to the proportion of ingested C used for growth, formulated as:

$$GGE_C = \frac{I_C * AE_C - R_C}{I_C} \quad (2)$$

where I_C is the C ingestion rate, AE_C the C assimilation efficiency and R_C the C respired.

In a comparable approach to Schmitz & Rosenblatt (2017), we define the thermal reaction norms for each process underlying the *TER*. The thermal response of daphnia ingestion rate has been largely documented (McMahon, 1969; Kibby, 1971; Yurista, 1999) and is known to present a temperature optimum below and above which process rate decreases generating a hump-shaped thermal performance curve (TPC) (Angilletta, 2006). We modelled I_C using a modified Gaussian as:

$$I_C = I_{CMIN} + \Delta I_{CMAX} * e^{-b(T-T_{OPT})^2} \quad (3)$$

where ΔI_{CMAX} is the difference between minimum (I_{CMIN}) and maximum ingestion rate. T is temperature, T_{OPT} the optimal temperature and b a coefficient determining the decrease rate around T_{OPT} . Here, we estimated the shape of C ingestion TPC parameters (b , $I_{CMIN}/\Delta I_{CMAX}$ ratio) by fitting eqn 3 to the measurements of Yurista *et al.* (1999) on the arctic cladoceran *Daphnia middendorffiana*. However, to adjust the TPC to our temperate *D. magna* we replaced the thermal optimum (T_{OPT}) to 22 °C, the acclimation temperature of our clones (Fig. 1). We also adjusted the maximum ingestion rate ($I_{CMIN} + \Delta I_{CMAX}$) to the measurement of Urabe & Watanabe (1991) on temperate *Daphnia* species fed on *Chlamydomonas reinhardtii*, which is also in accordance with Darchambeau *et al.* (2003). However, as data on thermal dependence of I_C are scarce for *Daphnia magna* (McMahon, 1969), we tested how the $TER_{C:P}$ thermal reaction is influenced by curvature of the I_C hump-shaped response by varying b in the sensitivity analysis (Fig. 1a).

In Schmitz & Rosenblatt (2017), respiration is modelled as a hump-shaped thermal reaction norm. Although this is valid, experimental evidence indicates that within the temperature range we used in our study, the metabolic rate of *D. magna* (i.e. respiration) rises exponentially. Hence, unlike Schmitz & Rosenblatt (2017), we modelled R_C as:

$$R_C = ae^{dT} \quad (4)$$

where d is the scaling exponent and a the proportionality constant. We measured *D. magna* metabolic rate (*see method below*) to confirm the exponential rise. Fitting our data to eqn 4 results in a scaling parameter $d = 0.035$ (Fig. S1). We also tested how the predicted $TER_{C:P}$ reaction norm is affected by variations in the scaling exponent d ranging from 0.01 to 0.07 (Fig. 1b).

Carbon and nutrient assimilation efficiency (*AE*) are typically assumed constant across temperatures in zooplankton *TER* models (Frost *et al.*, 2006; Anderson *et al.*, 2017) and experimental evidence seems to support this assumption (Yurista, 1999). Using previously reported values for *Daphnia* as reference we modelled AE_C and AE_P constant with temperature ($AE_C \sim 0.5$ (Urabe & Watanabe, 1991; Yurista, 1999) and $AE_P \sim 0.8$ (Anderson & Hessen, 2005; Frost *et al.*, 2006)). As levels of AE_C and AE_P reported in literature do not constitute a clear consensus, we tested how the $TER_{C:P}$ thermal reaction norm responds to increases and decreases in AE_C and AE_P levels around these reported values (Fig. 1c and d). *See Supplementary material for a model with AE thermal reaction norms* (Fig. S2).

We analysed the elemental content of our *Daphnia* clone (*see method below*) and showed no significant differences in body C:P ratio (Fig. S3) across dietary and temperature treatments, with an average value of 95. Such homeostatic regulation is not uncommon in daphnids fed stoichiometrically imbalanced. However, some studies on other *D. magna* clones and copepods suggested that body C:P can either increase (McFeeters & Frost, 2011) or decrease (Mathews *et al.*, 2018) with increasing temperatures. To test all these scenarios (constant, increasing and decreasing $Q_{C:P}$), we define $Q_{C:P}$ as:

