**Title**

Universal temperature dependence of nutritional demands in ectotherms

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**Abstract**

In light of ongoing climate change, it is increasingly important to know how nutritional requirements of ectotherms are affected by changing temperatures. Here, we analyse the wide thermal response of phosphorus (P) requirements via elemental gross growth efficiencies of Carbon (C) and P, and the Threshold Elemental Ratios in different aquatic invertebrate ectotherms such as the freshwater model species *Daphnia magna*, the marine copepod *Acartia tonsa*, the marine heterotrophic dinoflagellate *Oxyrrhis marina*, and larvae of two populations of the marine crab *Carcinus maenas*. We show that they all share a non-linear cubic thermal response of nutrient requirements. Phosphorus requirements decrease from low to intermediate temperatures, increase at higher temperatures, and decrease again when temperature is excessive. This universality in the thermal response of nutrient requirements is of great importance if we aim to understand or even predict how ectotherm communities will react to global warming and nutrient-driven eutrophication.

## Introduction

Temperature is among the most influential determinants of fitness in ectotherms, as it directly drives their metabolism (Clissold & Simpson 2015; Cross et al. 2015). Within biological relevant ranges, below the optimum temperature, ectotherm metabolic rates scale exponentially with temperature. Hence, even a small increase in environmental temperature may lead to strong changes in performance (Gillooly et al. 2001; Brown et al. 2004). Furthermore, to ensure the achievement of the Darwinian functions of survival and reproduction, all consumers must obtain a diet containing the appropriate balance of biochemical nutrients (Sterner & Elser 2002; Simpson & Raubenheimer 2012) including essential fatty acids, amino acids, and vitamins. On a more basic level, specific chemical elements, such as nitrogen (N) and phosphorus (P) are also essential components of the food (Sterner & Elser 2002), as they cannot be replaced by other elements or generated *de novo*. A tight connection between metabolism and food nutritional quality was recently shown by Ruiz et al. (2021), who reported increased resting metabolic rates in the freshwater cladoceran *Daphnia* facing food quality constraints (poly- and highly unsaturated fatty acids, and P limitation). Given the over-riding importance of temperature on metabolic rates of ectotherms, understanding the interactions of temperature and nutritional demands is critical, especially in the light of global change processes (Cross et al. 2015). The most straightforward way to assess temperature – food quality interactions is to investigate temperature effects on the Threshold Elemental Ratio (TER) (Urabe & Watanabe 1992; Sterner 1997; Frost et al. 2006). The $TER_{C:X}$ is the carbon:nutrient (C:X) ratio in the food that exactly matches the current physiological requirements for metabolism and growth of the consumer, with neither C nor X being limiting or in excess. The TER is a quantitative tool that integrates multiple responses of organism biochemistry and physiology. A low $TER_{C:X}$ is indicative of higher needs for the nutrient X relative to C, while a higher $TER_{C:X}$ means that consumers have a relatively low nutrient demand. Cross et al. (2015) noted that there were very few studies of how temperature affects the TER, and made a strong case to measure the $TER_{C:X}$ at different temperatures.

Existing studies on this topic are, unfortunately, rather equivocal, with reports of increasing, decreasing, and constant TER with increasing temperature (Persson et al. 2011; Wojewodzic et al. 2011; Boersma et al. 2016; Malzahn et al. 2016; Anderson et al. 2017; Ruiz et al. 2020). The most

parsimonious explanation for these findings is that the response of the nutritional demands to temperature is not monotonic and that the studies conducted so far have not covered a sufficiently broad temperature range. Indeed, based on theoretical considerations and experimental data, Ruiz et al. (2020) predict a U-shaped response of the $TER_{C:P}$ with temperature. Hence, the question remains whether there is a common response of ectotherm nutrient requirements to changing temperatures that would help in making predictions about secondary production responses to global change or whether the diversity of findings to date reflects true idiosyncrasies in temperature responses among taxa, consequently preventing general overarching predictions.

