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Metabolic dependence of phytoplankton species richness

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ABSTRACT

Aim To analyse the dependence of phytoplankton species richness on temperature within the framework of the metabolic theory of ecology (MTE) with explicit consideration of its assumptions and predictions.

Location Lakes from the Southern Hemisphere – South America (Argentinean Pampa to Tierra del Fuego) and Antarctica – and Northern Hemisphere – North America (USA) and Europe (Denmark to Spain).

Methods The MTE proposes that natural logarithm of species richness and the inverse of temperature are linearly related, with a slope equal to the activation energy. The MTE assumes that the total community abundance, average body size and per species average community productivity are independent of the temperature. These predictions and assumptions are here evaluated using *c.* 660 phytoplankton lake communities and a literature review of 281 experimental measures of growth rate. Linear, curvilinear and segmented models were contrasted with empirical trends.

Results Temperature–richness relationships showed a three-phase segmented form in two of the three continents. Generally, at temperatures above 17 °C and below 11 °C there was a weak relationship or none. Intermediate temperatures showed the expected positive association with richness, but with steeper slopes (*c.* 1) than MTE expectations (*c.* 0.3). Statistical models including total community abundance and average body size explained up to 64% of the variance in richness.

Main conclusions In its original formulation the MTE is not a satisfactory model for large-scale richness patterns in phytoplankton. However, the MTE is able to better explain richness patterns when the temperature dependence of abundances and body size are explicitly accounted for in the model. These temperature dependences improve the performance of MTE predictions but question the interpretation of the richness–temperature slope as a measure of activation energy. The balance among activation energy, abundance and body size produced the observed segmented pattern in temperature–richness relationships for lake phytoplankton.

Keywords

America, Antarctica, Europe, lakes, metabolic theory of ecology, phytoplankton biodiversity.

INTRODUCTION

The increase in species richness from the poles to the tropics was one of the first and best recognized ecological patterns, with a great number of proposed mechanisms but inconclusive support (Rohde, 1992; Willig *et al.*, 2003; Mittelbach *et al.*, 2007; Brown, 2014). Among different formulations, energetic hypotheses are supported by statistical analysis of geographic trends (Allen *et al.*, 2007; Hessen *et al.*, 2007; Pinel-Alloul *et al.*, 2013). Almost a decade ago, a quantitative relationship between richness and temperature was put forward (Allen *et al.*, 2002) in the framework of what has been called the metabolic theory of

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ecology (hereafter MTE; Brown *et al.*, 2004). The MTE proposes that the metabolism of individual organisms influences structure and dynamics at different organizational levels (Brown *et al.*, 2004). In its original form, the MTE predicts that the logarithm of species richness (S) will be linearly and negatively related to the inverse of absolute temperature ($1/T$; T in kelvin) as follows (Allen *et al.*, 2002):

$$\ln(S) = -E_a/kT + I \tag{1}$$

where ln is the natural logarithm, E_a is the activation energy for autotrophic metabolism and k is the Boltzmann constant ($k = 8.62 \times 10^{-5}$eV K^{-1}). E_a was proposed to be a constant for large taxonomic groups (Allen *et al.*, 2002; Brown *et al.*, 2004). However, subsequent empirical estimations reported large variation in its magnitude (Hawkins *et al.*, 2007), which brings into question the determinants of E_a as a main focus of research (Dell *et al.*, 2011). In equation 1, the intercept (I) comprises the following parameters:

$$I = \ln(J/A) + \ln(b_0 <M^{3/4}>) - \ln(<B_T>) \tag{2}$$

where J is community abundance in a given area or volume (A). The second term is the average over the community of body mass (M) scaled to ¾ with b_0 as a normalization constant. The third term represents the average per species energy use in the community ($<B_T>$). In its original formulation (Allen *et al.*, 2002), the exponential effect of temperature was expected to override the effects of the parameters in the intercept (equation 2) if two strong assumptions were met. These are, temperature invariance of (1) the total community abundance and (2) the average size of organisms. These two assumptions imply that equation 2 does not depend on temperature. Although the original formulation of the model was supported by empirical data (Allen *et al.*, 2002), it has been criticized because of departures from its predictions and assumptions (Algar *et al.*, 2006; Hawkins *et al.*, 2007; Cassemiro & Diniz-Filho, 2010).

The deviation from a linear relationship of inverse temperature and natural logarithm of species richness (Hawkins *et al.*, 2007; Cassemiro & Diniz-Filho, 2010) led some authors to suggest the MTE be abandoned. However, considering the set of explicit and implicit assumptions, a simple test of that bivariate relationship may not be appropriate (Storch, 2012). Some efforts towards a far-reaching evaluation of the model were made, introducing a consideration of model assumptions (Cassemiro & Diniz-Filho, 2010) and the effects of sampling area (Wang *et al.*, 2009), numbers of individuals and water availability (Šímová *et al.*, 2011). In this sense, it was proposed that a general improvement of metabolic theories should consider the role of community abundance – formerly assumed constant in the MTE – as a determinant of species richness (Allen *et al.*, 2007). This mechanism, known as the 'more individuals hypothesis' has long been recognized as a determinant of species richness (Rohde, 1992). Currently, the concomitant evaluation of model assumptions and predictions represents a crucial step in the analysis and construction of a metabolic theory of biodiversity. However, this evaluation demands large amounts of information about community richness, size structure, total abundance and productivity over spatial ranges large enough to show the action of proposed mechanisms.