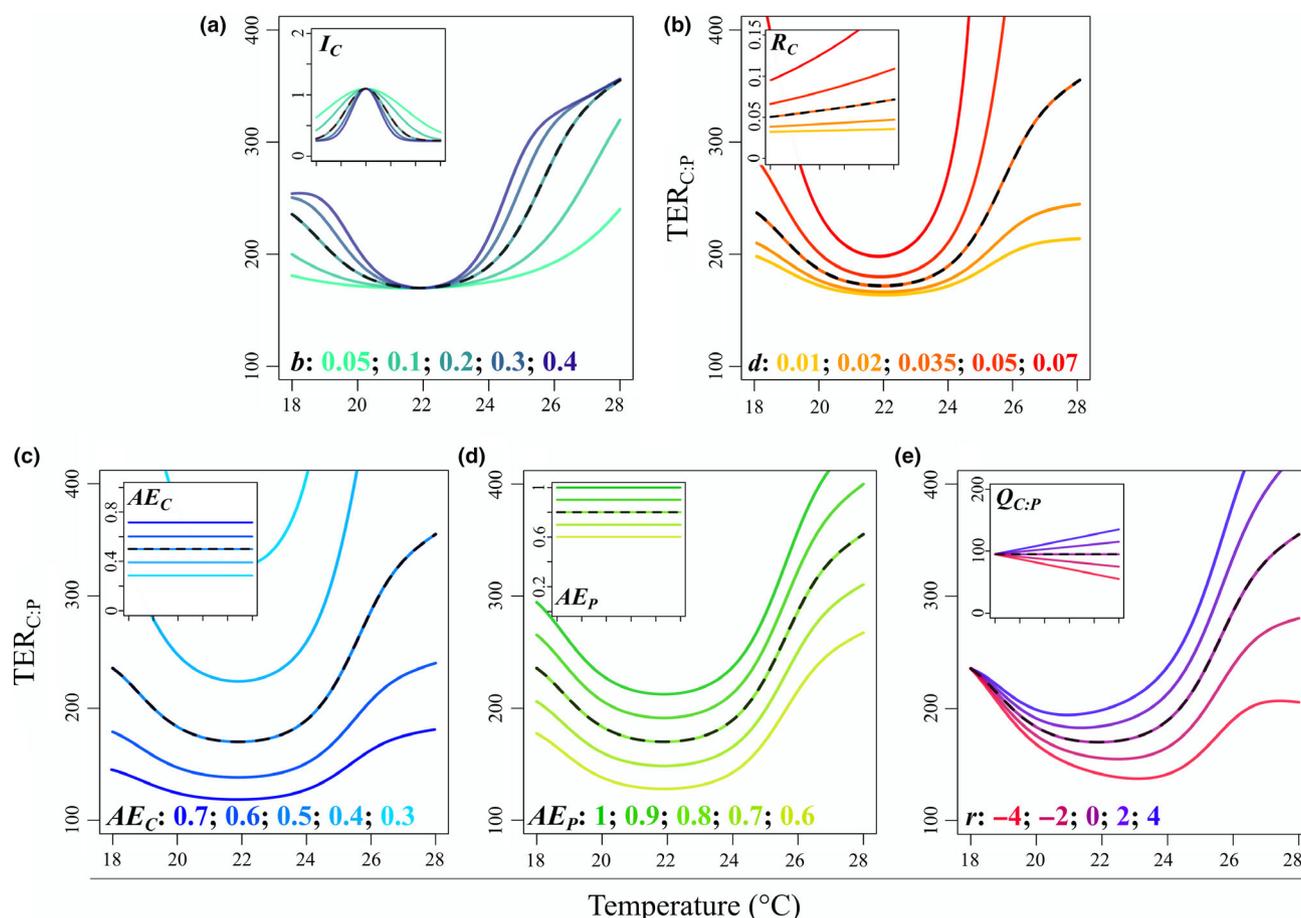


Figure 1 Effect of parameter values on the thermal reaction norm of TER. Each panel is associated with the variation in one specific parameter constraining: (a) The concavity (parameter b) of the carbon ingestion rate reaction norm I_C , (b) the temperature scaling (parameter d) of carbon respiration R_C , (c) the level of carbon assimilation efficiency AE_C , (d) the level of phosphorus assimilation efficiency AE_P and (e) the slope (parameter r) of consumer's body C:P response to temperature. Each parameter is varied individually while the others are kept constant at the values for *D. magna* (see Table 1). Upper left inserts present the temperature responses of physiological process associated with parameter variations. The dashed black line is the model prediction for *D. magna*. See Table 1 for details on the selected parameter ranges.

$$Q_{C:P}(T) = CP_0 + (r * (T - T_{MIN})) \quad (5)$$

where CP_0 and r , respectively, define the intercept and slope of the $Q_{C:P}$ versus temperature curve and T_{MIN} the minimal temperature observed (Fig. 1e). By setting r to 0, $Q_{C:P}$ becomes a constant ($Q_{C:P}=CP_0$), as it is the case for our *D. magna* clone.

To test the robustness of our predictions, we calculated the TER thermal reaction norm (from Eq.1), for 1000 random combinations of parameter values (parameters b , d , AE_C , AE_P , r) sampled from uniform distributions bound within the biologically relevant ranges defined in Table 1. For each obtained TER reaction norm, we calculated the average slope (at 0.5 °C intervals) below and above optimal temperature (set at 22 °C) to describe the shape of $TER_{C:P}$ thermal reaction. Only slopes above 5% were considered significant. When both slopes are positive or negative or when one of them is zero, we considered the $TER_{C:P}$ thermal reaction as monotonous. A U-shape (soft or strong) was assigned when the slope is initially negative and then positive. A strong-U $TER_{C:P}$ slope was assigned when both slopes are above 10% (Fig. 2).