In this study, we aim to shed light on the interactions between macronutrient requirements of ectotherms and temperature. The diversity of experimental conditions (e.g., different species, temperature, and food quality ranges) (Wojewodzic et al. 2011; Malzahn et al. 2016; Ruiz et al. 2020; Starke et al. 2020), in combination with limited data on the temperature dependencies of the parameters used to model TERs (Anderson et al. 2017; Ruiz et al. 2020), have so far prevented a synthetic view on how temperature affects nutritional demands of ectotherms. To overcome these limitations, we experimentally determined the response of the $TER_{C:P}$ to temperature for different aquatic invertebrates. We used two different clones of the freshwater cladoceran *Daphnia magna* with different thermal histories to determine the combination of temperature and diet C:P that maximizes growth. Further, we characterized the thermal response of fundamental parameters of the basic TER model (Frost et al. 2006; Doi et al. 2010) across ecologically relevant temperature ranges, using the marine copepod *Acartia tonsa*, the marine heterotrophic dinoflagellate *Oxyrrhis marina*, and larvae of two populations of the marine crab *Carcinus maenas*.

## Material and methods

We carried out two sets of experiments. First, we determined the thermal response of the $TER_{C:P}$ from growth rates as a function of temperature and food C:P, for two clones of *Daphnia magna*. The $TER_{C:P}$ is obtained from the diet C:P which maximizes growth, and this is done at different temperatures. Second, we determined the thermal response of body C and P contents and of ingestion (IR), respiration (RR), and growth (GR) rates, to obtain the thermal response of the gross growth

efficiency of C and P ($GGE_C$ and $GGE_P$) and of the $TER_{C:P}$ model proposed by (Frost et al. 2006; Doi et al. 2010), for the copepod *Acartia tonsa*, the dinoflagellate *Oxyrrhis marina*, and larvae of two different populations of the crab *Carcinus maenas*. With this second set of experiments we obtained information on how nutrient requirements of the studied organisms change with temperature, as well as the thermal response of all underlying variables that determine the TER.

1- **Thermal response of $TER_{C:P}$ from growth as a function of temperature and food C:P.** We determined the diet C:P which maximizes growth ($TER_{C:P}$) at different temperatures for two clones of *D. magna*.

*Culturing conditions.*

The first experiment was carried out at Arizona State University, USA, with a clone of *D. magna* (Clone US) that was maintained in the laboratory in COMBO media (Kilham et al. 1998) on a diet of the green alga *Scenedesmus acutus* (C:P~120) at room temperature (24°C). The experiment was carried out at 18, 23, and 28°C in a food quality gradient consisting of food with five C:P ratios ranging from 51 to 816. The second experiment was carried out at Universidad Nacional del Comahue, Argentina, with a clone of *D. magna* (Clone AR) that had been maintained in the laboratory in COMBO media (Kilham et al. 1998) on a diet of the green algae *Chlamydomonas reinhardtii* (C:P~150) at 20°C. The experiment was carried out at 15, 20, and 24°C in a food quality gradient consisting of food with five C:P ratios ranging from 40 to 746.

*Experimental design*.

Both experiments lasted for 3 days. To exclude the influence of indirect effects of P limitation on the nutritional quality of the algae, the gradients in food quality (C:P) were created with a short-term P-spiking technique following Rothhaupt (1995) and Plath and Boersma (2001) (See Appendix S1 in Supporting Information for details). The C:P ratios of the algal cultures were routinely determined by daily filtration through acid-washed precombusted (450°C, 2 h) GF/F Whatman filters and analyzed for particulate C using a Thermo Finnigan EA 1112 CN elemental analyzer (Thermo Scientific, Milano, Italy) and for particulate P via digestion with persulfate at 1.5 atm for 1 h, followed by a molybdate reaction (APHA 2005).

153 For both experiments, we placed one 24-h-old individual of *D. magna* per 30 mL beaker with 11
154 replicates in a factorial design of 3 temperatures and 5 food qualities. Food and media were replaced
155 daily at a concentration of 1.5 mg C $L^{-1}$. We determined the growth rate of each individual as the
156 difference in the natural logarithm of the lateral area (obtained with photographs and image analysis)
157 at the end and the beginning of the experiment divided by the time in days. In some cases, one or two
158 replicates were missing at the end of the experiment due to mortality during the daily handling of the
159 animals.

