The metabolism of lake plankton does not support the metabolic theory of ecology

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We tested if the metabolic theory of ecology (MTE) correctly predicts plankton metabolism in a temperate lake, based on a long-term (about 15 years), high-frequency dataset of body size, abundance and production, using two different techniques: least squares regression and maximum likelihood. For phytoplankton, the general fit was relatively poor ($r^2 = 0.53$). The assumption of the MTE on temperature dependence of metabolism was not supported, and the assumed value of $\frac{3}{4}$ of the allometric exponent was barely within 95% confidence limits. For some of the models, the value of b was significantly higher than $\frac{3}{4}$. When radiation was included as an additional predictor, it improved the model considerably ($r^2 = 0.67$). Including grazing by zooplankton reduced the model residuals during the summer period, when grazing is a dominant factor. The allometric exponent had virtually no effect for phytoplankton, due to little variability in average individual size. Zooplankton production, on the other hand, was better predicted by MTE, showing stronger effects of temperature and body size, the average of which varied by a factor of more than a hundred. However, the best-fitting value of the allometric exponent for zooplankton was 0.85, and significantly higher than the $\frac{3}{4}$ predicted by the theory. The ratio of observed production to biomass for the entire plankton community declined linearly with the body size (in log-log) with a slope corresponding to a value of b = 0.85. We conclude that the MTE has little predictive power for the metabolism of lacustrine plankton, in particular for phytoplankton, and especially at the scale of variability of this study, and that this could be improved by incorporating radiation into the model.

Since the initial works by Brown and colleagues (West et al. 1997, Gillooly et al. 2001, Brown et al. 2004), the metabolic theory of ecology (MTE) has received a lot of attention in recent years. The theory is based on individual body size and temperature, and makes general predictions on general characteristics of ecosystems, communities and organisms; such as: population growth rates, ontogenetic growth, survival and mortality, global patterns of species diversity and dynamics of the global carbon cycle, and has received support in some cases (Gillooly et al. 2002, Brown et al. 2004, Savage et al. 2004a, Allen et al. 2005, Gillooly and Allen 2007). It proposes that 'first principles of chemistry, physics, and biology' can be used to link the function of individual organisms to ecological processes. However, it has also been severely criticized on several grounds (Cyr and Walker 2004, Kaspari 2004, Tilman et al. 2004, Algar et al. 2007, Hawkins et al. 2007, O'Connor et al. 2007, Russo et al. 2007). The simplicity, apparent generality and predictive power of the MTE make it seductive as a tool to explain many emergent characteristics of populations and communities.

One of the main characteristics of any community (if not the most important) is its primary production (PP). The amount of energy that primary producers are able to fix is what sustains the rest of the food web. A good test of the usefulness and generality of the MTE would be its ability to predict the PP of an ecosystem, based on the size distribution of primary producers and temperature. These predictions will have to be compared with independent measurements of PP. Lopez-Urrutia et al. (2007) made such an attempt in a large-scale study of total plankton production and respiration for the Atlantic Ocean. The relationships between MTE-predicted community respiration and net primary production with in-situ measurements was highly significant in both cases. However, some of the correlation may have originated from the extremely large geographical scale of their study, and the correspondingly large variability of temperature and body size. Would the MTE also succeed in predicting the temporal variation of PP at a single location? After all, as Tilman et al. (2004) says 'It's a matter of scale'. The main proponents of the MTE claim it to be mechanistic, not statistical (West et al. 1997, Enquist et al. 2003, Allen and Gillooly 2007, but see O'Connor et al. 2007). If this is the case, the theory should predict equally well at any temporal or spatial scale. Large-scale, general predictions of all-encompassing theories are very attractive, but they lose much of their usefulness if they fail at the smaller-scale.

Another point of contention for the MTE is the true value of the allometric exponent (b) and if it is the same for all groups of organisms or if it is varies among groups. Some authors claim it to be $\frac{3}{4}$ for all organisms (West et al. 1997,

Enquist and Niklas 2001, Gillooly et al. 2001, Brown et al. 2004, Savage et al. 2004b, Allen and Gillooly 2007), while others defend a lower value of $\frac{2}{3}$ (Dodds et al. 2001, White and Seymour 2003, 2005, for mammals), and finally, others argue that there is no single true value, but that it varies within and between groups of organisms (Nagy 2005, Muller-Landau et al. 2006, Reich et al. 2006, White et al. 2006). The question of the true b is not trivial, since it is one pillar of the aspirations of mechanicism of the MTE. West et al. (1997) proposed that it derives from the fractal nature of distribution networks within organisms and how essential materials are transported through them, an assumption that has been criticized (O'Connor et al. 2007).

We tested the ability of the MTE to predict the productivity of phytoplankton and zooplankton in a large, open water body (Lake Constance) and its temporal variability, throughout almost fifteen years of measurements. We benefited from very detailed data of community composition, size spectra, environmental variables and estimates of primary and secondary production (Gaedke 1993, Bäuerle and Gaedke 1998, Gaedke et al. 2002). We also expanded the usual expression of the MTE to include the effect of other factors, both abiotic (radiation) and biotic (grazing). Finally, we examined the best-fitting value of b to asses if our data support the proposed value of $\frac{3}{4}$.