Daphnia maintenance

We tested our model predictions using a clonal line of *Daphnia magna* to exclude genotypic response variations. The stock cultures of daphnids were kept in Volvic water[®] at 22 °C on a 14:10 h day:night cycle and fed with *Chlamydomonas reinhardtii* (C:P 250 at 3 mgC L⁻¹) for more than three generations.

Preparation of food suspensions

Chlamydomonas reinhardtii was used as food in all experiments. We used three freeze-dried stock cultures with respective molar C:P ratio of 95 (high P), 331 (intermediate P) and 1014 (low P) previously prepared (See supplementary material for details). Food suspensions were prepared daily by mixing freeze-dried algae stocks diluted in Volvic[®] water (Table. S1). Despite a slight reduction in individual performance, freeze-dried algae were shown to be adequate food for *D. magna* for several generations (Naylor *et al.*, 1993). The P content of algae has no significant influence on their fatty acids composition (Table. S2) and the rehydration of freeze-dried algae had no effect on their elemental composition.

Table 1 Modelled thermal reaction norms of the physiological processes underlying the TER model in eqn 1 and associated parameters. Tested parameter ranges (see Fig. 1) are given in parentheses, values in bold are the values used for *Daphnia magna*. ¹(Yurista, 1999); ²(Urabe & Watanabe, 1991); ³(Anderson & Hessen, 2005); ⁴(Frost *et al.*, 2006).

C Ingestion		Equation: $I_C(T) = I_{CMIN} + \Delta I_{CMAX} * e^{-b(T-T_{OPT})}$
I_{CMIN}	0.25 ¹⁻² ($\mu\text{gC}\cdot\text{day}^{-1}\cdot\mu\text{gDW}^{-0.48}$)	
ΔI_{CMAX}	0.85 ¹⁻² ($\mu\text{gC}\cdot\text{day}^{-1}\cdot\mu\text{gDW}^{-0.48}$)	
b	(0.05, 0.4); 0.2 ¹ Unitless	
T_{OPT}	22 (°C)	
C Assimilation efficiency		Constant
AE_C	(0.3 ; 0.7) 0.5 ³⁻⁴ Unitless	
P Assimilation efficiency		Constant
AE_P	(0.6 ; 1) 0.8 ³⁻⁴ Unitless	
C respiration		Equation: $R_C = ae^{dT}$
d	(0.01-0.07) 0.035 this study Unitless	
a	0.0269 this study ($\mu\text{gC}\cdot\mu\text{gDW}\cdot\text{day}^{-1}$)	
Consumer body C:P		Equation: $Q^{C:P} = CP_0 + (r * (T - T_{MIN}))$
CP_0	95 this study Unitless	
r	(-4,4); 0 this study Unitless	
T_{MIN}	18 (°C)	

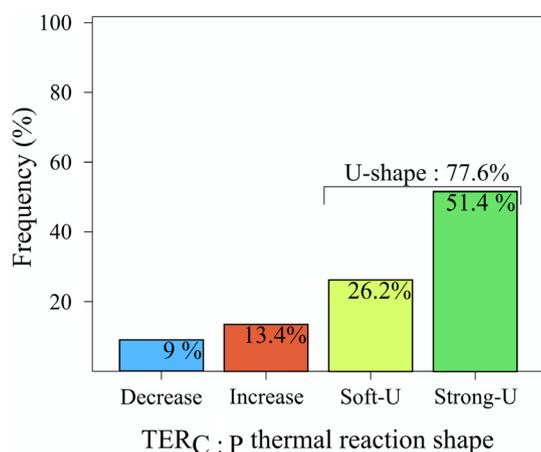


Figure 2 Frequency of occurrence of $TER_{C:P}$ thermal reaction norms across 1000 random model parameter combinations. Parameter values are sampled within the ranges given in Table 1 assuming uniform distributions. A U-shape is considered “strong” when both lines have a slope above 10%. *Decrease* and *Increase* indicate a monotonically increasing or regressing reaction norms respectively. Error on slopes estimates is set at 5%. See methods for details. See Figure S4 for detailed parameter distributions of each pattern.

Experimental procedure

We used a gradient experimental design to capture the nonlinearity of *D. magna* growth rate response to diet stoichiometry and temperature (Kreyling *et al.*, 2018). Daphnids were grown on a full factorial combination of six stoichiometric treatments (with C:P ratios of 95-172-250-331-553-1014, obtained by mixing the freeze-dried stock cultures) at six different temperatures (18-20-22-24-26 and 28 °C). For technical reasons, however, growth experiments were performed independently for each temperature using the same clonal line and a strictly identical protocol. At the beginning of the experiments, 8-h-old neonates obtained from the third or fourth clutch were randomly distributed in two replicate glass jars (250 mL, 12 ind/jar) per dietary treatments. The culture medium composed of Volvic® water with *ad libitum* food (3 mgC L⁻¹) was renewed daily to maintain food quantity above the saturating level during all the experiment. Individuals were raised until they reached maturity, which took from 5 to 9 days depending on temperature. They were then pooled by jar, dried 48h at 60°C, weighed (Sartorius-ME36S; Germany; precision:0.001mg) and stored until elemental analysis. The average growth rate in each jar was calculated as follows:

$$\text{Growth rate} = \frac{\ln(W_t) - \ln(W_0)}{t} \quad (6)$$

where W_t is the average weight (μg) at age of maturity t (days) and W_0 the average weight (μg) of neonates. The growth rate thus calculated refers to the juvenile growth rate which has been shown to represent the best proxy for population increase rate (Lampert & Trubetskova, 1996). After measuring the mean weight, pools of dried daphnids were divided into subgroups to measure their carbon and phosphorus content. In parallel, a subset of mature daphnids from the C:P 250 treatment was used to estimate their metabolic rate. As

variation in metabolic rate (a.k.a. respiratory rate) with diet is not included in TER model, we performed this measurement on a single dietary treatment which is the diet that daphnids used to consume. The mass-specific metabolic rates obtained by microcalorimetry (Ruiz *et al.* 2018) were plotted against temperature to determine the temperature dependence of metabolism (Fig. S2).

Elemental and biochemical analyses

Elemental composition of algae and daphnids was performed in a similar way. Carbon was determined using a CHN analyser (NA-2100-Protein, ThermoQuest-CE Instruments, Italy) and phosphorus content was estimated using a colorimetric determination based on potassium persulfate method (Ruiz *et al.*, 2018). Fatty acid of algae was extracted using chloroform/methanol method and then converted into fatty acid methyl esters by acid-catalysed transesterification (Denoux *et al.*, 2017). The fatty acid methyl esters were analysed by gas chromatography (6850-Network-GC, Agilent-technologies, USA).

Data analyses

To model the response surface of *D. magna* juvenile growth rate to experimental gradients of temperature and dietary C:P, we fitted a Generalised Additive Model (GAM) to the experimental growth rate data. The model of growth rate was constructed as follows:

$$GR_{T,C:P} = f(x_T) + f(x_{C:P}) + ti(x_T, x_{C:P}) \quad (7)$$

where $f(x_T)$ and $f(x_{C:P})$ are the smooth terms for temperature and diet stoichiometry, whereas $ti(x_T, x_{C:P})$ is the tensor interaction smooth term. The model was fitted to the data using the mgcv R package (Wood, 2001) and "Restricted Maximum Likelihood" REML method.

To assess the shape of the $TER_{C:P}$ thermal reaction norm, we first estimated the $TER_{C:P}$ at each experimental temperature separately. For each temperature, we fitted the following modified Gaussian function to the growth rate versus C:P data:

$$GR_T = a + b * e^{-E * \frac{x_{C:P} - c}{d^2}} \quad (8)$$

where $x_{C:P}$ is the resources C:P ratio, a the minimum growth rate, b is the height of the curve, c is the estimated $TER_{C:P}$ (i.e. curve's optimum), d the curve breadth and E a scaling parameter. The various parameters including c ($TER_{C:P}$) were estimated by nonlinear least squares regression. The confidence intervals around the $TER_{C:P}$ were estimated by non-parametric bootstrapping (Efron & Tibshirani, 1986). At each temperature the dataset was resampled 1000 times and the nonlinear regression procedure and $TER_{C:P}$ estimation reiterated. The obtained estimates are strongly robust against the number of bootstrap iterations (Table S3) and were used to calculate the mean $TER_{C:P}$ and the 95% confidence intervals ($CI_{95\%}$). A significant difference between $TER_{C:P}$ estimates at each temperature was concluded in the absence of overlap between their $CI_{95\%}$.

Finally, to determine whether the $TER_{C:P}$ reaction norm has a true U-shape we used the two-line test (Simonsohn, 2018) on the bootstrapped data. Briefly, the two-line test is used to statistically confirm or infirm a U-shape in data series. The test consists of estimating two regression lines, for “low” and “high” values of x separated at a specific breakpoint. The test confirms a U-shape if the two lines have opposite and individually significant slopes. All analyses were performed using the software R v.3.6.1 (R-Core Team 2019) with the alpha error set at 0.05.