160 *Data analyses*.

161 The shape of the $TER_{C:P}$ thermal reaction norm was obtained following Ruiz et al. (2020), fitting
162 the following modified Gaussian function to the individual growth rate versus the food C:P data for
163 each temperature:

164 Eqn. 1

165 $$GR = a + b * e^{-E*\frac{C:P-c^2}{d^2}}$$

166 where *C:P* is the food C:P ratio, *a* is the minimum growth rate, *b* is the height of the curve, *c* is
167 the estimated $TER_{C:P}$ (i.e. curve's maximum), *d* is the curve breadth and *E* is a scaling parameter. All
168 the parameters were estimated by nonlinear least squares regression (Baty et al. 2015). The confidence
169 intervals around the $TER_{C:P}$ were estimated by nonparametric bootstrapping (Efron & Tibshirani
170 1986). At each temperature the dataset was resampled 1000 times and the nonlinear regression
171 procedure and $TER_{C:P}$ estimation were reiterated. This was used to calculate the mean $TER_{C:P}$ and the
172 95% confidence intervals ($CI_{95\%}$) for each temperature. A significant difference between $TER_{C:P}$
173 estimates at each temperature was inferred in the absence of overlap between their $CI_{95\%}$.

174 **2- Thermal response of $TER_{C:P}$ model and underlying variables.**

175 *Culturing conditions.*

176 These experiments were carried out at the Biologische Anstalt Helgoland, Alfred-Wegener-
177 Institut, Helgoland, Germany. *O. marina* was obtained from the Göttingen culture collection (Strain
178 B21.89), and the stock culture used to inoculate the experimental containers was grown in batch
179 cultures at 18°C in the dark. Eggs of *A. tonsa* were obtained from a permanent laboratory culture at

18°C, and incubated in filtered seawater for hatching. Since hatching peaks between 24 h and 36 h of incubation, we collected the nauplii hatched only during this period to minimize age differences between individuals. Berried females of *C. maenas* were collected manually at the intertidal area of the bay of Cadiz (Spain), and Helgoland (Germany), during the reproductive periods. Animals were transported to the Biologische Anstalt Helgoland at AWI (Germany) in individual containers with seawater and constant temperature (15.7 and 12.5 °C for Cadiz and Helgoland, respectively). In order to ensure a successful embryonic development, and to reflect the temperature of the natural habitat at the time of hatching in summer, females were maintained in individual aquaria where temperature was gradually increased (0.2°C per day) until 18°C, when hatching occurred. We used four hatches from different females from the Cadiz population and three from Helgoland to account for maternal effects on the results (Torres et al. 2020), and each of them was considered as a replicate.

*Experimental design*.

Individuals of *O. marina*, or recently hatched *A. tonsa* (nauplii) and *C. maenas* (Zoea I) were placed in beakers with filtered seawater (0.2µm) and food *ad libitum*. *A. tonsa* and *O. marina* were fed *Rhodomonas salina*, and *C. maenas* was fed with *Artemia salina. A. tonsa* and *C. maenas* were distributed in temperature-controlled rooms, and *O. marina* beakers were placed in a gradient temperature table following Malzahn et al. (2016). Experiments with *A. tonsa* and *O. marina* were carried out with a photoperiod of 16 h light – 8 h dark and experiments with *C. maenas* with a photoperiod of 12 h light – 12 h dark. Media and food were replaced daily. The length of the experiment varied according to each species (for specific details on the experimental design see Appendix S2 Table 1).

*Carbon and phosphorus Analyses*.

At the beginning and the end of the experiments we determined C and P contents for individuals of *A. tonsa* and *C. maenas* and C and P contents per beaker of *O. marina*. C and P contents of *O. marina* and *R. salina* (food of *A. tonsa* and *O. marina*) were measured by filtering a known amount through acid-washed precombusted (450°C, 2 h) Whatman GF/F filters. Analyses for *Acartia tonsa* and *Artemia salina* (food of *C. maenas*) were carried out by placing a known number of individuals on GF/F filters and for *C. maenas* by placing individuals directly into pre-weighed tin capsules for C

analysis or in 1.5 mL Eppendorf tubes for P analysis, following by re-weighing before analysis. In all cases C was analyzed with a Vario MICRO cube CHNS analyzer (Elementar Analysensysteme), and P as orthophosphate after acidic oxidative hydrolysis with 5% $H_2SO_4$ (Grasshoff et al. 2009). Using these data we calculated initial and final body C:P ratios and growth rate in terms of C and P for each species (*GR*, see calculations).

