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The metabolic theory of ecology convincingly explains the latitudinal diversity gradient of Neotropical freshwater fish

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Abstract. In the context of diversity gradients, the metabolic theory of ecology (MTE) posits that the logarithm of species richness should decrease linearly with the inverse of temperature, resulting in a specific slope. However, the empirical validity of this model depends on whether the data do not violate certain assumptions. Here, we test the predictions of MTE evaluating all of its assumptions simultaneously. We used Neotropical freshwater fish and tested whether the logarithm of species richness varied negatively and linearly with temperature, resulting in the slope value specified by the MTE. As we observed that the assumption of the energetic equivalence of populations was not achieved, we also analyzed whether the energetic nonequivalence of populations could be responsible for the possible lack of fit to the MTE predictions. Our results showed that the relationship between richness and the inverse of temperature was linear, negative and significant and included the slope value predicted by the MTE. With respect to the assumptions, we observed that there was no spatial variation in the average energy flux of populations or in the body size and abundance of species. However, the energetic equivalence of populations was not achieved and the violation of this assumption did not affect the predictive power of the model. We conclude that the validity of the assumptions (spatial invariance in the average flux energy of populations and spatial invariance in the body size and abundance, especially) is required for the correct interpretation of richness patterns. Furthermore, we conclude that MTE is robust in its explanation of diversity gradients for freshwater fish, proving to be a valuable tool in describing ecological complexity from individuals to ecosystems.

Key words: abundance; assumptions; average flux energy of populations; body size; energetic equivalence rule; metabolic hypothesis; richness pattern; spatial invariance; temperature.

INTRODUCTION

One of the oldest recognized patterns in ecology is the increase in species richness from the poles towards the equator (Ricklefs 2004, Jablonski et al. 2006, Mittelbach et al. 2007). Although this pattern is widely accepted, there is still no consensus on the processes underlying this trend. Identifying the mechanisms that govern this pattern has become an important challenge for ecologists and has been the focus of intense debate (Willig et al. 2003, Currie et al. 2004, Rahbek 2005).

Many hypotheses have been proposed to explain biological diversity gradients at broad spatial scales (Rohde 1992, Hawkins et al. 2003, Willig et al. 2003, Mittelbach et al. 2007), but many of them are neither robust nor testable (Rohde 1992, Hawkins et al. 2003). Currently, one of the most widely accepted hypotheses is related to climate effects, which appears to be the strongest predictor of species richness at broad scales in a correlative sense (Hawkins et al. 2003). However, the mechanisms that link variation in species richness to climate have not yet been elucidated (Currie et al. 2004, Algar et al. 2007).

In the context of the climatic hypothesis, Allen et al. (2002) proposed the "metabolic hypothesis," based on the first principles of thermodynamics and biochemical kinetics, within the context of the more general metabolic theory of ecology (MTE hereafter; see Brown et al. 2004). The MTE proposes to address how the relationship between organismal metabolism, body size, and temperature scales up to population, community, and ecosystem properties. Specifically, metabolic theory, when applied to understanding diversity gradients, applies only to ectothermic organisms and proposes that log-transformed species richness, S, should decrease linearly with the reciprocal of temperature according to the formula $\ln(S) \propto 1/kT$, where k is the Boltzmann factor (8.62×10^{-5} eV/K) and T is temperature in Kelvin (see Allen et al. 2002). Note that the temperature is multiplied by k, which is a conversion factor between thermodynamic temperature and energy. Thus, in the

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MTE approach, kT is therefore a measure not only of temperature but also of energy. In this way, when temperature is mentioned herein, the value should also be interpreted as a measure of energy. Thus, the relationship between richness ($\ln S$) and temperature (kT) should display a linear slope between –0.6 and –0.7 eV (see Allen et al. [2002] and Brown et al. [2004] for a detailed explanation).

When dealing with diversity gradients, the model proposed by the MTE assumes that, at some level, species distribution patterns are related to temperature, which consequently affects the metabolism of organisms. According to this reasoning, the structure and dynamics of ecological communities are interconnected with the individual metabolism of organisms (Allen and Gillooly 2006). Thus, the MTE hypothesis differs from other because it provides a quantitative model with mechanistic explanations and a specific and direct prediction for the relationship between species richness and environmental temperature. This explicitly testable hypothesis, combined with its simplicity, generality, and apparent predictive power, is an attractive tool for explaining many emergent characteristics of populations and communities (O'Connor et al. 2007, del Rio 2008). Conversely, various studies have criticized the generality and validity of the theory, questioning the involved mechanisms and assumptions that underpin the theory (Cassemiro et al. 2007*a*, *b*, Hawkins et al. 2007*a*, *b*, O'Connor et al. 2007, del Rio 2008, Cassemiro and Diniz-Filho 2010, McCain and Sanders 2010, Rombouts et al. 2011). Thus, some authors have proposed alternatives to extend the MTE. For example, Allen et al. (2006) and Gillooly and Allen (2007) analyzed the effects of variation on evolutionary rates by including basic principles of population genetics in the metabolic hypothesis, and Stegen et al. (2009) and Ehnes et al. (2011) incorporated ecological traits in the equations of Allen et al. (2002). However, we would like to stress that this present study is focused on the model originally presented by Allen et al. (2002) and thereafter by Brown et al. (2004).