Evaluations of large-scale richness patterns in the framework of the MTE have been predominantly performed on terrestrial vertebrates (Hawkins *et al.*, 2007; Cassemiro & Diniz-Filho, 2010) or plants (Allen *et al.*, 2002; Wang *et al.*, 2009). Recently, the evaluation of the biogeography of microbial organisms has experienced a boost (Martiny *et al.*, 2006) with emphasis on aquatic microorganisms (Hessen *et al.*, 2007; Schiaffino *et al.*, 2011; Pinel-Alloul *et al.*, 2013) and on the relevance of understanding the biogeographic patterns in traits (Green *et al.*, 2008). Phytoplankton communities represent an exceptional model for studying the energetic determinants of community richness (Stomp *et al.*, 2011). Phytoplankton show high diversity even in small systems, and reports of community structure typically include measures of productivity, individual cell size and abundance (or proxies for it, such as chlorophyll *a* concentration (CHLA)). The main MTE scaling relationships tend to hold for phytoplankton (Kruk *et al.*, 2010). However, there are deviations with respect to the expected scaling that were related to local environmental conditions and to specific organism traits (Kruk *et al.*, 2012; Litchman, 2012). Lake phytoplankton are particularly interesting in this context due to the large range of local conditions, particularly temperature, to which they are exposed (Kruk *et al.*, 2012). However, in comparison with terrestrial or even marine communities, the main predictions of MTE have hardly been evaluated on lentic systems, there being some examples where no support to MTE was found (de Castro & Gaedke, 2008; Marañón, 2008).

Under the framework of the MTE, we hypothesize that environmental temperature, via its effect on biological rates and organism metabolism, determines the number of species when evaluated across the latitudinal gradient. This hypothesis will be valid only if community attributes, namely, average body size, abundance and productivity, remain independent of temperature. Alternatively, we expect that deviations in the temperature dependence of community attributes are able to generate systematic deviations in the relationship between richness and temperature. In this paper we analysed large-scale phytoplankton richness patterns among 686 lakes from the Southern and Northern Hemispheres in the framework of the MTE. We found that the richness–temperature relationship is nonlinear. But by contrasting these results with previous ones, a three-phase linear relationship was detected, demonstrating shifts in the relationship between temperature and species richness when examined across a broad temperature gradient.

METHODS

We compiled three separate data sets of phytoplankton community and environmental variables from lakes of South America and Antarctica, North America and Europe. Then we evaluated the shape of the relationship between the natural logarithm of richness and the inverse of temperature. Finally, we evaluated the assumptions of the MTE and linked them to observed patterns.

Data sources

We compiled a large database on species richness and environmental variables for freshwater lake phytoplankton. We gathered data from four continents in the Southern Hemisphere (South America and Antarctica, hereafter SAA) and in the Northern Hemisphere (USA, hereafter NA, and Europe, hereafter EU) (Fig. 1). We analysed each data set separately to keep the same standards for sampling design, species identification and counting.

We used phytoplankton data obtained from 48 freshwater lakes along a large latitudinal gradient from the Argentinean Pampa (34° S) to the Antarctic Peninsula (*c.* 63° S). In shallow lakes, samples were taken near the surface (*c.* 30 cm depth) in open waters, whereas in deep lakes integrated samples were collected within the epilimnetic region of the euphotic zone from the surface to a depth of 5 m. Temperature was measured *in situ* in the first metre of the water column with a Horiba D-54 meter. This set of samples (hereafter called SAA) was obtained in consecutive field trips from 2003 to 2008. In Pampean and Patagonian lakes samplings were conducted during the austral spring and in Antarctica during the austral summer (January–February). Phytoplankton analyses were carried out in all cases following the same protocol: samples were fixed with 1% acidified Lugol's iodine solution and counting was performed using an inverted microscope following Utermöhl (1958). Individual algae were considered as the unit, and in the case of colonial or filamentous species, the average cell numbers per colony or filament were estimated. The biovolume of individuals was calculated using geometric approximations according to their shape and mean dimensions (Hillebrand, 2004). Details of the sampling procedures and phytoplankton analyses corresponding to the Pampean shallow lakes were published in Allende *et al.* (2009) and Izaguirre *et al.* (2012). In the case of Patagonian and Antarctic lakes, most phytoplankton data used in this paper are unpublished, although sampling methodologies have been reported elsewhere (Schiaffino *et al.*, 2011; Tell *et al.*, 2011).

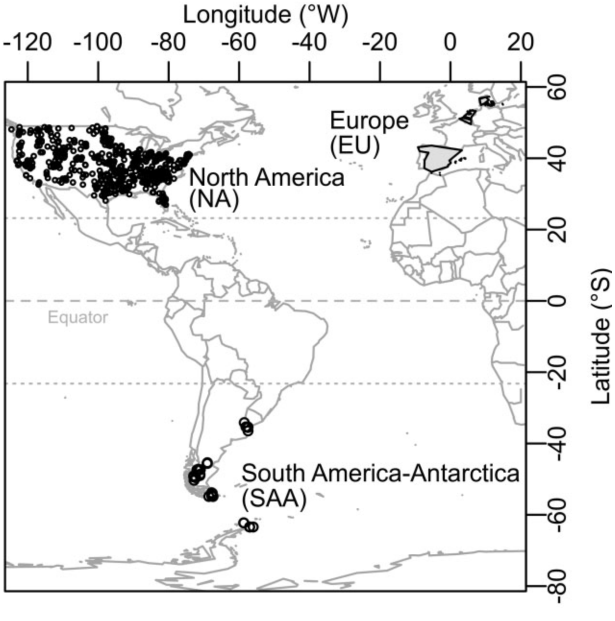

Figure 1 Lakes sampled (circles) in South America and Antarctica (SAA), North America (NA) and Europe (EU). Lack of exact lake positions in Europe precludes their inclusion in the map. Lakes were sampled in Denmark (55°48′–56°27′ N), Belgium, Holland (50°48′–52°41′ N) and southern Spain (36°10′–39°25′ N). These countries are filled in grey.