*Ingestion (IR) and Respiration (RR).*

At the end of the growth period, we determined ingestion rates in terms of C and P, and respiration rates of *A. tonsa*, *O. marina*, and both populations of *C. maenas*. To determine ingestion rates, organisms were placed in beakers with filtered seawater and a known amount and quality (C:P) of food. Consumers were removed after the feeding period, and the remaining food was quantified. The algal cells were counted with a CASY particle counter (SCHARFE SYSTEMS, Reutlingen, Germany) and *A. salina* were counted with a stereomicroscope. For *O. marina* and *A. tonsa* the feeding rate was determined by the number of algae remaining after the feeding period relative to a control with algae but without consumers (details for each species in Appendix S2 Table 2).

Respiration rates were determined by placing individuals in 4 mL glass vials in filtered seawater (0.2 µm) without food in a SDR SensorDish® 24- channel Reader (PreSens, Regensburg, Germany) in the dark, in temperature-controlled rooms set to the acclimation temperature. The system was calibrated at each temperature with filtered seawater at 100% and 0% air saturation following the manufacturer's protocol. Vials without organisms (n= 6) were used as a control to account for microbial oxygen consumption in seawater. To avoid oxygen stratification within the vials, reader and vials were placed on a rocking platform shaker (IKA Rocker 2D digital, Staufen, Germany) at 80 rpm. The oxygen concentration was recorded every 15 seconds in $mg \cdot L^{-1}$. Oxygen levels during measurement were monitored closely to avoid suboptimal levels (< 80% air saturation) inside the chambers. Oxygen consumption was determined by a linear regression of the change in $O_2$ concentration data plotted against time, and standardized to µgC (see details for each species in Appendix S2 Table 3).

*Calculations.*

We determined the $TER_{C:P}$ as a function of temperature for *A. tonsa*, *O. marina*, and *C. maenas* using the model of Frost et al. (2006) modified by Doi et al. (2010) (Eqn. 2).

Eqn 2.

$$TER_{C:P} = \frac{GGE_P}{GGE_C} \times \frac{Q_C}{Q_P}$$

Where $Q_C$ and $Q_P$ are the body C and P contents, $GGE_P$ and $GGE_C$ are the gross growth efficiencies of P and C calculated following Doi et al. (2010) as the ratio of the growth rate of P or C to the ingestion rate of P or C (for $GGE_P$ and $GGE_C$, respectively):

Eqn 3.

$$GGE = \frac{GR}{IR}$$

Where *GR* is the amount of C or P fixed as new biomass in a certain period, it is calculated as the difference in C or P content (per individual for *A. tonsa* and *C. maenas*, and per beaker for *O. marina*) at the end and the beginning of the experiment over time in days, and *IR* is the ingestion rate in terms of C or P in the same period.

*Data analyses.*

One-way analysis of variance (ANOVA) was performed to compare the organisms' C:P ratios, respiration, growth, and ingestion rates, GGE, and $TER_{C:P}$ values with temperature as a factor, followed by Holm-Sidak post hoc tests. To meet the assumption of normality for the ANOVA, the C:P ratio of *A. tonsa* was log transformed prior to the analysis. When assumptions were not met by transforming data, Kruskal-Wallis ANOVA on ranks was used. The temperature where the $TER_{C:P}$ is the highest ($MaxTER_{C:P}$ temperature) and its $CI_{95\%}$ for *A. tonsa*, *O. marina* and *C. maenas* were obtained through nonlinear regression fit of the thermal response of the $TER_{C:P}$ to a Gaussian function, with the R package nlstools (Baty et al. 2015). All analyses were performed using the software R v.4.1.0 (R-Core-Team 2021).