A reliable test of the MTE model applied to richness patterns depends on important assumptions: (1) the energy flux of populations per unit area is independent of body size (energetic-equivalence of populations); (2) the metabolic rate varies with body size and environmental temperature; (3) the average energy flux of populations; (4) the mean derived from the body size distribution; and (5) the total number of individuals in the community per unity area does not vary with the temperature gradient (see Allen et al. [2002] and Brown et al. [2004] for details). In addition to these assumptions, it is important note that the domain of the model encompasses groups of organisms with large phylogenetic groups and high taxonomic diversity (see Hawkins et al. 2007*a*, Cassemiro and Diniz-Filho 2010). Furthermore, it is necessary to assume that the time over which different speciation rates have been acting is similar.

To date, no studies have tested the predictions of MTE explicitly taking into account all of these assumptions. Surprisingly, even proponents of the theory (Allen et al. 2002, Brown et al. 2004) have not adequately tested the MTE and argue that the model is robust and works well even when the assumptions are not fully met (but see Hawkins et al. 2007*a*, *b*). Consequently, many studies have criticized the theoretical and methodological structure of metabolic theory as applied to the understanding of richness gradients (Cassemiro et al. 2007*a*, *b*, Hawkins et al. 2007*a*, *b*, O'Connor et al. 2007, del Rio 2008, McCain and Sanders 2010, Rombouts et al. 2011). However, a model can be genuinely evaluated by validating its critical assumptions; i.e., a critical assumption determines the qualitative behavior of the model, and if the assumption proves false, the model predictions should also be considered false (Angilletta 2009).

Here, we are the first to test the predictions of MTE correctly, evaluating all assumptions simultaneously. We used an extensive database of Neotropical freshwater fish to assess whether the latitudinal variation in species richness can be explained by temperature according to the MTE. We first assessed whether the energy flux of populations per unit area is independent of body size (energetic equivalence assumption). We also investigated whether our data fulfilled the assumptions of temperature invariance of the average energy flux of populations, body size, and abundance of species. Subsequently, we examined support for the two key predictions of MTE by testing whether the logarithm of the species richness varied linearly with the reciprocal of temperature and whether the slope value corresponded to that specified by MTE. We observed that the energetic equivalence assumption was not achieved; therefore, we also analyzed whether the energetic nonequivalence of populations could be responsible for the possible lack of fit to the predictions of MTE. We are aware of no other study assessed the empirical validity of MTE taking into account all of its assumptions. In doing so, this study improves our understanding of the predictive power of this theory.

METHODS

Study area

To assess the relationship between species richness of Neotropical freshwater fish and temperature in the context of the MTE, we analyzed assemblages from reservoirs widely distributed through Brazil along a latitudinal gradient (see the Appendix). Fish communities in reservoirs arise as a result of the restructuring of communities that previously occupied the local river segment, accurately reflecting the original fish fauna (Agostinho et al. 2008). Although this process is influenced by the characteristics of the river segment, these communities display marked historical-regional characteristics (Agostinho et al. 2008). The reservoirs are located between 2° and 29° S latitude and 38° and 55°

TABLE 1. Comparative information for the biological data sets used to test MTE.

Metric	Data set I	Data set II
Number of assemblages	39	100
Data gathering method	sampled data	compiled data
Sampling effort control	control of sampling effort	without control of sampling effort
Capture methods	one capture method in a single reservoir region	several capture methods in multiple reservoir regions
Richness data	observed and estimated richness	observed richness only
Size and abundance data availability	body size and abundance data available	body size and abundance data unavailable
Type of richness data	richness data restricted to the adopted sampling criteria	more comprehensive richness data

W longitude and the total area of lakes covers approximately 23 275 km^2.

Biological data

To evaluate how freshwater fish species richness correlates with temperature, we analyzed information from the two data sets (Table 1), which we describe in detail here.

Data set I included 39 assemblages and was used to assess whether Neotropical freshwater fish achieve the assumptions of the MTE. The richness of this data set was obtained by taking samples using control of capture effort in the lacustrine region of the reservoirs (see Thornton 1990) near the dam. The lack of sampling effort control has been commonly pointed out as a problem in ecological studies (see Gotelli and Colwell 2001). With this in mind, we used this data set also to ensure that the richness pattern found was not related to differences in sampling effort. Thus, this data set provided great reliability to the cause–effect relationship investigated by the MTE. The sampling effort was controlled by standardizing the method of fish capture in each reservoir as follows: (1) the fish were captured using a single type of device (i.e., gill nets of different mesh sizes); (2) the exposure time of nets was standardized (24 hours); (3) the nets were examined in the morning, afternoon, and night; and (4) the nets were installed at a single sampling station located within the lacustrine region of the reservoirs near the dams. This approach also allowed us to obtain accurate species abundance data.