The NA data set was generated by the Environmental Protection Agency (EPA) from 1973–75 and included 540 lakes sampled in the USA (*c.* 49 to 26.9° N; Fig. 1). Sampling was performed in spring, summer and autumn and the lakes were visited one to four times (Taylor *et al.*, 1979). The depth-integrated water samples were taken from the surface to a depth of 4.6 m, or from the surface to the depth at which light intensity fell to 1% of the surface light intensity, whichever was greater, at the deepest point in each lake and reservoir (Taylor *et al.*, 1979; Stomp *et al.*, 2011). If the depth of the sampling site was less than 4.6 m, the sample was taken from just above the sediment to the surface. Subsamples for phytoplankton counts were preserved with Lugol's solution, and phytoplankton were identified to species or genus level and enumerated by microscopes at 400× using a Neubauer counting chamber. Detailed identification of some diatom species was performed in a phase contrast microscope. The count was stopped when a minimum of 100 fields had been viewed or when the dominant species had been observed a minimum of 100 times. Phytoplankton richness was defined as the total number of phytoplankton species encountered in each lake over the course of a year. This data set was previously used to determine large-scale richness patterns in a different framework (Stomp *et al.*, 2011; data set available in the referred work). Mean annual water temperature and annual average CHLA were used in the analysis. Further details on sampling protocols are available in Stomp *et al.* (2011) and at the EPA Legacy and Storage and Retrieval data system (http://www.epa.gov/storet).

EU lakes were sampled within the framework of the European Union project BIOMAN (Muylaert *et al.*, 2010) during 2000 and 2001 and included 98 water bodies. They included three geographic zones from Denmark to Spain (55–36° N; Fig. 1). The lakes were sampled every month for 6 months throughout the boreal summer. In each lake, subsurface water samples were pooled from 8 (in lakes < 5 ha) or 16 (in lakes > 5 ha) randomly chosen sites within each lake (Muylaert *et al.*, 2010). At the end of the sampling season, samples from different months were mixed at equal volumes to yield one composite sample that was analysed for phytoplankton richness (Muylaert *et al.*, 2010). Phytoplankton were identified and enumerated using standard inverted microscopy methods. A different scientist analysed samples from each European region, but genus lists and identification were thoroughly compared between the scientists involved to avoid bias when comparing richness between regions (Muylaert *et al.*, 2010). Original identification in the European region was to genus level. Identification in Denmark was carried out to species level, and it was found that generic richness was a good predictor of species richness (linear regression, slope = 1.16, $n = 32$, $P < 0.001$; Fig. 1 in Muylaert *et al.*, 2010). We converted genus data to species data using that conversion factor for the European data set. Detailed sampling protocols can be found in Muylaert *et al.* (2010). Water temperature for each lake was calculated as the average during sampling visits. To keep data set comparable, we only used freshwater lakes and excluded some brackish and saline shallow lakes from the southern Spanish region with conductivity values above 2000 μS cm^{-1}.

The predictions and assumptions of the MTE

We evaluated the prediction of a linear relationship of the inverse of temperature times Boltzmann constant ($1/kT$) and the natural logarithm of richness (equation 1) in the three data sets. We also evaluated the statistical associations between density of individuals in the community (J/A), mean community body size (M) and population maximum growth rate with the inverse of temperature ($1/kT$). Average community body size was only analysed in SAA, for which data were available. For the variable total community density (J/A), in NA and the EU we used CHLA as a proxy as both are well correlated (log–log; Spearman's $r = 0.7$, $n = 54$, $P << 0.01$ for SAA; see also Reynolds, 1984). For the maximum growth rate ($\mu_{\max}$), we searched for experiments in freshwater phytoplankton aimed to estimate $\mu_{\max}$ under non-limiting conditions and covering an ample gradient of temperatures (> 10 °C) in the same experimental set-up. We revisited 42 experiments comprising 281 measures of $\mu_{\max}$ in a temperature range from 2 to 40 °C, with representative species from different taxonomic classes and morphology-based functional groups (Kruk *et al.*, 2010; species and data values can be found in Appendix S1 in Supporting Information).

Three different statistical models were considered: a linear model (Allen *et al.*, 2002), a quadratic polynomial model (Hawkins *et al.*, 2007) and a piecewise relationship model (Muggeo, 2003). In a regression model in which the effect of the predictor variable on the response variable changes abruptly at an unknown value, the relationship between the response and the explanatory variable is said to be segmented, broken-line, or piecewise linear (Muggeo, 2003). Compared with other nonlinear regressions, such as splines or polynomials, piecewise regression benefits from interpretable parameters, slopes and breakpoints, having direct and biological meaning (Muggeo, 2003).