**Results**

**1- Thermal response of $TER_{C:P}$ from growth as a function of temperature and food C:P.**

Growth rates of both clones of *D. magna* showed a hump-shaped response to the food C:P gradient fit to the modified Gaussian equation (Eqn. 1) at all temperatures (Fig. 1 a and b), except for *D. magna* (US) at 15 °C (lowest temperature), which showed a nearly flat response of growth rates to changing food quality (Fig. 1 a). The $TER_{C:P}$, that is the food C:P that maximizes growth at each temperature (maximum of the gaussian function, except for *D. magna* (US) at 15°C), showed a hump-shaped relation with temperature. The maximum $TER_{C:P}$ was found at intermediate temperatures for both clones (Fig. 1 c and d), with decreasing $TER_{C:P}$ at low and high temperatures (Fig. 1 c and d).

**2- Thermal response of TER model and underlying variables.**

*Ingestion, respiration, and growth rates in the thermal gradient.*

Ingestion and respiration rates showed the same response to temperature within species, but were not consistent between them (Fig. 2 a-d and g-j). Ingestion and respiration rates showed a hump-shaped response to temperature for *A. tonsa* and *O. marina*, with maximum values in the range of 18-21°C for *A. tonsa*, and at 18°C for *O. marina* (Fig. 2 a-d). In contrast, both populations of *C. maenas* presented a consistent increase in ingestion and respiration rates with temperature in the thermal range of the analyses (Fig. 2 g-j). Growth rates had an increasing trend in the thermal gradient for all species (Fig. 2 e, f, k, l). However, growth rates of both populations of *C. maenas* seemed to reach a maximum growth at around 21°C (Fig. 2 k, l).

*Body C:P in the thermal gradient.*

We found similar effects of temperature on body C:P ratio in all organisms tested (Fig. 3). C:P of *A. tonsa*, *O. marina* and *C. maenas* (Cadiz) had a U-shaped response to temperature, with minimum values at intermediate temperatures (around 18°C) (Fig. 3 a-c). C:P ratios of *C. maenas* from Helgoland showed the same U-shaped response to temperature as the other organisms but also had a second minimum in the highest temperatures resulting in an "inverse N-response" of body C:P to temperature (Fig. 3 d).

*Gross growth efficiency of C and P ($GGE_C$ and $GGE_P$) in the thermal gradient.*

The thermal response of gross growth efficiency of carbon presented some differences between species (Fig. 4 a-d). $GGE_C$ in *A. tonsa* increased at the highest temperature (Fig. 4 a), had a U-shaped

response to temperature in *O. marina* (Fig. 4 b), and had no variation with temperature for both *C. maenas* populations (Fig. 4 c and d). On the other hand, $GGE_P$ showed larger responses to temperature with patterns that differed among the study taxa (Fig. 4 e-h).

*Nutrient requirements as a function of temperature ($TER_{C:P}$ model).*

Using the $TER_{C:P}$ model approach, we obtained a hump-shaped response of the $TER_{C:P}$ to temperature for *A. tonsa*, *O. marina*, and *C. maenas* Cadiz (Fig. 5 a-c), with a maximum $TER_{C:P}$ ($MaxTER_{C:P}$) at intermediate temperatures. This $MaxTER_{C:P}$ is indicative of the organism's lowest P-requirements. The unimodal shape of the $TER_{C:P}$ relationship indicates that at both lower and higher temperatures animals need food with higher P content relative to C to grow maximally. In the case of *C. maenas* Helgoland, we obtained an N-shaped response of the $TER_{C:P}$ as a function of temperature (Fig. 5 d). The lower temperatures of the gradient (12-18 °C) resulted in a hump-shaped response for this population similar to the other taxa, while in the warmer temperatures (18-24 °C) formed a U-shaped thermal response of the $TER_{C:P}$ (Fig. 5 d).

## Discussion

In the recent literature, significant effort has been devoted to understanding the independent role of nutrients and temperature influencing ecological processes, however it still remains largely unclear how temperature and nutrients interact driving organisms' growth and metabolism (Cross et al. 2015). Here, we analyzed the nutrient requirements of four different invertebrates in a consistent way, including two clones and two populations with different thermal history and found common thermal responses in their nutrient requirements.