To correct the effects of different numbers of samplings, we also estimated the species richness for each reservoir using presence/absence data based on ICE, Jackknife 1, Jackknife 2, Chao 2, and Bootstrap (S_{ICE}, S_{Jack1}, S_{Jack2}, S_{Chao2}, and S_{Boot}, respectively) estimators using EstimateS 8.2 software (Colwell 2009).

In order to evaluate the assumptions of energetic equivalence of populations, temperature invariance (spatial invariance) of the average energy flux of populations as well as spatial invariance in body size and species abundance, we collected data on the body size and abundance of each species caught in each assemblage of data set I. Body size data were based on the total mass (g) and the standard length (distance between the snout and the last vertebra; cm) of all captured individuals. The abundance was based on the values of catch per unit effort (CPUE), expressed as the number of individuals per 1000 m^2 of net per 24 hours of exposure.

Although data set I was appropriate for accurately assessing all of the MTE assumptions, species richness can be limited (underestimated) by the use of a single method of capture (gill nets) in a sole reservoir region. To deal with this issue, we also tested the predictions of the MTE for data set II, which contained more comprehensive information about species richness based on data compiled from literature or provided by collaborating institutions. Data set II encompassing a total of 100 assemblages and included information from lacustrine, transitional and fluvial zones of the reservoirs (see Thornton 1990), based on captures using several sampling methods without effort control.

Environmental data

We used the mean water temperature as a predictor variable for species richness for data set I. The water temperature was measured using a thermometer in the limnetic and littoral regions of the reservoirs in the same locations where the fish were sampled. Due to lack of water temperature data for all reservoirs in data set II, we used the mean atmospheric temperature as predictor variable, using data from New et al. (1999), which provides historical temperature series from 1961 to 1990 with a spatial resolution of 0.5° of latitude and longitude. Thus, for data set I, we used temperature data at the local scale and, for data set II, we used temperature data at the regional scale. Because these two variables were linearly and positively related and exhibited a high coefficient of determination and a Pearson's r value close to unity ($r^2 = 0.76$; $P < 0.0001$; $y = -34.58 + 1.22x$; SE = 0.11; 95% CI = 0.91–1.33; Pearson's $r = 0.87$), we expected that atmospheric temperature had the same effects on fish richness as did water temperature for modeling purposes.

Evaluation of MTE assumptions

Allen et al. (2002) demonstrated that to test the metabolic hypothesis, it is necessary to respect implicit and explicit assumptions. First, Allen and his colleagues mentioned that the model is an extension of the energetic equivalence rule (EER) and assumed that the total energy flux of populations does not vary with body size. Then, considering that the metabolic rate varies with body size and temperature (see Gillooly et al. 2001), the authors added the environmental temperature to the EER model to demonstrate that the average energy flux of populations is temperature invariant. Based on this latter statement, the authors finally considered species richness in the model and explicitly reported that the body size and abundance of species should be invariant of temperature. Thus, a reliable test of the MTE, when applied to richness patterns, requires five important assumptions: (1) the energy flux of populations per unit area is independent of body size (energetic equivalence of populations); (2) the metabolic rate varies with body size and environmental temperature for ectotherms; (3) the average energy flux of populations is invariant along the temperature gradient; (4) the mean derived from the body size distribution is temperature invariant; and (5) the number of individuals in the community per unity area is temperature invariant (see Allen et al. 2002).

Thus, to evaluate whether our data achieve the first assumption, we tested the predictions of the EER (Damuth 1981) for each fish assemblage. Specifically, we used ordinary least squares (OLS) regression to assess whether the population abundance (log-transformed) is a function of the body size (log-transformed), as predicted by EER, and to verify whether the slope (*b*) of the relationship between body size and abundance fit the parameter proposed by Damuth ([1981]; $b = -0.75$; also see Nee et al. [1991] for details). However, it is important to stress that the aforementioned regression model is applicable only when the relationship between the logarithm of body size and abundance is linear. To address to this issue, when a polygonal relationship was observed, we used the technique of OLS regression of the upper right bound of the envelope (URB; Blackburn et al. 1992). The observations were clustered into of 5 to 10 size classes; the first size class corresponded to the value from which the abundance decreases with increases in this variable. The parameters from this negative linear regression were then compared to the EER predictions. Slopes steeper than predicted by the EER (–0.75) indicated that smaller fish tended to control a disproportionately greater amount of energy in the assemblages. By contrast, slopes shallower than predicted by the EER or those positive implied that populations of large species utilized, on average, a greater amount of energy than populations of species with small body sizes. We considered the assumption to have been met if the mode of observations covered the slope predicted by EER.