Material and methods

The data

Lake Constance is a temperate, large (476 km^2) and deep ($z_{max} = 252$ m), warm-monomictic lake, north of the European Alps, where plankton biomass and the growth regulating factors exhibit strong seasonality (Sommer et al. 1986). During winter and early spring physical factors like irradiance and temperature dominate, whereas during summer, trophic interactions, nutrient depletion and low food quantity and quality are of major importance for most populations (Simon and Tilzer 1987, Müller 1989, Weisse 1991, 1997, Bäuerle and Gaedke 1998, Gaedke et al. 2002, Tirok and Gaedke 2007).

Water temperature was measured at several depths (we used mean temperature from 0 to 20 m). Temperature data was interpolated to daily values using a piecewise cubic hermite interpolating polynomial procedure, when required. Daily data of incident radiation (Watt m^{-2}) were obtained from the local weather station. Plankton sampling and measurements of primary production were carried out weekly during the growing season (except 1981, in which phytoplankton biomass was measured every 2–3 days, and 1987 on two consecutive days per week) and approximately every two weeks in winter, at a central sampling site (depth 147 m) in the northwestern arm of the lake (phytoplankton, crustaceans, primary production: 1980–1982, 1986–1997 or longer; ciliates: 1987–1998; rotifers: 1984–1985, 1987–1996). We maximized the temporal and vertical resolution of sampling at the cost of the horizontal one since in the off-shore water body vertical gradients and temporal changes were much more pronounced than horizontal variability. The water column from the depth of 0–20 m was sampled with a 2 m long tube sampler ten times, and 2–5 integrated

samples were counted. In accordance with numerous previous studies, we considered the average value of the uppermost 20 m, which roughly correspond to the epilimnion and the euphotic zone (Tilzer and Beese 1988). Crustaceans which mostly feed in the uppermost 20 m but may migrate vertically to larger depth during daytime, were collected with a Clarke-Bampus sampler (mesh size 140 μm) by vertical hauls from 140 m depth.

The abundance of all eukaryotic plankton organisms ranging from small phytoplankton (10^{-11} g C cell^{-1}) to large crustaceans (10^{-4} g C ind^{-1}) was assessed by microscopy using different counting techniques appropriate for the size and fragility of the organisms (DAPI-staining and epifluorescence microscopy for heterotrophic flagellates, Weisse 1997, settling chambers and inverted microscopy for phytoplankton and ciliates, Müller 1989, Gaedke and Schweizer 1993, stereo-microscopes for rotifers and crustaceans, Geller 1989). Individual body sizes were established by measuring either size frequency distributions (e.g. heterotrophic flagellates), or average cell volumes of individual taxa or morphotypes (phytoplankton, ciliates, rotifers), or the individual length of the organisms (crustaceans). Original measurements of body size were converted to units of C using measurements from Lake Constance or from the literature (details in Gaedke 1993). For phytoplankton, we assumed a constant carbon content (C) of 14% of cell volume (V) for cells larger than 1600 μm^3, and C $= 0.433$ V$^{0.866}$ (pg C cell^{-1}) otherwise (Verity et al. 1992). The carbon content provides a measure closely related to metabolic activity, in contrast to fresh weight, which is influenced by the variable water content of the various organisms. The mean body mass of the individual plankton groups (phytoplankton, heterotrophic nanoflagellates, ciliates, rotifers and herbivorous and carnivorous crustaceans) was calculated as the geometric mean, weighted by the biomass within each size class.

Photosynthetic rates were determined using a radiocarbon method: duplicate light bottles and one dark bottle were filled with water collected at 15 depths covering the euphotic zone and, after an addition of ^{14}C incubated in situ at the respective sampling depths for four h around local noon time. The samples were filtered onto membrane filters after withdrawing an aliquot for measuring the added activity and the radioactivity incorporated into particles > 0.8 μm was measured with a liquid scintillation counter. During the incubation period, a concomitant profile of the photosynthetically available radiation was recorded by an underwater scalar irradiance meter. Daily photosynthetic rates were extrapolated from the vertical integrals of the four hour incubations using Talling's light division hours (Tilzer and Beese 1988). When comparing phytoplankton and zooplankton data we assured the comparability of measurements of primary and secondary production by calculating net PP. This was done by subtracting dark respiration (20% of gross PP) and exudation (5–15% of gross PP depending on the season) from gross PP (Gaedke and Straile 1994a).

Production of heterotrophic flagellates was measured in situ by the dilution technique (24 h of incubation) in clean plexiglas diffusion chambers of 3.8 l volume after predators had been removed by filtration (Weisse 1991, 1997). The same in situ technique was used to obtain production estimates for ciliates (Weisse and Müller 1998) which were