We found a hump-shaped $TER_{C:P}$ among all organisms, except for the Helgoland *C. maenas* population, which indicates that phosphorus (P) requirements are high at low temperatures, decrease when temperatures increase to intermediate values, and increase again when temperatures are higher. Our results may seem contradictory to the study of Ruiz et al. (2020), which reported a U-shaped $TER_{C:P}$ to temperature. It is important to note, however, that Ruiz et al. (2020) focused on temperatures that were clearly above the optimal temperatures of the animals they studied. As we aimed to study ecologically relevant temperatures, our experiments did not include these high

316 temperatures. It is possible that, above a certain temperature threshold, physiological stress increases
317 C-demands for respiratory and catabolic processes, resulting in higher TER. The observation that the
318 $TER_{C:P}$ of the Helgoland population of *C. maenas*, a colder environment than the population from
319 Cadiz, increases at the very high temperatures, linked to a strong increase in respiration (Fig. 2 j),
320 indeed suggests that this mechanism may come into play at too high temperatures. At lower
321 temperatures, which were not studied by Ruiz et al. (2020) we observed a decreasing $TER_{C:P}$.
322 Interestingly, when we extended the model of Ruiz et al. (2020) to lower temperatures (below 18°C),
323 we obtained an N-shaped TER response to temperature. In fact, exactly the shape of the response of
324 the Helgoland *C. maenas* population (Fig. 5 d), providing experimental support for the extension of
325 the model by Ruiz et al. (2020) to lower temperatures. Thus, we suggest that, when encompassing the
326 entire operating temperature range of a species, hump-shaped $TER_{C:P}$ responses as observed here can
327 be combined with the U-shape found by Ruiz et al. (2020), resulting in a cubic thermal response of the
328 $TER_{C:P}$ (as seen for *C. maenas* from Helgoland). In this response, the hump-shaped part of the function
329 encompasses cold to optimal temperatures while the U-shaped of Ruiz et al. (2020) largely pertains to
330 the range above optimal temperatures (Fig. 6).
331 In agreement with previous work (Woods et al. 2003; Kendrick & Benstead 2013; Balseiro et al.
332 2021), but in contrast to Ruiz et al. (2020), we observed that body C:P stoichiometry changes
333 significantly with temperature. Body C:P had a nonlinear U-shaped thermal response, with a minimum
334 C:P located at the $MaxTER_{C:P}$ temperature. In addition, for *C. maenas* (Helgoland) we found a second
335 body C:P minimum at the highest temperature of the range we studied. Thus, for all species tested,
336 body C:P increased above the $MaxTER_{C:P}$ temperature, while a decrease was observed only for the
337 colder population of *C. maenas* at the highest temperature (Fig. 3d). Strikingly, the response of body
338 C:P to temperature showed the opposite pattern compared to the thermal response of the $TER_{C:P}$ (Fig.
339 3 and 5). This means that, at temperatures for which P requirements are higher (low $TER_{C:P}$),
340 organisms had a lower body C:P ratio than at temperatures where P requirements are lower (high
341 $TER_{C:P}$). This complex thermal response of body C:P suggests that the influence of temperature on P
342 metabolism might play a role in the $TER_{C:P}$ thermal response, as suggested by Cross et al. (2015). It is
343 important to note that, even though body C:P changed considerably with temperature, these changes