RESULTS

Model

When parameterised for *Daphnia* (Table 1), the model predicts that the $TER_{C:P}$ thermal reaction norm is U-shaped, decreasing between 18 and 22 °C and then increasing up to 28 °C (Fig. 1, dashed line). The $TER_{C:P}$ is thus minimised at 22 °C which constitutes the threshold minimising temperature (TMT). Among the model parameters, b , which controls the concavity of the C ingestion rate thermal reaction norm (I_C) has a strong effect on the convexity of $TER_{C:P}$ thermal response (Fig. 1a). Similarly, the temperature scaling exponent of respiration (R_c), d , has a strong effect on the $TER_{C:P}$ (Fig. 1b). The lowest d value used here (0.01), which corresponds to a $Q_{10} = 1.2$ for R_c , yields a nearly flat $TER_{C:P}$ reaction. Increasing the temperature scaling of R_c up to a $Q_{10} = 2$ (parameter $d = 0.07$) strongly increases the convexity of the $TER_{C:P}$ thermal reaction norm and shifts the minimal value of $TER_{C:P}$ toward higher C:P ratios. The level of C assimilation efficiency (AE_C) also constrains both the level and the convexity of the $TER_{C:P}$ thermal reaction norm. Reducing AE_C from 0.7 to 0.3 strongly increases the level and convexity of the reaction norm (Fig. 1c). The assimilation efficiency of P (AE_P) also modulates the thermal reaction of $TER_{C:P}$ in a lesser extent. Increasing AE_P values from 0.6 to 1 only shifts $TER_{C:P}$ thermal reaction norm to higher C:P values without altering its convexity (Fig. 1d). The scaling of consumer body composition ($Q_{C:P}$) with temperature (defined by parameter r) only affects $TER_{C:P}$ at higher temperatures. Increasing $Q_{C:P}$ with temperature (parameter $r = 4$) leads to a rapid increase in $TER_{C:P}$ at higher temperatures. Oppositely, decreasing $Q_{C:P}$ at high temperature ($r = -4$) tends to limit $TER_{C:P}$ to lower values, slightly reducing the convexity of its thermal response without flattening the U-shape (Fig. 1e).

The sensitivity analyses of the model show that across a large number ($n = 1000$) of random parameter combinations (selected within biologically relevant ranges, see Table 1), the U-shape $TER_{C:P}$ thermal reaction norm is predominant with a frequency of occurrence of 77.6% (Fig. 2). More precisely, softly convex U-shapes occur in 26.2% of observations and strongly convex U-shapes in 51.4%. The remainder of simulation outcomes are monotonic increases (13.4%) or decreases (9%) of $TER_{C:P}$ with temperature. These monotonic thermal reactions of the $TER_{C:P}$ only emerge when I_C thermal response is the less concave or when R_C thermal reaction is flattened. In such situations, it is the thermal reaction norm of $Q_{C:P}$ that dictates the direction of the $TER_{C:P}$ thermal response (Fig. S4).

Experiments

The surface response of daphnids growth across the factorial combination of dietary treatment and temperature is given in Fig. 3. The GAM used to generate the surface fit well with our data explaining 97.9% of total variance (Figs S5 and S6). The GAM also indicates a significant effect of the temperature and dietary C:P interaction on *D. magna* growth ($n = 72$; $F = 15.9$; $P < 0.001$). The response of growth to temperature is typically unimodal for diet C:P ratios ranging from 95 to 331, with an optimum ranging from 0.36 at 22 °C to 0.39 at 24 °C, respectively (Fig. 3, Fig. S4). For C:P ratio above 500, the response of growth appears to be almost flat. Growth rate responded unimodally to diet at all temperatures (Fig. 3, Fig. S4) but the $TER_{C:P}$ (i.e. the peak of the unimodal response) changed across temperatures forming a U-shape with a minimum value at 22 °C. The $TER_{C:P}$ decreased from 338.4 (CI_{95%}[326.9, 349.9]) at 18 °C down to 193.4 (CI_{95%}[176.6, 210.2]) at 22 °C. The latter can be considered as our *Daphnia* clone's TMT as it represents the lowest $TER_{C:P}$ observed in our thermal range. Further temperature increases above this TMT lead to an increase in the $TER_{C:P}$ up to 414 (CI_{95%} [370.8, 457.2]) at 28 °C (Fig. 3). The two-line test of Simonsohn (2018) confirmed that $TER_{C:P}$ significantly decreases between 18 and 22 °C ($P < 0.0001$), reaches a breakpoint at 22 °C after which $TER_{C:P}$ significantly increases up to 28 °C ($P < 0.0001$) (Fig. 3), thus generating a significant U-shape.

DISCUSSION

Both our model and growth experiments showed that the $TER_{C:P}$ of *D. magna* responds non-monotonically to temperature generating a “U-shaped” thermal reaction norm. The $TER_{C:P}$ decreases between 18 and 22 °C indicating a rising demand in P with rising temperature. At 22 °C, the $TER_{C:P}$ reaches its

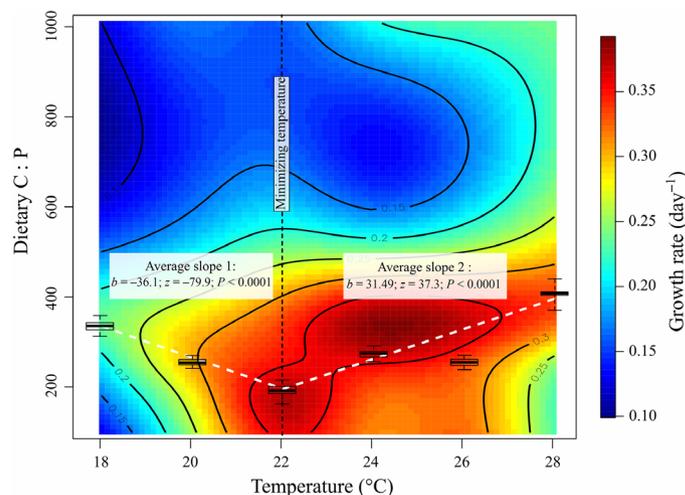


Figure 3 Generalised Additive Model (GAM; $n = 72$, see also Fig. S3) of *D. magna* growth rate exposed to a factorial combination of dietary molar C:P ratio and temperature. The boxplots at each temperature are the bootstrapped $TER_{C:P}$ mean values and confidence intervals ($n = 1000$). The shape of $TER_{C:P}$ thermal reaction norm (white line) has been assessed with the two-line test (Simonsohn, 2018). Decreasing and increasing sections are both significant ($P < 0.0001$)