Piecewise segmented regression models allow us to objectively determine the range of explanatory variables with different response behaviours. According to Muggeo (2003) a segmented relationship between the mean response $\mu = E[Y]$ and the variable Z, for observation $i = 1, 2, \ldots, n$ can be modelled by adding in the linear predictor the following terms:

$$\beta_1 z_i + \beta_2 (z_i - \psi)_+ \quad (3)$$

where $(z_i - \psi)_+ = (z_i - \psi) \times I(z_i > \psi)$ and $I(\cdot)$ is the indicator function equal to one when the statement is true. According to such parameterization, β_1 is the left slope, β_2 is the difference in slopes and ψ is the breakpoint (Muggeo, 2003). The method only depends on equation 3 and it can be applied to any general lineal model (GLM) including additional linear covariates (Muggeo, 2003). Muggeo (2003) showed that the nonlinear term (equation 3) has an approximate intrinsic linear representation which, to some extent, allows the translation of the problem into the standard linear framework: given an initial guess for the breakpoint (ψ') the method attempts to estimate model (3) by iteratively fitting the linear model with a linear predictor

$$\beta_1 z_i + \beta_2 (z_i - \psi)_+ + \gamma I(z_i > \psi')^- \quad (4)$$

where $I(\cdot)^- = -I(\cdot)$ and γ is the parameter which may be understood as a re-parameterization of ψ and therefore accounts for the breakpoint estimation (Muggeo, 2003). At each iteration, a standard linear model is fitted, and the breakpoint value is updated via $\psi' = \psi' + \gamma'/\beta'_2$; note that γ measures the gap, at the current estimate of ψ, between the two fitted straight lines coming from model (4) (Muggeo, 2008). Due to its computational facility, the algorithm is able to perform multiple breakpoint estimation in a very efficient way as implemented in R using the segmented package (Muggeo, 2008). For a detailed description of the piecewise regression fitting method we refer to Muggeo (2003) and for examples of biological applications to Segura *et al.* (2013) or Toms & Lesperance (2008).

Model selection was made on the basis of the Akaike information criteria (AIC; Burnham & Anderson, 2002), explained variance (R^2) and parameter significance ($P < 0.05$). When using the AIC criterion, the best model is that with the lowest AIC value; models with ΔAIC values less than 2 ($\Delta\text{AIC} = \text{AIC}_i - \text{AIC}_{\text{best}}$) are considered competitive models and those with values greater than six are considered inferior (Burnham & Anderson, 2002). When models were competitive ($\Delta\text{AIC} < 2$), explained variance and parameter significance were used to select the better model. If the slope's 95% confidence interval of the relationship between any variable and $1/kT$ overlapped zero it was assumed that the variable was temperature invariant. The statistical models evaluated allow the description of a wide range of ecological relationships including the expected patterns from MTE, as well as both gradual and abrupt deviations from theoretical assumptions and predictions.

RESULTS

Species richness and temperature ranges were similar among SAA, NA and EU data sets (Table 1). SAA presented the coldest temperatures (0 °C) and the widest latitudinal (*c.* 29°) and temperature ranges (*c.* 27 °C). In turn, NA and EU data sets presented larger ranges in CHLA than SAA.

The relationship between the natural logarithm of phytoplankton richness and the inverse of temperature ($1/kT$) was negative in all three datasets, as predicted by the MTE (Fig. 2). Linear models were significant ($P < 0.05$) and presented E_a

Table 1 Description of lake variables for the three data sets used. Median [minimum–maximum] are presented.

	Number of lakes	Latitudinal range	Temperature (°C)	Species richness	Chlorophyll *a* ($\mu g\ l^{-1}$)	Depth (m)
South America and Antarctica (SAA)	48	34–63° S	8.7 [0–27]	24 [8–60]	1 [0–101]	4 [< 1–200]
North America (NA)	540	26–49° N	18.8 [7–28]	27 [3–84]	10 [0–691]	6 [< 1–307]*
Europe (EU)	74	36–55° N	17.0 [13–25]	21 [6–53]	22 [1–175]	1 [< 1–7]

*There was only one lake deeper than 70 m. The 95% quantile was 25 m and more than 70% of the lakes were less than 9 m deep.

within the expected ranges according to the MTE (range –0.28 to –0.70; Table 2) but with moderate to low explained variance ($R^2 = 0.13–0.42$). Nonlinear models (quadratic or piecewise) showed better congruence with data according to the AIC criterion (Table 2). Within each data set, piecewise regression models frequently presented higher explained variance and lower AIC than quadratic models, except for EU where the AIC difference between piecewise and quadratic model was less than one (Table 2).

Segmented regression models identified two breakpoints in SAA and NA data sets and one in the EU data set (Fig. 2). Segmented models showed similarities among data sets. First, all data sets showed a middle temperature region with a negative slope close to unity (average slope for the three continents ± SD = –1.08 ± 0.07). At warm temperatures (SAA > 15 °C, NA > 20 °C and EU > 17 °C) there was a shift in the relationship towards a positive slope in SAA, and flat slopes (95% CI overlapping zero) in NA and EU. In the lower temperature range (SAA < 10 °C and NA < 12 °C) the relationship was not significant (NA) or negative but with a flatter slope than in the middle region (SAA). In summary, the relationship of richness and temperature presented two extreme zones where the effect of temperature was small and a central region with significant temperature effects.

In the SAA data set the inclusion of total community density (*J*/*A*) and average body size ($<M^{3/4}>$) in the segmented regression improved model fit (Table 3). The multivariate segmented model including *J*/*A* in the SAA data set explained up to 64% of variance. In the NA and EU data sets, systematic effects of total community density (where CHLA was used as a surrogate for *J*/*A*) along the temperature gradient were detected. In NA, the best model explaining richness was a multiple regression with two breakpoints in the inverse of temperature and the covariate ln(CHLA) (Table 3). In EU, the better model was segmented with respect to the inverse of temperature and included ln(CHLA) (Table 3). In EU the nonlinear models with ln(CHLA) as the covariate were similar in AIC ($\Delta AIC < 1$) and similar in the explained variance ($R^2_{seg} = 0.40$ and $R^2_{quad} = 0.39$) but quadratic model parameters were non-significant ($P > 0.05$). For all three data sets, best models included the inverse of temperature and community abundance (or CHLA) as important explanatory variables (Table 3), and in SAA and NA the structure of the models was markedly nonlinear. All fitted model statistics and goodness of fit measures are detailed in Appendix S2.