344 are in *opposite direction* of the $TER_{C:P}$, and hence body C:P was not the driver for the observed
345 changes in TER with temperature. Hence, differential changes in the growth efficiencies of P and C
346 with temperature seem to drive the thermal response of nutrient requirements.
347 Growth efficiencies of P and C are determined by how P or C are retained after ingestion. Ruiz et
348 al. (2020) stated that the differential response of ingestion and respiration generated their U-shaped
349 TER thermal reaction norm in their model and they assume that the reaction norms of ingestion and
350 respiration are unimodal and exponential, respectively. However, we show that this response is not
351 universal. Within the thermal range we tested, ingestion rates were unimodal for *A. tonsa* and *O.*
352 *marina* but increased monotonically for both *C. maenas* populations. Respiration rates, on the other
353 hand, seemed to be exponential in both *C. maenas* populations but were unimodal in *A. tonsa* and *O.*
354 *marina*. Hence, we are not able to predict the thermal response of the $TER_{C:P}$ from only these two
355 variables. Studies analyzing carbon growth or use efficiency in thermal gradients are limited, mainly
356 carried out in microbes, and show that all types of thermal responses are possible: decreasing,
357 increasing, and no change (Hagerty et al. 2014; Ye et al. 2019; Zheng et al. 2019; Smith et al. 2021).
358 Indeed, these studies show that the thermal response of carbon use efficiency can change depending on
359 substrate quality (Steinweg et al. 2008; Öquist et al. 2016), making generalizations and predictions
360 even more difficult. In our study, *A. tonsa* and *O. marina* fed on the algae *R. salina* and their $GGE_C$
361 had a similar increasing response to temperature, while both *C. maenas* populations that fed on
362 *Artemia* had the same flat response to temperature. This possible effect of resource quality in driving
363 $GGE_C$, as suggested by Steinweg et al. (2008) and Öquist et al. (2016), deserves further exploration.
364 Understanding the thermal dependencies of nutrient GGEs may open the path towards integrating
365 metabolic theory and ecological stoichiometry. In an attempt to do so, Doi et al. (2010) compiled data
366 from the literature spanning from unicellular organisms to vertebrates, but were not able to find a
367 universal relationship between $GGE_P$ and temperature. They concluded that it is difficult to make
368 robust inferences due to limited number of assessments of $GGE_P$. As we previously stated, the ratio
369 between the GGEs of C and P shaped the thermal response of the $TER_{C:P}$, over-riding effects of
370 changes in body C:P ratio. $GGE_P$ was the main driver of this ratio for three (*A. tonsa* and both *C.*
371 *maenas*) of the four organisms in the analyses and we found that, in general, $GGE_P$ seems to be high at

372 the $MaxTER_{C:P}$ temperature. We still do not know whether there is a universal pattern for the thermal
373 dependence of GGEs. However, it is clear that the nonlinear thermal responses of $GGE_C$ and $GGE_P$
374 result in a complex nonlinear thermal response of nutrient requirements in different organisms.
375 Nevertheless, $GGE_P$ seems to be the main driver of the thermal response of $TER_{C:P}$.
376 We propose that the $MaxTER_{C:P}$ observed for all species at intermediate temperatures reflects the
377 lower P requirements at temperatures that are close to the species' thermal environment in nature
378 (Schulte et al. 2011) (Fig. 6), a response that would optimize P metabolism given that this element is
379 often limiting in aquatic environments. McFeeters and Frost (2011), observed that the negative effect
380 of low P-food on *D. magna* was weakest at intermediate temperatures, which is a good indication that
381 P is used most efficiently at those temperatures.
382 Our results imply that the sensitivity of ectotherms to nutrient limitation increases at low and high
383 temperatures. Apart from globally increasing temperature, altered nutrient availability is one of the
384 greatest anthropogenic disturbances of our aquatic ecosystems (Bindoff et al. 2019). Thus, for
385 organisms living at temperatures around their $MaxTER_{C:P}$ temperature, warming would increase their
386 sensitivity to nutrient limitation (or C excess). Although the temperature range we used in our
387 experiments is much wider than is expected to be produced by global warming, we show that,
388 depending on where the thermal environment sits relative to the $MaxTER_{C:P}$ temperature and the
389 direction of change in nutrient availability, even relatively small predicted temperature increases can
390 have strong effects on consumer performance. However, the effects of these temperature changes
391 might be counteracted if changes in nutrient availability move in the same direction as the thermal
392 response of nutrient requirements, i.e. further eutrophication of waters would increase P-availability,
393 and at the same time warming would increase organism P-requirements. Based on our results, we
394 predict that nutrient reductions that are ongoing in many water bodies globally may ironically
395 exacerbate the impacts of warming temperatures. Hence, we propose that an accurate mechanistic
396 understanding of the complex relationship between temperature and stoichiometric requirements of
397 consumers is essential if we are to predict how ectotherms will respond to ongoing changes in nutrient
398 supplies and environmental temperature.
399