We used Neotropical freshwater fish to achieve the second assumption, since in this group the metabolic rate varies with body size and environmental temperature (see Clarke and Johnston 1999, Ohlberger et al. 2012).

To evaluate our data with respect to the third assumption, we tested whether the natural logarithm of mass-corrected population density is a linear function of 1000/environmental temperature (E), with the positive slope of approximately $E/1000k$ ($\approx$9.0 K), as proposed by Allen et al. (2002).

Finally to evaluate whether our data achieve the fourth and the fifth assumptions, i.e., spatial invariance of body size (mass in grams and length in centimeters) and abundance (CPUE), we conducted OLS regressions between these variables (dependent variables) and latitude and temperature (independent variables). The r^2 values were used as indicators of the spatial structure in body size and abundance along the temperature gradients (see Cassemiro and Diniz-Filho 2010). As previously mentioned, we used data set I to assess the first, third, fourth, and fifth assumptions due to the availability of body size and abundance data.

Testing the metabolic hypothesis

To evaluate the influence of temperature on species richness according to the MTE (Brown et al. 2004), we tested the metabolic hypothesis proposed by Allen et al. (2002). According to this hypothesis, the logarithm of species richness varies inversely with temperature multiplied by the Boltzmann constant: $\ln(S) \propto 1/kT$, where S is the number of species, k is the Boltzmann constant (8.62×10^{-5} eV/K) and T is the local temperature in Kelvin (K). The metabolic hypothesis relates the temperature to the individual metabolic rates of organisms through the Boltzmann factor ($e^{-E/kT}$, in which E is the activation energy of metabolism equal to ~0.78 eV) to establish the relationship between temperature and the species richness specifically for ectothermic organisms. In this context, if the linear relationship between the natural logarithm of species richness ($S_{\mathrm{Obs\ I}}$, S_{ICE}, S_{Chao2}, S_{Jack1}, S_{Jack2}, S_{Boot}, and $S_{\mathrm{Obs\ II}}$) and the inverse of the temperature exhibits a slope (*b*) between –0.6 and –0.7 eV, the test corroborates the hypothesis (Brown et al. 2004). These analyses were performed for both data sets I and II.

Fish species richness in reservoirs is strongly influenced by the age and area of these habitats; accordingly, is expected a greater number of species in larger and younger reservoirs (see Agostinho et al. [2007] for details). Therefore, we included the area and age of the reservoirs (log-transformed) as predictors in the MTE model through multiple regression. In doing so, we were able to control the effect of the age and area of the reservoirs on the relationship between temperature and species richness.

The originally proposed test for the metabolic hypothesis used OLS regression. However, this regression method does not consider the problem of spatial autocorrelation (Diniz-Filho et al. 2003). Biological data for species richness are frequently spatially autocorrelated (Diniz-Filho et al. 2003, Dormann 2007), typically leading to autocorrelated residuals in OLS models. We analyzed spatial autocorrelation in the richness data using spatial correlograms of Moran's I coefficients (see

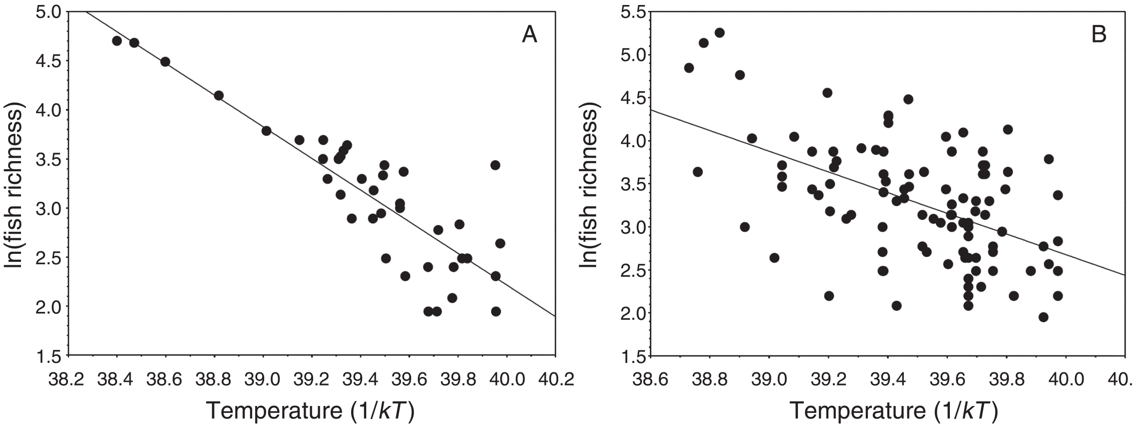

FIG. 1. Relationship between the natural logarithm of the species richness of Neotropical freshwater fish and the inverse of temperature (*kT*; where *k* is the Boltzmann factor [8.62×10^{-5} eV K^{-1}] and *T* is temperature in Kelvin) for (A) data set I and (B) data set II.