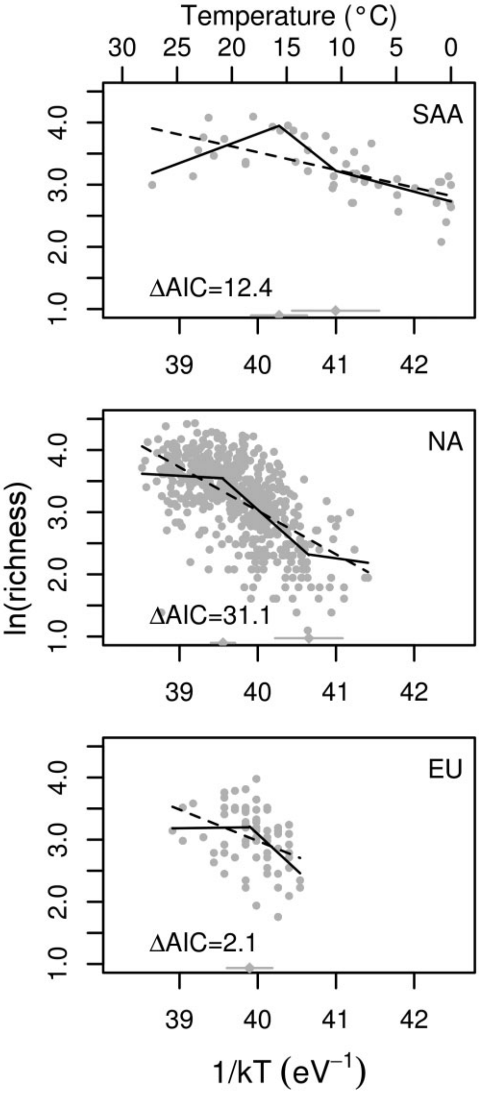

Figure 2 Large-scale patterns of freshwater phytoplankton richness and temperature from 48 South American and Antarctic lakes (SAA), 540 lakes from North America (NA) and 74 European lakes (EU). Differences in the Akaike information criterion (ΔAIC) between linear (dashed line) and segmented models (continuous line) is shown. Segmented models explained the data better than linear and quadratic models. Diamonds with horizontal bars represent the breakpoint estimate and 95% CI respectively. Detailed information on competing models can be found in Appendix S2.

Table 2 Temperature–richness models for the South American–Antarctic, North American and European freshwater lake plankton data sets.

	Linear	Quadratic	Segmented	
South America-Antarctic (SAA)	$\ln(R) = -0.28X + 14.87$	$\ln(R) = -0.13X^2 + 10.2X - 199$	$\ln(R) = 0.47X - 14.97$	If $X < 40.27$
			$\ln(R) = -1.00X$	If $40.27 > X > 40.99$
			$\ln(R) = -0.33X$	If $X > 40.99$
Goodness of fit	$R^2 = 0.42$; d.f. = 45	$R^2 = 0.52$; d.f. = 44	$R^2 = 0.62$; d.f. = 41	
	ΔAIC = 12.4	ΔAIC = 5.6	ΔAIC = 0	
North America (NA)	$\ln(R) = -0.7X + 31$	$\ln(R) = -0.25X^2 + 19.39X - 369$	$\ln(R) = -0.07X + 6.29$	If $X < 39.55$
			$\ln(R) = -1.12X$	If $39.55 > X > 40.65$
			$\ln(R) = -0.18X$	If $X > 40.65$
Goodness of fit	$R^2 = 0.36$; d.f. = 537	$R^2 = 0.38$; d.f. = 536	$R^2 = 0.40$; d.f. = 533	
	ΔAIC = 31.05	ΔAIC = 14.07	ΔAIC = 0	
Europe (EU)	$\ln(R) = -0.51X + 23.16$	$\ln(R) = -0.66X^2 + 52.22X - 1026$	$\ln(R) = -0.03X + 1.99$	If $X < 39.88$
			$\ln(R) = -1.13X$	If $X > 39.88$
Goodness of fit	$R^2 = 0.14$; d.f. = 72	$R^2 = 0.19$; d.f. = 71	$R^2 = 0.21$; d.f. = 70	
	ΔAIC = 2.9	ΔAIC = 0	ΔAIC = 0.8	

The natural logarithm of richness is evaluated with respect to the inverse of temperature (in kelvin) times Boltzmann constant ($X = 1/kT$). The slope of the linear model is an estimate of the activation energy (E_a; Allen *et al.*, 2002). The difference in AIC (ΔAIC) with respect to the best model within a geographic region (row) is shown.

Table 3 Selected models on the temperature dependence ($X = 1/kT$) of species richness (R), total community density (J/A) and average body size (M) in phytoplankton communities from South America and the Antarctic, North America and Europe.