related to estimates derived from maximum laboratory growth rates (Montagnes et al. 1988), the observed net biomass increase in spring, and the balance between primary production and herbivore demands (Gaedke et al. 2002). In situ growth rates were comparable to laboratory measurements conducted at the same temperature in spring and autumn and distinctly lower during summer (Weisse and Müller 1998). This fits with the observed net biomass increase in spring which can only be achieved when assuming maximum growth rates, and also with the balance between primary production and herbivore demands. During spring, measured primary production is sufficiently high to maintain maximum ciliate growth which is not the case during summer (Gaedke and Straile 1994b, Gaedke et al. 2002). Production estimates for rotifers and crustaceans were obtained with the 'growth increment method' (Geller 1989, Wölfl 1991, Gaedke et al. 2002), which relies on size- or stage-resolved counts of in situ abundances, measurements of the weight increments between stages, and experimentally determined growth curves (Lampert and Sommer 1997). The validity of these estimates under in situ conditions was cross-checked for rotifers carrying eggs, using egg ratios and egg development times (Lampert and Sommer 1997), and for crustaceans using some in situ grazing experiments (Pinto-Coelho 1991). All techniques to measure production including those conducted in situ involve some degree of uncertainty and some of the above-mentioned estimates of zooplankton production are based on laboratory measurements which, for part of the year, are unlikely to be reached in the field due to food limitation. However, at Lake Constance zooplankton is mostly under severe predation pressure (Gaedke and Straile 1994b). This implies that growth rates are typically relatively high (i.e. close to laboratory measurements) or predation is not compensated by growth, leading to low in situ abundances. In addition, we cross-checked results with different techniques, and we established temporally resolved models of balanced production of the entire food web, which accounted for the available food quantity and quality simultaneously (Gaedke et al. 2002). By these means we obtained likely values of zooplankton production in situ which were used in this study. Accounting for food quantity and quality modified the ratio between production and biomass by a factor of up to 5, but typically much less, whereas biomass changed by 2 orders of magnitude or more throughout the season. We restricted our analysis to the eukaryotic plankton as it is well-established that pelagic in situ bacterial production strongly deviates from what is expected from their cell size and biomass (Simon and Tilzer 1987). Biomass data were expressed in g C m^{-2} and production in g C m^{-2} d^{-1}.

The models

The standard model of the MTE is (Brown et al. 2004):

$$I = i_0 M^b e^{E/kT} \qquad (1)$$

where I is the individual metabolic rate, i_0 is a normalization constant independent of body size and temperature, M is body mass (g), b is the allometric exponent, E is the average activation energy of metabolic reactions (eV), k is the Boltzmann constant (8.61×10^{-5} eV K^{-1}), and T is temperature (K).

To fit this model to data, usually it is first transformed to mass-corrected (or, alternatively, temperature-corrected) metabolism and then linearized by taking natural logarithm of both sides, as:

$$\ln \frac{I}{M^b} = \ln(i_0) + E\frac{1}{kT} \qquad (2)$$

Equation 2 is then fitted to data by least-squares linear regression. In this way, we can calculate i_0 and E as the intercept and slope of the regression, respectively. However, this approach has two problems. Most importantly, it does not allow to fit the three parameters (i_0, b and E) simultaneously, but we have to first assume a value for b (or a value for E for temperature-corrected metabolism) before the other two parameters are determined. Hence, the values obtained for i_0 and E may not be the best possible fitting values, but only the best fitting given the chosen b value. As a way around this, we fitted Eq. 2 with increasing values of b from 0 to 1 (obtaining different values for i_0 and E). The predicted production values for each b were then compared to observed values by linear regression (log–log), the r^2 of which reflects the effect of changes in b. Second, the value of the proportionality constant, calculated by exponentiating the estimated intercept, will be a biased predictor of its arithmetic mean (see for instance Smith 1993). For our data set this bias was negligible, and would only become relevant if the fitted values are used to make predictions. To our knowledge, this is never addressed in the context of MTE.

Since our measured data represent the production of the whole community (primary production for phytoplankton or secondary production for zooplankton), rather than of individuals, we expressed the community metabolic rate (P) as the sum of the metabolic rates of all individuals in each species or size class present in the community at a given sampling date (Enquist et al. 2003).

$$P = i_0 \left[\sum_{j=1}^{S} N_j M_j^b \right] e^{E/kT} \qquad (3)$$

where P is production (either of phytoplankton or zooplankton), S is the number of species or size classes, N_j is the number of individuals of species or size-class j, and M_j is the average body mass of individuals of species or size class j. For brevity, we refer to the expression between square brackets in Eq. 3 as M_c.

Since least-squares linear regression on the log-transformed model is the most used method to fit the MTE model, we also applied it for comparison with previous works. However, the limitations of this method can be avoided by using maximum likelihood estimation (ML). Using ML we can fit the three parameters simultaneously in the original, non-linear form of the model, without the need to assume a particular a priori value for b. To apply ML, we assumed a gamma distribution for the stochastic component of the models, because of its flexibility and because observed production (both primary and secondary) were strongly skewed to the right. To minimize the log-likelihood function we used the MATLAB function fminsearch, which incorporates a Nelder–Mead simplex

search method. As starting values for parameters i_0 and E, we used those obtained from the linear fit described above, while b was initially set to 0.75, the value most frequently proposed in the literature. To calculate confidence intervals we produced likelihood profiles for a reasonable range of values for each parameter in turn, allowing the other two parameters to vary in the minimization. From these profiles, confidence intervals were determined using the likelihood ratio test. In some cases, approximate confidence intervals can be derived more directly than using likelihood profiles, from the variance–covariance matrix, which is estimated as the inverse of the Hessian of the model (second-order derivatives for the log-likelihood function) evaluated at the best estimates of each parameter. Unfortunately, the Hessian of our models was undefined in most cases and prevented us to apply this method. We used ML to fit Eq. 3 to phytoplankton and zooplankton data separately, and compared the parameter values obtained with those from least-squares linear regression (Table 1). We also tested the ability of each set of parameters to predict the observed values using linear regression of measured versus predicted values, in log-log scale.