Diniz-Filho et al. 2003) calculated at 12 distance classes. When significant residual autocorrelation was found, we used simultaneous autoregressive models (SAR, see Haining 2003) to deal explicitly with the spatial component. In this analysis, area and age of reservoirs were also used as predictors in addition to temperature.

Finally, as the assumption of energetic equivalence of populations was not achieved, we analyzed whether the energy nonequivalence of populations could be responsible for the possible lack of fit of the MTE predictions for data set I. In this case, we included the *b* values of the EER relationship (i.e., abundance × body size) as predictor variable in the MTE model through multiple regression and confirmed whether the slope was distinct from the value predicted by MTE. All analyses were performed in SAM 4.0 (Rangel et al. 2010).

RESULTS

Fish species richness for the two data sets showed a general spatial pattern along the temperature gradient, with higher species richness at lower latitudes (Fig. 1A, B).

Regarding data set I, we observed that the frequency distribution of the slopes of EER was bimodal. The modes occurred at values steeper (between −1.48 and −1.73) and shallower (between −0.43 and −0.69) than predicted by EER (Fig. 2). Thus, the assumption of energetic equivalence of populations was not achieved; i.e., in the fish assemblages of data set I, there were different amounts of energy available to species of different sizes.

In contrast, the natural logarithm of mass-corrected population abundance showed a positive and linear

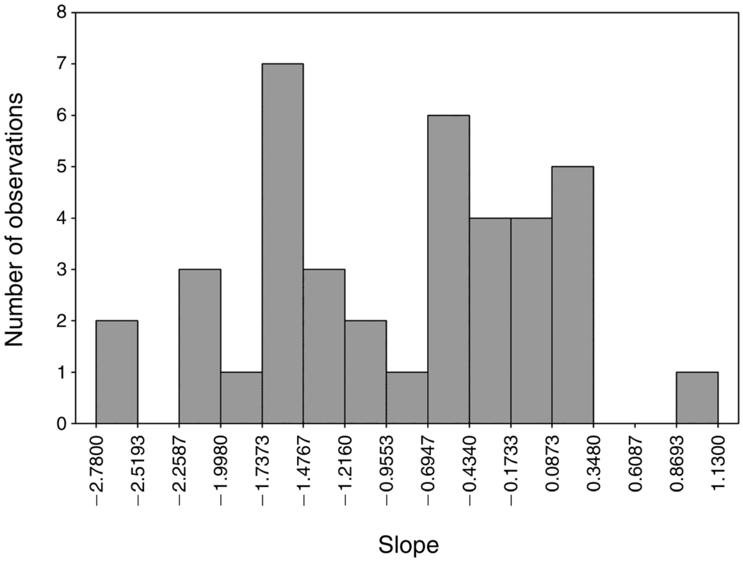

FIG. 2. Frequency distribution of the slopes for the relationship between abundance and body size of freshwater fish species using data set II.