Zone, model and response variable	Equation		R^2	d.f.
South America Antarctic (SAA)	$\ln(R) = 0.49X + 0.07(J/A) + 0.03\ln(M^{3/4}) - 16.6$	If $X < 40.31$	0.65	37
Segmented model	$\ln(R) = -0.91X + 0.07(J/A) + 0.03\ln(M^{3/4})$	If $40.31 < X < 40.97$		
Species richness (R)	$\ln(R) = -0.20X + 0.07(J/A) + 0.03\ln(M^{3/4})$	If $X > 40.97$		
Density (J/A)	$\ln(J/A) = -0.98X + 47.79$		0.27	46
Body size (M)	$\ln(M) = -0.16X + 11.28$	If $X < 40.91$	0.42	41
	$\ln(M) = -0.83X$	If $X > 40.91$		
North America (NA)	$\ln(R) = -0.16X + 0.22\ln(\mathrm{CHLA}) - 3.32$	If $X < 39.33$	0.52	532
Segmented model	$\ln(R) = -0.83X + 0.22\ln(\mathrm{CHLA})$	If $39.33 < X < 40.69$		
Species richness (R)	$\ln(R) = -0.043X + 0.22\ln(\mathrm{CHLA})$	If $X > 40.69$		
Density (CHLA)	$\ln(\mathrm{CHLA}) = 0.17X - 4.03$	If $X < 39.86$	0.12	536
	$\ln(\mathrm{CHLA}) = -1.40X$	If $X > 39.86$		
Europe (EU)				
Segmented model	$\ln(R) = -0.18X + 0.20\ln(\mathrm{CHLA}) + 9.77$	If $X < 39.94$	0.39	69
Species richness (R)	$\ln(R) = -1.13X + 0.20\ln(\mathrm{CHLA})$	If $X > 39.94$		
Density (CHLA)	No significant model; Spearman correlation $r = 0.08$; P-value 0.50			72

All models for richness included total community density or its surrogate chlorophyll *a* (CHLA) as a significant covariate. Explained variance (R^2) and degrees of freedom (d.f.) are shown. Detailed descriptions and associated statistics of these and competing models are given in Appendix S2.

We then analysed the relationship of the attributes of the community (abundance, body size and population growth rate) with temperature. In SAA and NA, community density varied significantly along the temperature gradient. SAA presented a significant negative relationship between log density (organisms ml^{-1}) and the inverse of temperature [$\ln(J/A) = 47.8 - 0.98(1/kT)$; $n = 46$; $P << 0.01$]. For NA lakes we found a piecewise relationship between temperature and ln(CHLA) with negative slopes for temperatures lower than 18 °C (slope = −1.397, 95% CI −1.83 to −0.97) and no relationship for higher temperatures (slope = 0.168, 95% CI −0.178 to 0.514) (Fig. 3). In EU lakes, $1/kT$ and CHLA were not related (slope = 0.23, 95% CI −0.47 to 0.95). The average organism size in SAA lakes decreased with temperature (Fig. 4). The linear [$\ln(<M^{3/4}>) = 23.8 - 0.46(1/kT)$; d.f. = 43; $R^2 = 0.36$; $P < 0.01$; Fig. 4] and the segmented model were equally well fitted (ΔAIC < 1) but the latter explained a slightly higher amount of variance. The segmented model indicated no relationship for temperatures above 10 °C (slope = −0.15; 95% CI −0.63 to 0.30) and a negative slope (−0.826, 95% CI −1.29 to −0.36) for colder temperatures. Finally, the relationship between experimental maximum population growth rate and the inverse of temperature suggested the

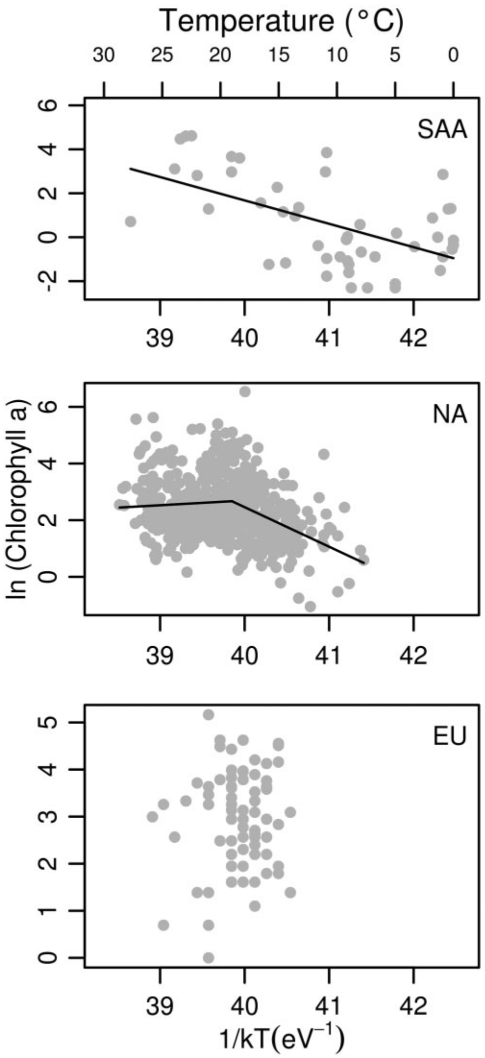

Figure 3 Effect of temperature on Chlorophyll *a* concentration used as surrogate for organism density (*J*/*A*) in data sets from South America and Antarctica (SAA), North America (NA) and Europe (EU) . Lines represent the best fitted model (see the main text for parameter values).

existence of two linear relationships with a breakpoint at *c.* 28 °C (Fig. 5). At low to medium temperatures (2–28 °C), estimated E_a was 0.497 (95% CI −0.62 to −0.38). At higher temperatures (28–40 °C) we found a significant decline of E_a ($E_a = -0.89$; 95% CI = −0.38 to −1.39; Fig. 5). The quadratic model presented a similar AIC but explained a lower amount of variance. All fitted model statistics and goodness of fit measures are detailed in Appendix S2.