Other factors: radiation and grazing in phytoplankton

Temperature is the only environmental factor considered in the MTE model. However, temperature is obviously not the only factor affecting the metabolism of individuals. For primary producers in particular, radiation is a determining factor. To compare the explanatory power of temperature and radiation in the metabolic model and its ability to predict temporal variations, we extended Eq. 3 as:

$$P = i_0 M_c e^{E/kT} PAR^c \qquad (4)$$

where c is a fitted constant and PAR is photosynthetically active radiation. We also tried two other functions to incorporate the effect of radiation on production, a Michaelis–Menten type model $(PAR/PAR + K_m)$ as in Lopez-Urrutia et al. (2007), and a more complex one parameterized specifically for Lake Constance (Tirok and Gaedke 2007), which accounts for factors like turbidity, density-dependent self-shading, and vertical integration of production (using the formulation of Steele 1962). The power function, the Michaelis-Menten function and the Tirok-Gaedke model produced almost identical results when using the fitted value (c = 0.45). Furthermore, the latter model did not improve the fit of the MTE model over the other two, so we chose the power function for simplicity.

Photosynthetically active radiation and temperature were correlated only to some extent (Pearson correlation coefficient was 0.34, p $\ll$ 0.001), and the results of the analysis indicated that the effect of temperature on phytoplankton metabolism was very weak (Results). This enabled us to test their effects separately by analyzing a reduced model including only PAR and dropping the temperature factor $e^{(E/kT)}$ (usually called the Boltzmann factor). This reduced model is:

$$P = i_0 M_c PAR^c \qquad (5)$$

Table 1. Values of the parameter estimates of each model. The column 'Group' indicates the group of plankton the model refers to (phytoplankton or zooplankton). Column 'Method' indicates the estimation procedure: LS for least-squares regression of the linearized model, and ML for maximum likelihood estimation. Column 'Factors' indicates the factors included into the model: T =temperature, R =radiation and G =grazing. For each parameter the 95% CI is indicated between parenthesis. The estimates of b obtained with LS have no CI (see text for details). The CI for parameter d in the model with temperature, radiation and grazing could not be calculated due to the flatness of the likelihood profile. Column AIC shows the value of the Akaike information criterion for each model, with the percent increment over the minimum value between parenthesis.

Group	Method	Factors	i_0	b	E	c	d	Adj. r^2	n	AIC (%Δ)
Phyto	LS	T	0.017 (2.4 ×10⁻⁴,1.17)	0.65	−0.062 (−0.17,0.04)	–	–	0.53	519	
Phyto	ML	T	0.12 (0.09,0.14)	0.86 (0.74,0.98)	−0.04 (−0.15,0.12)	–	–	0.53	519	740 (30%)
Phyto	ML	T+R	3 ×10⁻⁶ (2.9 ×10⁻⁷,7.5 ×10⁻³)	0.90 (0.78,1.02)	0.20 (0.08,0.36)	0.45 (0.35,0.55)	–	0.67	519	611 (15%)
Phyto	ML	R	3.22 ×10⁻³ (−0.005,0.01)	0.85 (0.74,0.95)	–	0.41 (0.33,0.49)	–	0.68	519	621 (16%)
Phyto	ML	T+R+G	3.98 ×10⁻⁸ (3.9 ×10⁻⁸,4.3 ×10⁻⁸)	0.90 (0.84,0.96)	0.23 (0.15,0.40)	0.45 (0.34,0.56)	0.21	0.66	482	520 (0%)
Phyto	ML	R+G	1.73 ×10⁻³ (−2 ×10⁻³,4.95 ×10⁻³)	0.82 (0.72,0.92)	–	0.41 (0.33, 0.48)	0.11 (0.01, 0.21)	0.66	482	596 (13%)
Zoo	LS	T	709 (16,31864)	0.85	−0.32 (−0.41, −0.22)	–	–	0.82	258	
Zoo	ML	T	28 (−75,131)	0.85 (0.83,0.87)	−0.19 (−0.28, −0.1)	–	–	0.80	258	

Grazing

During early summer, cladocerans, and in particular daphnids, graze heavily on phytoplankton. This selects for small, fast growing species, reduces competition by increasing resource and light availability, and so maintains the remaining phytoplankton at a high rate of renovation, increasing the observed weight-specific metabolic activity (Sommer et al. 1986, Gaedke et al. 2002). These effects are not captured by Eq. 3, 4 and 5. To introduce the effect of grazing we assumed the following. First, as predictor of grazing intensity (D), we used the natural logarithm of the total biomass of the herbivorous cladocerans, with a minimum limit of 1 mg C m^{-2} (whenever the biomass is lower than that limit, we used 1 instead). Second, grazing by daphnids is only a strong factor in May and June, thus we set all values of D outside this period to 1. Third, we assumed a power function for the effect of grazing on phytoplankton production. Thus, the model is:

$$P = i_0 M_c e^{E/kT} PAR^c D^d \qquad (6)$$

Finally, we tested a model including PAR and grazing but not temperature as:

$$P = i_0 M_c PAR^c D^d \qquad (7)$$

Due to mismatch in the sampling dates of the different variables, the number of data points for these models is lower than for the others (n = 482 instead of 519). Models in Eq. 3 to 7 were fitted to the phytoplankton data using ML. Then, all models were compared with the Akaike information criterion (AIC). For zooplankton, only Eq. 3 was used.