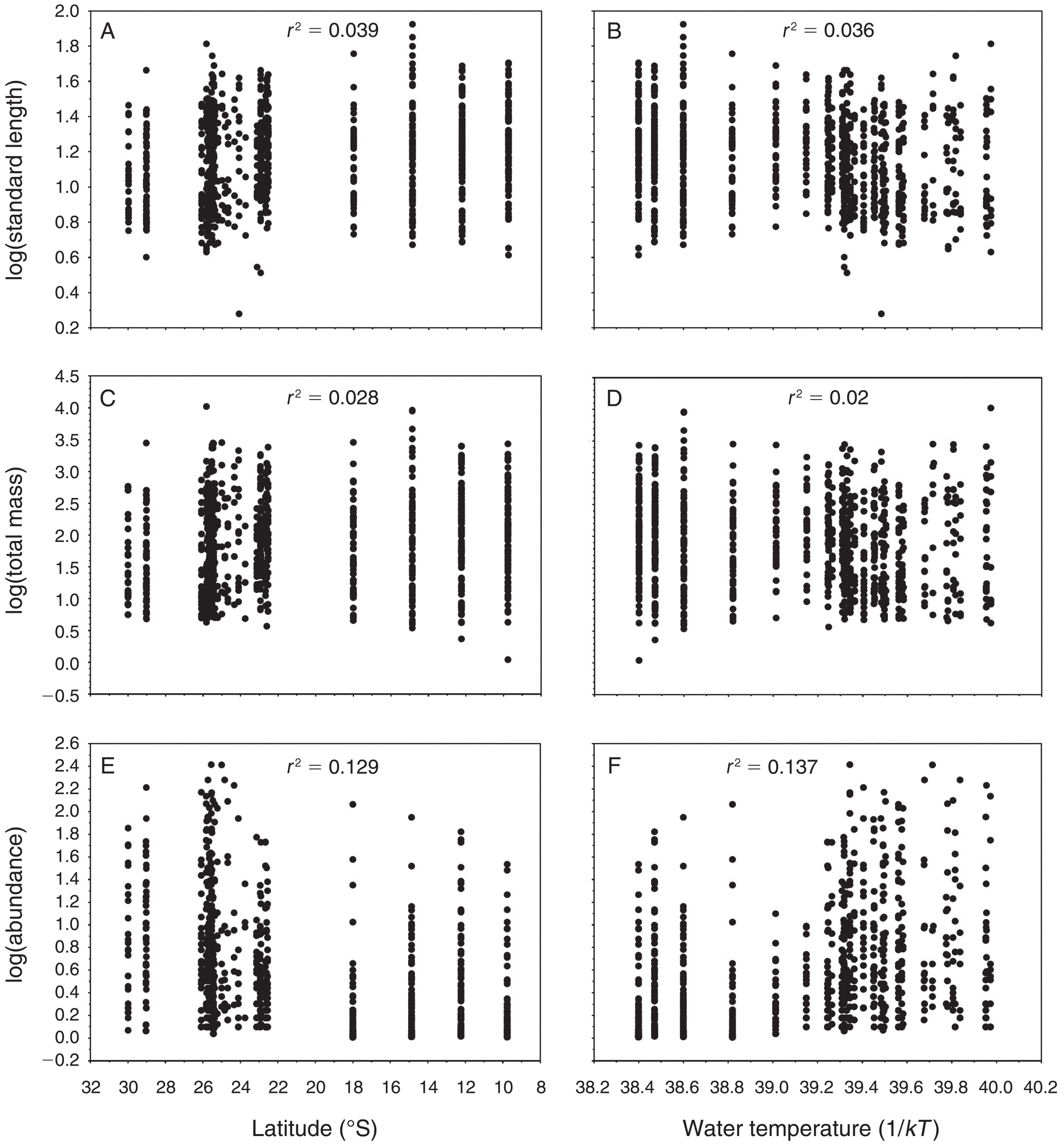

FIG. 3. Relationships between latitude and (A) standard length, (C) total mass, and (E) abundance and relationships between water temperature (kT) and (B) standard length, (D) total mass, and (F) abundance for Neotropical freshwater fish for data set II. Body size data were based on the total mass (g) and the standard length (distance between the mouth and the last vertebra; cm) of all captured individuals. Abundance was based on the values of catch per unit effort (CPUE), expressed as the number of individuals per 1000 m^2 of net per 24 hours of exposure.

relation to inverse absolute temperature, and the CI of the slope encompassed the predicted value of ≈9.0 K ($y = 18.17 + 6.7x$; 95% CI = 4.5; 9.0 K). These results demonstrate that by controlling for the body temperature, the average energy flux of populations is temperature invariant. Additionally, we observed that there was no spatial variation in length ($r^2 = 0.039$ and $r^2 = 0.036$; Fig. 3A, B), mass ($r^2 = 0.028$ and $r^2 = 0.02$; Fig. 3C, D) or abundance ($r^2 = 0.129$ and $r^2 = 0.137$; Fig. 3E, F). These results likely correspond to the general pattern found for freshwater fish assemblages; therefore, we assumed this same scenario for data set II.

The relationship between the species richness and the inverse of temperature (with age and area of reservoirs included as predictor variables) was linear, negative, and significant for data set I ($b = -0.906$, $P < 0.001$, $r^2 = 0.876$). The 95% CI (−0.58 to −1.23 eV) included the predicted slope value (between −0.6 and −0.7 eV) in accordance with the MTE predictions. We did not use spatial statistic due to the absence of positive autocor-

Table 2. Parameter values of regressions between the natural logarithm of modeled species richness (S) and the inverse of temperature (kT) for two data sets.

	Data set I	Data set II
Number of assemblages studied	39	100
Assumptions		
Energetic equivalence of populations	no	no†
Spatial invariance of AEFP	yes	yes†
Spatial invariance in body size	yes	yes†
Spatial variance in abundance	yes	yes†
Spatial autocorrelation	no	yes
OLS regression		
S_{Obs} × temperature	**−0.906**‡ (0.876)	**−0.538**‡ (0.537)
S_{Chao2} × temperature	**−1.015**‡ (0.819)	observed richness only
S_{Jack1} × temperature	**−1.014**‡ (0.86)	observed richness only
S_{Jack2} × temperature	**−1.067**‡ (0.85)	observed richness only
S_{Boot} × temperature	**−0.957**‡ (0.87)	observed richness only
S_{Obs} × temperature and EER b	**−0.907**‡ (0.89)	EER b unavailable
SAR regression		
S_{Obs} × temperature		**−0.59**‡ (0.56)

Notes: In all tests, the age and area of reservoirs were included as predictor variables. To correct the effects of different numbers of samplings, we also estimated the species richness for each reservoir using presence/absence data based on ICE, Jackknife 1, Jackknife 2, Chao 2, and Bootstrap (S_{ICE}, S_{Jack1}, S_{Jack2}, S_{Chao2}, and S_{Boot}, respectively) estimators. Values are the slope (b) for temperature; values in parentheses are r^2. Abbreviations are: AEFP, average energy flux of population; and EER, energetic equivalence rule. Boldface type indicates a significant relationship between richness and temperature.