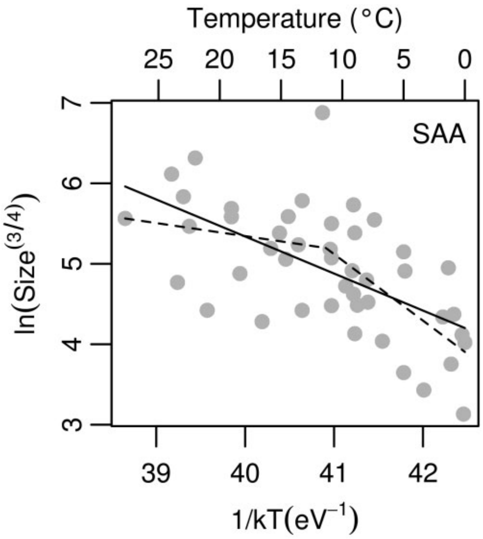

Figure 4 Decrease of average organism body size towards cold temperatures in South America and Antarctic lakes (SAA). Linear (continuous) and segmented (dashed) models are shown. See text and Appendix S2 for model parameters.

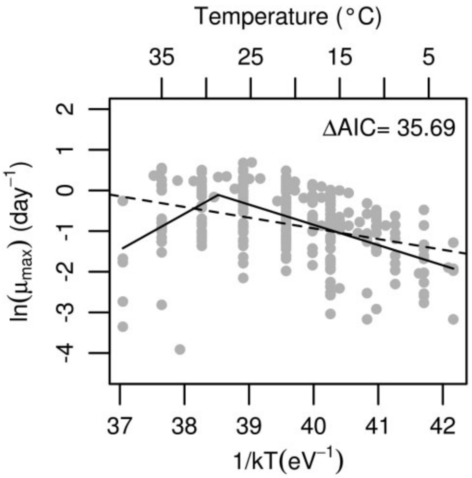

Figure 5 Piecewise segmented relationship (continuous line) between maximum growth rate (μ_{max}) and temperature in freshwater phytoplankton. The shift implies a change in the activation energy at high temperatures (28 °C). Linear model (dashed line) and the difference in the Akaike information criterion (ΔAIC) between both models is shown.

DISCUSSION

The large-scale pattern of species richness and its causal mechanisms were evaluated in the framework of the MTE for phytoplankton communities from SAA, NA and EU lakes. The expected negative relationship between the inverse of temperature and the natural logarithm of richness was found. However, segmented models were better in describing the pattern, and total community abundance was an important explanatory variable improving the fit in all geographic zones. Expected temperature–richness associations under the MTE were not observed at extreme temperatures, where weak patterns or patterns opposite to those expected were revealed. In lakes from the middle range, temperature effect was more pronounced (slope *c.* 1) than expected by the MTE (slope *c.* 0.3). This suggests that departures from MTE assumptions related to temperature dependence of E_a, size structure or abundance have an important influence on trends in species richness. The latter two were suggested in the original formulation of the model (Allen *et al.*, 2002) and in its revised version (Allen *et al.*, 2007) but were rarely considered in later evaluations (Algar *et al.*, 2006; Allen *et al.*, 2007; Hessen *et al.*, 2007; Cassemiro & Diniz-Filho, 2010; Pinel-Alloul *et al.*, 2013). We showed that when abundance and average body size are explicitly incorporated, a better understanding of the richness–temperature relationships at a global scale is gained. Similar results have been reached in a different framework (Stomp *et al.*, 2011).

The negative relationship between the inverse of temperature and the natural logarithm of species richness was evident in all the analysed datasets, suggesting generalities in temperature dependence of species richness (Allen *et al.*, 2002; Hessen *et al.*, 2007; Fuhrman *et al.*, 2008). Deviations from the linear relationship were previously detected for several taxa using polynomial or similar models, suggesting departures from MTE predictions at all temperatures (Algar *et al.*, 2006; Hawkins *et al.*, 2007). While a description of nonlinearity in such relationships is not new, the segmented regression improves the biological interpretation of parameters, as the regression slopes can be interpreted as estimates of E_a. The present results systematically point to the occurrence of ranges of temperatures with constant E_a. Further, a higher E_a than former expectations was found at intermediate temperatures, which appears to conform qualitatively to MTE predictions. Similarly, it should be noted that our findings of systematic variation in E_a within a broad range of temperatures (Fig. 5) is in accordance with recent empirical syntheses (Dell *et al.*, 2011).

The present results showed that the slope of log richness versus $1/kT$ is an estimation of E_a only if the original assumptions of the MTE are met. The concomitant negative trend in abundance, body size and growth rate, each one with a putative effect on richness, no longer supports the former interpretation of the regression slope of log richness versus $1/kT$ as an estimate of E_a. According to the original formulation, the effect of temperature evaluated in ample gradients should overwhelm any effect caused by total community abundance or size structure because of its exponential nature (Allen *et al.*, 2002). Phytoplankton communities can present changes of orders of magnitude in abundance or average size associated with resource availability, hydrological washout, sedimentation and trophic interactions (Kruk *et al.*, 2010). Thus, the expected positive effect of temperature on richness can be attenuated because of a decrease in total community density (J/A) or average size ($<M^{3/4}>$; see equation 2) caused for example by increased grazing pressure towards tropical zones or by Bergmann's rule and the temperature–size rule (Angilletta, 2009). This effect, coupled with the observed shift in E_a at high temperatures (Knies & Kingsolver, 2010), may explain the negative deviation from MTE expectations at warm temperatures.