Results

The values of all parameter estimates (with 95% CI) are presented in Table 1. The results for the proportionality constant (i_0) are not discussed in detail, since it depends on the group of organisms and the units used. We therefore focus on the values of the allometric exponent (b), the activation energy (E) reflecting the temperature effect, and the exponents of PAR and grazing (c and d respectively).

Standard MTE model: temperature

The parameter estimates obtained by linear least-squares regression (LS) and by maximum likelihood (ML) were similar, except for the value of i_0 which was one order of magnitude larger in the ML estimate. The value of E was −0.06 with LS and −0.04 with ML, both not significantly different from 0 and none of the estimates included the assumed value of −0.32 for autotrophic processes (Allen et al. 2005) in their confidence intervals. This, together with the scatter in the data (Fig. 1A), clearly suggest that temperature, contrary to MTE predictions, plays only a minor role (if any) in regulating the metabolic activity of the autotrophic community.

With respect to the allometric exponent (b), we do not have a true estimate when using LS, since we have to assume an a priori value to fit the model. The r^2 profile obtained
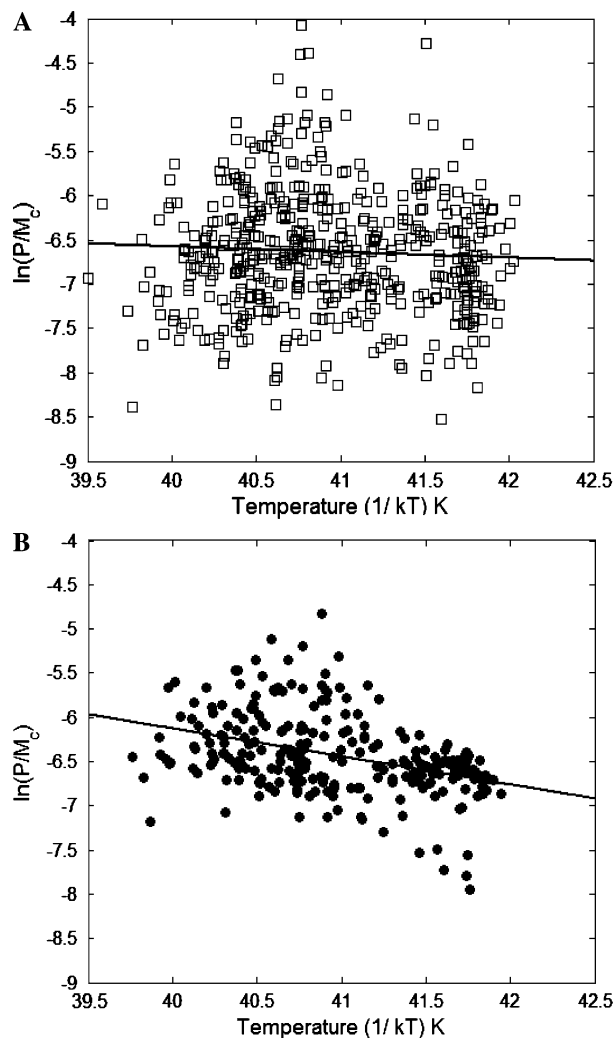

Figure 1. (A) and (B): mass-corrected community metabolic rate vs inverse of temperature for phytoplankton (A) and zooplankton (B) fitted line: $\ln(P/M_c) = \ln(i_0) + E(1/kT)$ (Eq. 3).

using increasing values of b (Fig. 2), indicated that the best fitting value was 0.65, but this value is of little significance, given the flatness of the curve and the absence of confidence intervals (which would be very wide). The estimate of b obtained with ML was 0.86, higher than the value of ¾ assumed by the MTE model, which is barely included in the confidence interval.

When comparing observed and predicted production by linear regression (log–log) using the parameter estimates from LS, Eq. 3 reached an r^2 of 0.53 with both methods.