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**Figure legends:**

**Figure 1:** (a) and (b) Growth rate as a function of food C:P ratio in the thermal gradient for clone US (a) and clone AR (b) of *Daphnia magna*. Dots are individual data, and the lines are the best fit to the Gaussian Function (Eq. 1) estimated by nonlinear least squares regression, the maximum of the gaussian function is the C:P threshold elemental ratio ($TER_{C:P}$). (c) and (d) $TER_{C:P}$ as a function of temperature and the 95% confidence intervals ($CI_{95\%}$) estimated by nonparametric bootstrapping (n=1000) for clone US and clone AR respectively. Lower-case letters inside the graphs indicate homogeneous groups according to overlapping $CI_{95\%.}$ Lines and dots colours represent different temperatures (see reference in figure).

**Figure 2:** Thermal response of ingestion rate (a, b, g, h), respiration rate (c, d, i, j), and growth rate (e, f, k, l), for *Acartia tonsa* (a, c , e), *Oxhirris marina* (d, c, d), and both populations of *Carcinus maenas* (g-l). Symbols are mean values and bars SE. In some cases the error bars are not visible, because they are smaller than the symbols. Lower-case letters inside the graphs indicate homogeneous groups according to the post hoc Holm-Sidak multiple comparison test results.

**Figure 3:** Thermal response of the body C:P ratios of (a) *Acartia tonsa*, (b) *Oxyrrhis marina*, (c) *Carcinus maenas* Cadiz, and (d) *C. maenas* Helgoland. Dots and bars are mean values and SE. Lower-case letters inside the graphs indicate homogeneous groups according to the post hoc Holm-Sidak multiple comparison test results.

**Figure 4:** Gross growth efficiency of carbon (a-d) and phosphorus (e-h). (a and e) *Acartia tonsa*, (b and f) *Oxyrrhis marina*, (c and g) *Carcinus maenas* Cadiz, and (d and h) *C. maenas* Helgoland. Dots and bars are mean values and SE. In some cases the error bars are not visible, because they are smaller than the symbols. Lower-case letters inside the graphs indicate homogeneous groups according to the post hoc Holm-Sidak multiple comparison test results or Kruskal-Wallis ANOVA on ranks (a and c).

**Figure 5:** C:P Threshold Elemental Ratio ($TER_{C:P}$) as a function of temperature for (a) *Acartia tonsa*, (b) *Oxyrrhis marina*, (c) *Carcinus maenas* Cadiz, and (d) *C. maenas* Helgoland. In (a-c) dashed vertical lines represent the mean $MaxTER_{C:P}$ temperature and the shaded areas the $CI_{95\%}$ obtained through non-linear regression fit of the thermal response of the $TER_{C:P}$ to a Gaussian function, with the

R package nlstools (Baty et al. 2015). Dots and bars are mean values and SE. Lower-case letters inside the graphs indicate homogeneous groups according to the post hoc Holm-Sidak multiple comparison test results Kruskal-Wallis ANOVA on ranks (c).

**Figure 6:** Proposed N-shaped response of the $TER_{C:P}$ to temperature. Light blue, green, and orange areas, until the vertical dashed line represent temperatures within the ecological environment of the species, the red area represents temperatures beyond the thermal optimum (Pörtner & Farrell 2008; Pörtner 2012) that might be experienced by the species only in rare conditions. In this proposed concept, the increasing demands of C relative to P when temperatures increase from cold (light blue) to intermediate (middle green) are the result of increasing respiration rates and P use efficiency, until the $MaxTER_{C:P}$ is reached. When temperatures increase above the normal thermal environment of the organism (from green to orange area), the increasing demands of P relative to C are the result of the decrease in P use efficiency, and might prevent an excessive increase in metabolism that can result from the combination of low P diets (Ruiz et al. 2018; Ruiz et al. 2020) and increased temperatures. The increase in C demands relative to P when temperature is excessive may reflect the physiological stress that amplifies C-demands for respiratory and catabolic processes (Schmitz 2013).

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