Our results suggest strong deviations from MTE predictions in extreme temperature ranges. These deviations were recognized in previous works (Hawkins *et al.*, 2007; Hessen *et al.*, 2007; Cassemiro & Diniz-Filho, 2010) and can be observed in the results for the original formulation of the model (Allen *et al.*, 2002). However, a segmented regression model allows a novel pattern to be disentangled, as it showed that the relationship of richness with temperature is mild at the extremes. Deviations from MTE predictions at extreme temperatures could be expected in light of the range of physiological processes operating at extreme temperatures (Karasov & Martínez del Rio, 2007; Angilletta, 2009; Knies & Kingsolver, 2010). Further, the breakpoint observed in community abundance for NA (Fig. 3) and in maximum population growth rate from independent experiments (Fig. 5), could be directly related to the discrete transitions detected for richness (Fig. 2). Also, the accelerated decrease of individual body size at temperatures colder than 9 °C in SAA (Fig. 4) could help to explain the change in the slope of log richness versus $1/kT$ relationship. Finally, a reduction in average productivity per species ($<B_T>$) in cold lakes could also account for the observed deviation in cold environments. Unfortunately there are no data on total community production for any of these data sets to evaluate this hypothesis. This hypothesized reduction in productivity could be related to extreme temperatures and nutrient stress (Markager *et al.*, 1999). The thermal buffering effect of water can generate differences in the extreme temperatures among lakes in the same latitudinal band caused by lake morphology and mixing processes that should be accounted for when analysing latitudinal richness patterns. In this work, we used *in situ* measured temperatures, and thus we avoided that effect. As stated in our working hypothesis, the effect of environmental temperature on the metabolism of ectothermal organisms will influence community richness patterns. Temperature range in aquatic ecosystems is less pronounced than in terrestrial ones, which can lead to systematic differences between ecosystem types. We cannot discard this effect, but given the inverse relationship of water column stratification and nutrient supply it is suggested that it is nutrient limitation rather than the thermal buffering effect that is responsible for the contrasting large-scale richness patterns between terrestrial and aquatic ecosystems.

It is worth noting that despite significant differences in the sampling schemes, geographic locations and biotas among SAA, NA and EU, the richness–temperature relationships were similar. In this sense, the use of segmented regressions could change the empirical suggestion of additional mechanisms operating along the whole set of temperatures (e.g. changes in mutation rates; Allen *et al.*, 2007), to a view in which specific mechanisms within the MTE dominate at different ranges of environmental conditions (e.g. Arim *et al.*, 2007). The biodiversity patterns found for lake phytoplankton suggest that the MTE could be improved by the relaxation of its main assumptions. Our analysis indicates that the combined effect of temperature (T), density (J/A), body size structure ($<M^{3/4}>$) and productivity (B_T) determine the expected richness in a lake, and that the balance among them can change systematically among geographic zones. The balance among T, J/A, $M^{3/4}$ and B_T gives raise to the observed segmented pattern in lake phytoplankton temperature–richness relationships and provides a plausible explanation of the variability in the temperature–richness relationship observed for other taxa (e.g. Algar *et al*., 2006; Hawkins *et al*., 2007; Hessen *et al*., 2007; Pinel-Alloul *et al*., 2013). These results reinforce the paradigm of multiple mechanisms interacting to directly or indirectly shape biodiversity patterns (Stomp *et al*., 2011). Temperature affects richness by increasing biological metabolism, mutation rates and thus increasing speciation rates. This was summarized as 'the red queen runs faster when she is hot' (Brown, 2014). However, in deep lakes, higher temperatures also imply a strong stratification and a decrease in the nutrient supply to the illuminated zone, which decreases total community density and species richness. This is in accordance with the differential effect of temperature and CHLA in species richness found in a previous analysis (Stomp *et al*., 2011). This independent trend in abundance and temperature is rarely observed in other ecosystems, making aquatic systems excellent models for disentangling the mechanisms that determine species richness. Another consequence of high stratification and low nutrient concentration is the dominance of smaller organisms, which in turn could lead to higher richness as more small species can partition the same amount of resources as a few larger ones (Brown *et al*., 2004). Its is remarkable that the community attributes that have long been recognized as determinants of richness, namely abundance of organisms, body size and energy use, were already formally incorporated into the original MTE model but with the assumption that they were not dependent on temperature. We showed here that when the temperature dependence of community attributes is explicitly considered, a better description of the richness–temperature relationships at a global scale is obtained, and a putative domain of operation of the MTE is visualized.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix S1 Temperature dependent growth rate in freshwater phytoplankton.

Appendix S2 Fitted model statistics and goodness of fit measures for the temperature dependence of richness, density, size and growth rate.

BIOSKETCH

Angel Segura is a PhD student studying biodiversity patterns at multiple scales and supervised by **Matías Arím**, **Hugo Fort** and **Danilo Calliari**. Angel Segura, Danilo Calliari and **Carla Kruk** are part of the Aquatic Functional Ecology Group-UdelaR (http://www.efsa.edu.uy). Their main interests are the description of patterns and the understanding of the processes generating biodiversity, productivity and energy flow of aquatic communities. They combine field work, experiments and modelling. Matías Arím is interested in discovering the empirical connections of community and food web theories.

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