For zooplankton, the value of E was considerably higher than for phytoplankton (−0.32 with LS and −0.19 with ML), indicating a stronger influence of temperature on its metabolism (Fig. 1B). However, the value for heterotrophic processes assumed by the MTE, approximately −0.6 (Gillooly et al. 2001, Allen et al. 2005), is not included in the confidence interval. The value of b that produced the best fit to the data (using the same procedure as for phytoplankton) was b = 0.85 (Fig. 2), a value considerably higher than the one proposed by the MTE of 0.75. In this case, the model showed a much stronger response to changes in b. The ML estimate of b was 0.85, identical
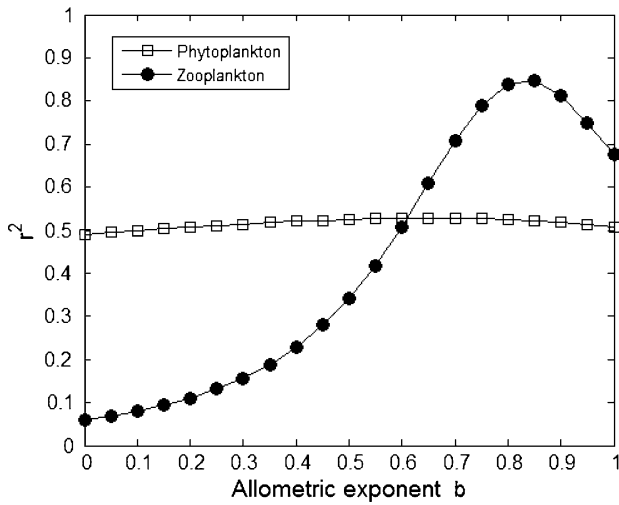
Figure 2. Relationship between the r^2 of the regression predicted vs observed P, and the allometric exponent b for Eq. 3 fitted by least-squares, for phytoplankton and zooplankton.

to the the value obtained by the r^2 profile. The narrow confidence interval also suggest a stronger effect of this parameter on model results. The model predicted zooplankton production much more accurately than primary production. The r^2 of the log-log regression between predicted and observed values was 0.82 (LS), and 0.80 (ML). For the remaining of this section we refer only to phytoplankton models, since the additional factors we examined (radiation and grazing) do not apply to zooplankton.

The effect of radiation and grazing

Obviously, the values of the parameters differ among the different models, so their values are not directly comparable among them. Including radiation into the model besides body mass and temperature (Eq. 4) increased the estimate of b to 0.90, and E to 0.20 making it positive, instead of negative. Adding radiation improved the goodness of fit compared to the model including only temperature, reaching an r^2 of 0.67. On the other hand, removing temperature and leaving only radiation led to very similar results (the relationship of radiation with mass-corrected metabolism is shown in Fig. 3). This reinforces the idea that temperature plays a negligible role in the prediction of phytoplankton metabolism. Adding grazing by cladocerans as an additional factor, besides temperature and radiation, added complexity to the model but it did not improve the r^2. Again, removing temperature and leaving only radiation and grazing as predictors did not reduce the r^2 of the model, and changed little the estimates of the parameters (b = 0.82, c = 0.41, d = 0.11). Finally, leaving only radiation as predictor (besides body mass) resulted in the best fit, r^2 = 0.68 (b = 0.85 and c = 0.41). In general, the value of c hardly changed by including or excluding other variables, which points to a strong direct effect of radiation and little confounding with other factors. Estimated with ML, the value of b was consistently high in all models, and the assumed value of $\frac{3}{4}$ was hardly included in the confidence intervals. The value of E differed greatly among
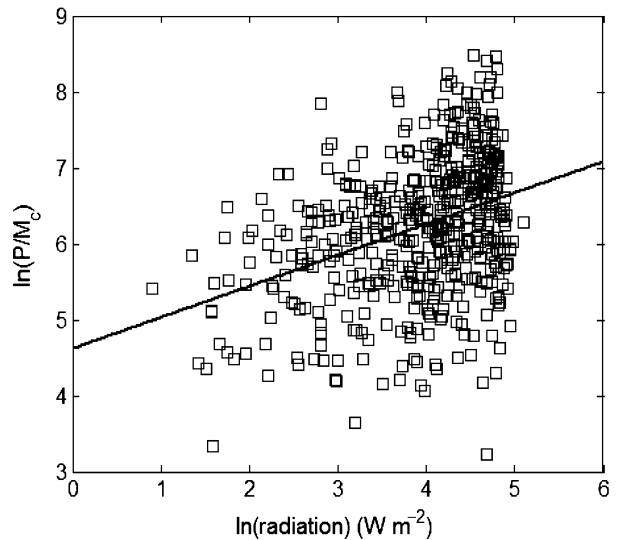

Figure 3. Mass-corrected community metabolic rate vs radiation for phytoplankton. Fitted line: $\ln(P/M_c) = \ln(i_0) + c\,PAR$.

models and was either indistinguishable from 0 or positive. This implies that higher temperatures are related to lower metabolic rates, once the effect of radiation is taken into account, which is most likely due to indirect effects. The small values of d (especially when considering that they refer to the logarithm of the variable) might be attributable to the restricted period of time considered (clear water phase) where the density of cladocerans is generally high. Accounting for grazing did not improve the r^2, but reduced the residuals during this period (Fig. 4).

The Akaike information criterion (Table 1), which takes into account maximum likelihood and penalizes models with more parameters, suggests that the best model overall (the one with the minimum AIC) is the most complex one, including temperature, radiation and grazing (AIC = 520), while the worst is the standard MTE model (AIC = 740), the other models reaching intermediate values between
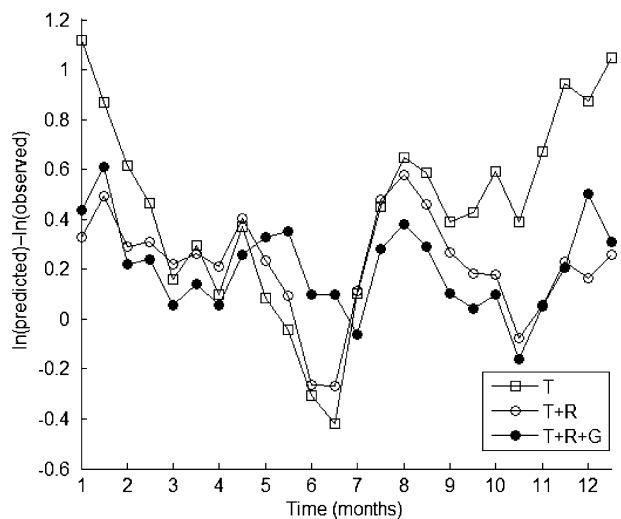