minimal value, the Threshold Minimising Temperature (TMT) where the dietary C requirements relative to those of P are minimised. Above the TMT, and at least up to 28 °C, the $TER_{C:P}$ increases again indicating a rising demand in dietary C. Consequently, for the same *D. magna* clone, both temperature-driven decrease and increase in the TER are possible, depending on the temperature range within which observations are performed. Provided that this $TER_{C:P}$ U-shaped reaction norm can be generalised across consumers, our results suggest that previous apparent contradictory predictions of ectotherm consumers requirements in a warming context (Persson *et al.*, 2011; Wojewodzic *et al.*, 2011; Boersma *et al.*, 2016) could be reconciled within a single framework.

Our model differs from those based on MTE that often assume that consumption is a monotonous function of temperature that has the same or higher Q_{10} than metabolism (Anderson *et al.*, 2017). Such assumptions can only be valid within the lower part of consumer's thermal tolerance range. All else kept equal in our model, such assumptions will yield a monotonously decreasing $TER_{C:P}$ thermal reaction norm. On the other hand, focusing only on the upper part of the consumers' thermal tolerance range where metabolism increases faster while consumption decreases will yield a monotonously increasing $TER_{C:P}$ thermal reaction norms.

However, understanding ectotherm consumers' responses to climate change requires considering their full thermal tolerance range (Kingsolver & Buckley, 2017). Within this range, C ingestion (I_c) and respiration rates (R_c) respond differently to temperature, both in terms of shape and scaling. It is these differential responses of I_c and R_c that generate the U-shaped TER reaction norm in our model. In agreement with empirical data on *Daphnia* (McMahon, 1969; Kibby, 1971; Yurista, 1999) and numerous other ectotherms (Dell *et al.*, 2011; Lemoine & Burkepile, 2012), we assume that within the full thermal tolerance range, the reaction norms of I_c and R_c are unimodal and exponential respectively. At the lower temperature range of the modelled I_c reaction norm, ingestion rate rises faster than metabolic rate does (Yurista, 1999; Lemoine & Burkepile, 2012). Under these circumstances and given a constant C assimilation efficiency (AE_C), higher temperatures increase C gross growth efficiency (GGE_C ; eqn 2). The biological interpretation is that at higher body temperatures, the consumer "perceives" the food as being richer in C relative to P. This manifests as a decrease in the $TER_{C:P}$ with temperature until it reaches the TMT. In the model, the TMT indicates the tipping point where R_c begins to rise faster than I_c . From this point, the GGE_C starts decreasing with temperature and the consumer "perceives" the food as being increasingly "poorer" in C relative to P as temperature rises. Consequently, above the TMT, the $TER_{C:P}$ rises with temperature. Hence, when considering a sufficiently wide thermal tolerance range, the $TER_{C:P}$ exhibits a U-shaped response to temperature.

The ingestion and respiration rates might not be the only model components affected by temperature. Although much less consensual, there is evidence that the assimilation efficiencies for C and P (AE_C and AE_P) as well as the consumer's body stoichiometry ($Q_{C:P}$) may also respond to temperature (Bobka *et al.*, 1981; Tsai *et al.*, 2008; Prater *et al.*, 2018). Depending on whether they increase or decrease with temperature, strong thermal responses could either "flatten" the U-shape, or one of

its sides thereby yielding a quasi-monotonous $TER_{C:P}$ thermal reaction norms. For example, unlike our *D. magna* clone, Prater *et al.* (2018) found that the body stoichiometry of *D. magna* in two lakes tends to decrease with increasing temperature. In the model, such a decrease tends to flatten the rising part of the $TER_{C:P}$ reaction norm. However, the range of $Q_{C:P}$ in Prater *et al.* (2018) (from 100 at 15 °C to 65 at 25°C) is not enough to cause a monotonous decrease in $TER_{C:P}$ with temperature in the model (Fig. 1e).

More generally, based on the model parameter ranges from literature, the simulation shows that 77.6% of random parameter combinations result in a U-shaped $TER_{C:P}$ thermal reaction norm with 51.4% producing strongly convex U-shapes. This result suggests that U-shaped TER thermal reaction norms might be quite common among ectotherm consumers. Obviously this is a rough estimate and it needs to be considered with caution. First, given the scarcity of data in literature, we assumed a uniform distribution for all parameter values. Improving the estimates of parameters distribution may potentially lead to different predictions but this is beyond the scope of this study. Second, parameter combinations in our simulation are random, and some of them might be biologically aberrant. For example, we could expect that in nature, higher ingestion rates are systematically associated with higher respiration rates in agreement with the increased intake hypothesis (Burton *et al.*, 2011) and lower $Q_{C:P}$ in agreement with growth rate hypothesis (Elser *et al.*, 2003). Keeping only the biologically relevant combinations could alter the predicted frequency of occurrence of a U-shaped $TER_{C:P}$.