† We understand that this condition is a pattern for Neotropical freshwater fish and therefore is maintained.

‡ The 95% CI includes the slope predicted by the metabolic theory of ecology (MTE).

relation in the regression residuals (Moran's $I_{residuals} = -0.02$) for the first class of distance in the spatial correlogram (0 to 55.09 km). In general, the same pattern was observed using the estimated richness (S_{ICE}, S_{Jack1}, S_{Jack2}, S_{Chao2}, and S_{Boot}) as dependent variable (Table 2).

Incorporating b values of the abundance–body-size relationships, which were distinct from EER expectations as predictor variable in the OLS model did not result in a slope different than that predicted by MTE ($b = -0.907$, 95% CI = −0.60 to −1.22, $r^2 = 0.89$, $F_{38} = 64.88$, $P < 0.001$, Moran's $I_{residuals} = 0.082$).

Considering data set II, the OLS regression of the relationship between the species richness and the inverse of temperature (with age and area of reservoirs included as predictor variables) was also linear, negative and significant ($b = -0.538$, $P < 0.001$, $r^2 = 0.537$). The CI also included the value of the slope predicted by MTE (95% CI = −0.16 to −0.91).

For data set II, Moran's I for the first class of distance in the spatial correlogram (0–92.4 km) was equal to 0.392 ($P < 0.05$), thereby confirming the strong spatial structure of the richness data. The value of Moran's I for the residuals of the OLS regression of richness against temperature was also high and significant ($I = 0.168$, $P < 0.05$). To eliminate the effect of autocorrelation on the OLS regression residuals, we used SAR. This procedure reduced the autocorrelation (Moran's $I_{residual} = 0.049$) and improved the model fit, increasing the R^2 to 0.56 ($F_{99} = 36.74$, $P < 0.001$). The slope value in the SAR model was −0.59 ($P < 0.05$), which was close to the predicted value (between −0.6 and −0.7 eV), and the confidence interval also included the predicted slope (95% CI = −0.19 to −0.99). All of these results are summarized in Table 2.

Discussion

Our results showed that MTE convincingly explains the richness pattern of freshwater fish. In this study, the two central predictions of MTE were achieved, i.e., the logarithm of species richness varied negatively and linearly with the inverse of temperature and the slope was consistent with the predicted value. Several studies have refuted these two predictions (see Algar et al. 2007, Cassemiro et al. 2007*a*, *b*, Hawkins et al. 2007*a*, Terribile and Diniz-Filho 2009, McCain and Sanders 2010, Rombouts et al. 2011); however, their conclusions are not well-supported because the assumptions required for the correct fit of the model were either ignored or not achieved. In this regard, Price et al. (2012) reported that the overwhelming majority of tests have evaluated the MTE predictions instead of directly evaluating the model's internal consistency and/or its assumptions. Indeed, a model can be genuinely evaluated only by validating its critical assumptions (Angilletta 2009). Thus, despite widespread criticism, the predictions of MTE were not truly falsified.

Two crucial considerations for the correct interpretation of MTE emerged from our analyses using data set I. First, for this data set there was no positive spatial autocorrelation in the regression residuals, unlike what has been observed in other studies (Cassemiro et al.

2007*b*, Terribile and Diniz-Filho 2009), conferring reliability to the results obtained by the original regression model proposed by Brown et al. (2004). However, it is known that spatial autocorrelation is a prevalent problem in species richness data (see Diniz-Filho et al. 2003, Dormann 2007). Thus, the spatial autocorrelation in tests of MTE must not be ignored, mainly because (1) this theory provides precise predictions based on a specific slope value, and (2) autocorrelation generates biased regression parameters, which, in principle, cannot be interpreted (see Diniz-Filho et al. 2003). Following this reasoning, spatial autocorrelation may quantitatively alter the obtained results and qualitatively change the interpretation of results in the context of MTE, as has been observed in other studies that rejected MTE predictions after neutralizing the effects of autocorrelation on the regression residuals (see Cassemiro et al. 2007*b*, Terribile and Diniz-Filho 2009). Thus, we call attention to the fact that Allen et al. (2002) and Brown et al. (2004) proposed an oversimplified methodology and, in principle, a model inappropriate for predicting ectothermic species richness at large spatial scales, since they did not take spatial structure into account.

The second crucial consideration is the absence of spatial variation in the average energy flux of populations and in the body size and abundance of the species, which are requisite for evaluating MTE. To date, no study has tested the MTE in simultaneous agreement with these three assumptions. Studies have only evaluated spatial invariance of body size and abundance of species separately. In contrast to body size (see Terribile and Diniz-Filho 2009, Cassemiro and Diniz-Filho 2010), spatial invariance of abundance was not achieved (see McCain and Sanders 2010) in tests of MTE. In fact, the majority of empirical tests of MTE ignore the aforementioned assumptions due to the paucity or absence of reliable information on body size and abundance on large spatial scales. Thus, a robust test of MTE requires variables that are extremely hard to determine on a broad spatial scale and that often do not meet the expectations of the MTE assumptions.