Figure 4. Residuals of Eq. 3 (temperature), 4 (temperature and radiation) and 6 (temperature, radiation and grazing), fitted to the data with ML. In the fitting we assumed a gamma distribution, which leads to a sum of residuals larger than 0.
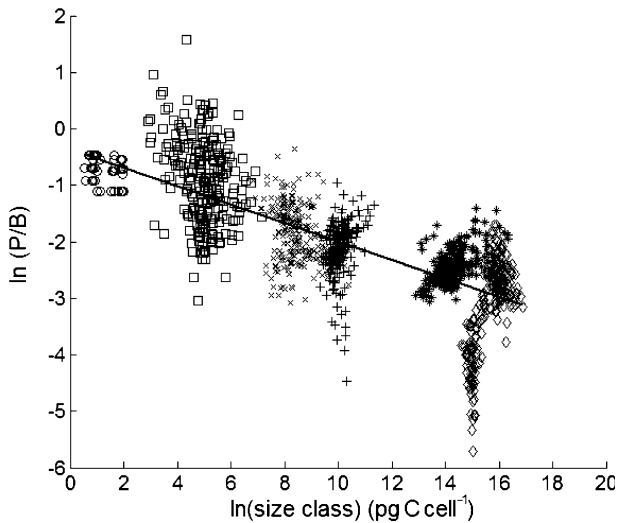
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Figure 5. Relationship between mean body mass and the ratio production to biomass (P/B) for each group of organisms. The slope (−0.15) is equivalent to (b−1). Legend: circles = heterotrophic nanoflagelates; squares = phytoplankton; x-shape = ciliates; cross = rotifers; diamonds = herbivorous crustaceans; asterisks = carnivorous crustaceans.

these two. However, the relative differences of the AIC between all models (except the standard MTE) is relatively small, approximately 15%.

In Fig. 5, the combined data from phytoplankton and zooplankton are represented as the ratio of observed production to biomass (P/B ratio) with respect to body size. Fitting a linear regression to these data (in log–log), we obtained a value of b = 0.85 (actually, the slope of the regression line was −0.15, which would be equal to b–1), very close to the values of the allometric exponent from the other models described above.

Discussion

We fitted the standard model of the MTE to an extensive data set (comprising approximately 15 years of data of primary production and 12 years of secondary production) of lacustrine plankton, and compared the parameter values with those assumed by the MTE, namely that the allometric exponent, b, is ∼3/4 and that the activation energy of basic metabolic reactions, E, is ∼0.3 eV for autotrophic metabolism and ∼0.6 eV for heterotrophic metabolism. The fitting was made with two methods: linear least-squares regression (LS) on the log-transformed MTE model, and maximum likelihood (ML). We also extended the MTE model for phytoplankton to include two other factors: radiation and grazing by zooplankton, and analysed how these factors influenced the predictive capabilities of the model.

Our data did not fit the predictions of the MTE for primary producers, independently of the fitting method used: LS or ML (Table 1). The slope of the mass-corrected metabolic rate with respect to temperature (E) was far from the assumed value of −0.3 for autotrophic processes, in fact it was undistinguishable from 0. The strong scattering around the tendency line suggests that other, more

influential processes obscure a potential role of temperature, and stress its relatively small role in regulating the metabolic activity of this group of organisms. Moreover, the annual pattern of the residuals of Eq. 3 showed a systematic overestimate of primary production during winter, implying that other factors limit primary production more than temperature. It should be noted that the present data set comprises a considerable temperature range (from 3 to 20°C), which is, however, smaller than that in Gillooly et al. (2001), approximately from 5 to 40°C for unicellular organisms. A broader temperature range might have produced a stronger relationship. However, Marbá et al. (2007) showed recently that the allometric scaling of birth and death rates of plants, ranging from unicellular phytoplankton to large trees, was also independent of temperature. Also, Clarke (2006) argues that the relationship between metabolic rate and temperature is not truly mechanistic, since metabolism is the result of a large number of processes, and should be considered only a statistical description of a number of different evolutionary optimizations.