A fundamental challenge in experimental ecology is to capture nonlinearities of ecological responses to environmental drivers. Our results underscore the importance of carefully selecting the temperature range and increments in any experimental assessment of temperature-dietary stoichiometry interaction on ectotherm consumers. In the case of strongly convex $TER_{C:P}$ responses, large temperature increments (> 4 °C) might miss the U-shape and thus yield misleading conclusions. From an ecological perspective, strongly convex $TER_{C:P}$ responses imply that small changes in temperature can drastically change consumer nutritional requirements. A diet of a given stoichiometry can shift towards one or the other side of $TER_{C:P}$ within a range of few degrees Celsius. In agreement with the "knife-edge" theory (Elser *et al.*, 2016), our experimental data show that both sides of the $TER_{C:P}$ imply physiological costs which manifest as decrease in growth rate. Hence, consumers with strongly convex $TER_{C:P}$ responses to temperature might be more susceptible to thermally induced changes in their dietary preferences (Boersma *et al.*, 2016). Following the same reasoning, such consumers might also be more prone to behavioural thermoregulation to optimise nutritional outcomes (Coggan *et al.*, 2011; Clissold *et al.*, 2013). Furthermore, the U-shape of the $TER_{C:P}$ thermal reaction norm implies that, for a given resource C:P, climate change-induced nutritional limitations could differ at the borders of the species geographical range. At the lower latitudinal border where the species is at the "warmer" part of its thermal breadth, an increase in environmental temperature could drive it towards a limitation by C. On the other hand, the same temperature increase could lead to P limitation at the

higher latitudinal border where the species is at the “colder” part of its thermal breadth.

In the model, the U-shape of the $TER_{C:P}$ is strongly influenced by the shape of the I_c thermal reaction norm. A wide I_c thermal reaction norm breadth (*thermal generalist*) will lead to a “flat” $TER_{C:P}$ U-shape. From this point on, one can explore possible links between thermal and stoichiometric traits. For example, thermal biology tells us that in some cases the wide thermal breadth of a trait (here, I_c) could trade-off with a low maximum performance (i.e. *specialist–generalist trade-off* (Angilletta, 2006)). All else kept equal in model, a low maximum I_c implies a low maximum consumer growth rate. On the other hand, reaching a high I_c peak performance and consequently a higher maximum growth rate could come at the cost of a narrow thermal performance breadth (*thermal specialist*) and a strongly convex $TER_{C:P}$ reaching lower values (Fig. 4). Hence, the higher growth rate potential of a thermal specialist (reached at the TMT) has also a lower $TER_{C:P}$ than that of the generalist. Biologically, this means that the specialist may require relatively more P to reach its full growth

potential as compared with the thermal generalist. It is interesting to note how this prediction connecting high growth rate potential to higher P demand resembles that of the growth rate hypothesis (GRH-(Elser *et al.*, 2003)). The GRH is based on the observation that taxa with high growth rate potential have lower body C:P ratios. Here, we show that this positive relationship between growth rate potential and P requirements persists even when assuming a constant body C:P ratio. Another common pattern found in thermal biology is the “hotter is better” continuum (Angilletta *et al.*, 2010)) in which warm-acclimated organisms reach higher maximal performances (here I_c) than cold-acclimated ones. Applying this into our $TER_{C:P}$ model also yields a prediction that reminds the GRH: warm-acclimated organisms achieve higher peak I_c and growth rates at lower $TER_{C:P}$ than cold-adapted ones (Fig. 4).

Another influential model component is AE_C . A low AE_C shifts the $TER_{C:P}$ towards higher C:P ratios and increases the convexity of the U-shaped response to temperature. Biologically, AE_C reflects the digestibility of ingested C. Detritivores or