Patterns of geographic variation in body size have been observed for different groups of ectothermic organisms, including freshwater fish (Ashton and Feldman 2003). Many organisms exhibit an increase in body size with latitude as proposed by Bergmann's rule (Ashton and Feldman 2003, Cruz et al. 2005, Olalla-Tárraga and Rodrígues 2007), whereas others exhibit the opposite pattern (Knouft 2002, Ashton and Feldman 2003, Olalla-Tárraga and Rodrígues 2007). Likewise, patterns of geographic variation are also reported for abundance, which can show a positive correlation with latitude (Johnson 1998, Symonds et al. 2006, Barnes 2010). These evidences indicate that violation of spatial invariance in body size and abundance assumptions is often inevitable, making MTE a fairly restrictive theory.

The assumption of the energetic equivalence of populations appears to follow this same tendency. In natural communities, it is very unlikely that all species in the community, regardless of body size, have access to the same amount of resources, a fact clearly evident for different animal groups (see Blackburn et al. 1993). In the present study, energetic equivalence did not prevail in the analyzed fish populations, as stated by MTE. We observed that in part of assemblages smaller fish tend to control a greater amount of energy as reported for other aquatic communities, including fish, from different regions of the world (Cyr et al. 1997). Conversely, to the other part of the studied assemblages we registered that larger species utilize on average a greater amount of energy, which also has been observed for fish communities (Knouft 2002, Ackerman and Bellwood 2003). A recent study treated the EER not as a rule but as a flawed concept plagued by circular reasoning and argued that the energetic paradigm can be strengthened by considering alternative, non-energetic, hypotheses (Isaac et al. 2013).

When based on unrealistic assumptions, which are often not met, MTE may fail to predict correctly the observed empirical patterns and can be rejected not because of any failure of its reasoning but due to a lack of the generality required for its proper fit (Cassemiro and Diniz-Filho 2010). Brown et al. (2004) assumed such risk when they established that for MTE tests, the data should be previously fitted to quantitative predictions of another theory, namely EER. Nevertheless, proponents of MTE argue that the theory is relatively robust and works well even when the assumptions are not fully satisfied (but see Hawkins et al. 2007*b*). In fact, we observed that violation of the energetic equivalence assumption did not compromise MTE predictions for freshwater fish since that the inclusion of the slopes of abundance-body size relationships (distinct from EER expectations) in the MTE model did not modify the slope value found in the relationship between species richness and temperature. This would not be expected since the metabolic hypothesis is an extension of EER. Empirical data indicate that pure three-quarters scaling for metabolic rate does not hold across the full size range for different groups (Price et al. 2012). Consequently, unlike the EER posits, it is possible that the energy flux of populations varies with body size in the communities. Thus, predicting species richness as a function of temperature based on the alternative hypothesis of non-energetic equivalence would be a great challenge for future studies.

Although data set I was appropriate for the accurate assessment of all of the assumptions of MTE, the species richness might have been underestimated. To deal with this issue, we used data set II, which contained more comprehensive information of species richness. The results corroborated the pattern found using data set I, supporting the predictions of MTE. After removing the positive autocorrelation in the regression residuals, we also obtained results consistent with the predictions of MTE. In contrast, other studies reported that by removing the spatial autocorrelation, the predictions of MTE were not achieved (see Cassemiro et al. 2007*b*, Terribile and Diniz-Filho 2009).

The importance of standardized sampling effort in species richness studies has been discussed in literature (Gotelli 2001, Gotelli and Colwell 2001). In the MTE context, the results can be biased by discrepancies in sampling effort because species richness can be higher or lower in a region not by effect of a causal variable, as postulated by MTE, but purely by differences in sampling effort. However, our analyses demonstrated that sampling effort differences did not affect the explanatory power of temperature, since there was no qualitative difference in the fit when using species richness data that were obtained without controlling for sampling effort.

After considering all of the necessary conditions for a reliable test of MTE, our results indicate that the theory is robust in explaining the diversity latitudinal gradient of freshwater fish. The fulfillment of the assumptions (spatial invariance in the average energy flux of populations, body size and abundance of the species in a greater instance) is required to explain the latitudinal variation of species richness according to MTE. In fact, Cassemiro and Diniz-Filho (2010) argue that most deviations from predictions of the MTE can be explained by violations of these assumptions. However, one of the main challenges of ecological studies that test the empirical validity of the theory is finding the necessary biotic conditions in natural communities under which temperature effectively acts in the structuring of species richness pattern in the context of the MTE.

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SUPPLEMENTAL MATERIAL

Appendix

Additional information about the fish assemblages of the studied reservoirs (*Ecological Archives* E095-048-A1).