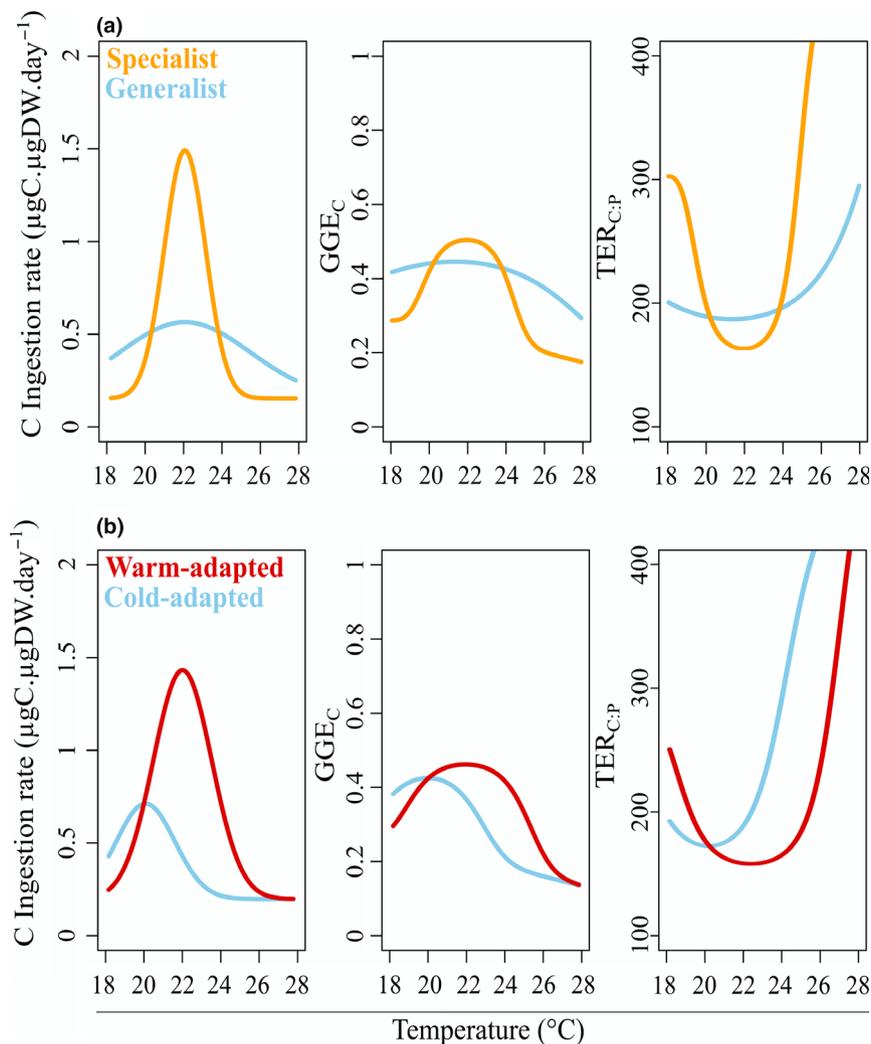


Figure 4 Comparison of hypothetical GGE_C and $TER_{C:P}$ thermal reaction norms of: (a) thermal specialist (orange lines) versus thermal generalist (blue lines) consumers and (b) cold-acclimated (blue line) versus warm-acclimated (red line) consumers. Changes are induced by modifying parameters b , $\Delta I_{C_{MAX}}$ and T_{OPT} (see Table 1) while keeping all other parameters constant at the values for *D. magna*. See text for discussion.

terrestrial herbivores feeding on diets rich in refractory carbon should have lower AE_C than aquatic herbivores consuming microalgae. Hence, when considering only AE_C , it is tempting to speculate that detritivores and terrestrial herbivores might be more susceptible to thermally induced dietary preference shifts or dietary-induced behavioural thermoregulation than aquatic herbivores because of their more convex $TER_{C:P}$ U-shape. However, further studies are necessary to determine how the other model components vary among feeding guilds of terrestrial and aquatic ectotherms and whether they can compensate predicted effects of AE_C on the $TER_{C:P}$ U-shape.

In conclusion, we demonstrate that in *D. magna* the $TER_{C:P}$ responds to temperature in a U-shaped fashion. Our model simulations suggest that such a U-shaped reaction norm might be common among ectotherm consumers thereby offering a potential common explanation to previous contradictory results indicating either increases or decreases in consumer P demand at higher temperatures. However, the shape of thermal responses can be highly variable among traits and species (Dell *et al.*, 2011) and the generality of the U-shaped TER remains to be tested for other species having different thermal response shapes and positions than the one provided in Table 1. Caution is also required when transposing our finding to other nutrients. The present formulation of $TER_{C:P}$ assumes negligible P metabolic losses (Frost *et al.*, 2006). This does not hold for N for which nitrogenous metabolic waste excretion represents an important fraction of the nutrient budget. Temperature accelerates metabolism and N excretion with unknown consequences on the $TER_{C:N}$ thermal reaction norm (Anderson *et al.*, 2017). Finally, body size will also differentially affect the different model components and thus potentially the shape of the $TER_{C:P}$ response. Including the allometric size scaling in the model is clearly a promising perspective. Despite these potential caveats, our study unveils novel potential links between variation in thermal and stoichiometric traits and contributes to the on-going unification of thermal ecology and ecological stoichiometry (Hillebrand *et al.*, 2009; Cross *et al.*, 2015; Rosenblatt & Schmitz, 2016).

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AUTHOR CONTRIBUTIONS

AB, AMK, MD and TR conceived the idea and developed the experimental protocol. TR ran the experiment and acquired the data. AMK and TR developed the model and statistical analysis. TR, AB and AMK led the writing of the manuscript. All authors contributed critically to the draft and gave the final approval for publication.

COMPETING INTERESTS

Authors declare no competing interests.

DATA AND MATERIALS AVAILABILITY

The data supporting the paper conclusions and R scripts associated are available on Figshare.

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