The second assumption of the MTE model, that the exponent of body mass is ¾, was not supported by our data either, at the level of community metabolism. Fitting the model with increasing values of b revealed that the effect of varying the allometric exponent was negligible in the case of phytoplankton. Moreover, the value of b estimated from ML was 0.86, and the assumed value of ¾ was just included in the 95% confidence interval. The low response of community metabolism to b may suggest a weak dependency of metabolism on body size, which may originate from the fact that the temporal variability of mean phytoplankton cell size was relatively small, compared with the variability of biomass. The daily geometric mean of individual size (weighted by biomass) changed by a factor of 7 (calculated as the ratio of the 95th to the 5th percentiles), while the daily total biomass changed by a factor of 11, and measured primary production, in turn, by a factor of 21. This, added to the fact that variability in size is dampened by the allometric exponent, allows biomass to explain more of the variance than does individual size. Another point to consider is that the theoretical basis for the ¾ value (West et al. 1997) is mainly the volume-filling, fractal nature of distribution networks in organisms, a rationale that has been criticized by Painter (2005) and van der Meer (2006). Finally, when observed and predicted values are compared with log-log regression, using the best estimates of the parameters, the MTE model showed a low r^2 (0.52).

When radiation was introduced into the model, the fit improved considerably ($r^2 = 0.67$), as would be expected for photosynthetic organisms. Almost the same result was obtained using only radiation and removing temperature as predictor ($r^2 = 0.68$), which reinforces the conclusion that temperature has little effect on phytoplankton community metabolism, contrary to the assumption of generality of the metabolic theory of ecology. To our knowledge, only López-Urrutia et al. (2007) incorporated radiation to the MTE model, but they did not compare the extended model (including radiation) with the standard one. Radiation decreases exponentially, not only in the water column, but also in well developed plant canopies (de Castro 2000),

making it a likely limiting resource in aquatic and some terrestrial ecosystems alike, irrespective of temperature. Photosynthesis, in turn, is more limited by radiation availability than by temperature as it decreases when radiation falls below its saturation point, which is often the case in the field. Including radiation into the model strongly reduces the residuals during winter (Fig. 4), showing that radiation limitation explains to a considerable extent the overestimation of Eq. 3 in that part of the year. Consequently, we suggest that including radiation would improve the MTE in the case of photosynthetic organisms. Considering other abiotic factors may also improve the model, for instance Algar et al. (2007) suggested that including water availability would improve the accuracy of predictions of latitudinal diversity patterns. Besides abiotic factors, also biotic ones may play a role in the regulation of individual and community metabolic activity, e.g. by influencing resource availability and selecting for distinct functional types. The underestimation of phytoplankton production by Eq. 3 and 4 during the period of most intense grazing was reduced when we included the effect of grazing on phytoplankton, although it did not improve the general fit. The goodness of fit of the models emphasized the dominant role of radiation for predicting primary production.

The Akaike information criterion (AIC) suggested that the 'best' model overall is the most complex one, including temperature, radiation and grazing as predictors (apart from abundance and body size, which are obviously included in all models), although it penalizes it for having more parameters. The model with the highest r^2 was the one including only radiation, but the difference with the AIC 'best' model was very small. The worst model, according to the AIC and the r^2, was in fact the standard MTE model. The other three models were very close to each other in their AIC values, with only a difference between 13% and 16% with respect to the best one.

For zooplankton, the fit of the MTE model was much better than for phytoplankton, the r^2 of model predictions vs observations was 0.82 using the LS estimates and 0.80 using ML estimates. The value of E was negative (-0.32 with LS and -0.19 with ML) as assumed by the MTE, but significantly lower than the assumed value of -0.6, which was, by far, not included in the confidence intervals. The allometric exponent was b = 0.85 with both LS and ML, significantly higher than the assumed invariant ¾. In contrast to phytoplankton, the allometric exponent had a strong effect on the model fit. This was caused by the larger variability of mean body size (108-fold) compared to the variability in total biomass (6 fold) which increased the influence of body size on the calculation of community metabolism. The large temporal variation in mean body size originated, in turn, from the sequential dominance of plankton groups of very different sizes during the annual cycle. More generally, we would expect that, in any community where mean body size exhibits little variability, either because species composition is constant, or because species are substituted by similarly-sized ones, individual size will be of little importance when scaling-up metabolic activity from individuals to the community, and vice versa.

Considering together the entire eukaryotic plankton community (i.e. phytoplankton and zooplankton) and regressing directly the weight-specific production against body size, we obtained again a value of 0.85 for the allometric exponent (Fig. 5). This implies a less pronounced decrease of the mass-specific metabolic activity with increasing body mass than expected from MTE predictions for the plankton community, ranging over 7 orders of magnitude in body size. A weak size-dependency was also found for marine plankton by Moloney et al. (1991). The P/B values of the individual plankton groups declined largely linearly along the size gradient. The scatter around the tendency line arises mostly from including high and low temperature and resource conditions. The effect of different life history strategies between mostly carnivorous copepods (slow growth, temperature sensitive, prevailing in winter/ spring) and herbivorous cladocerans (fast growth, most abundant in summer) is also reflected in the residuals around the tendency line.

In conclusion, we found that the MTE has a limited predictive power at the scale of temporal variability of lake plankton. The MTE did not predict well the metabolic activity of the autotrophic community, nor its temporal variability, and its two major assumptions were not supported by the data. Other authors also pointed out that plants do not accommodate to several predictions or assumptions of the MTE (Russo et al. 2007). The zooplankton community metabolism fitted relatively better to the MTE, but we still found strong differences to the assumptions of the theory. This reflects an obvious and fundamental difference between photosynthetic and non-photosynthetic organisms. While temperature affects the metabolism of both, photosynthetic organisms are further (and typically more) affected by radiation limitations, which are not considered in the MTE. We suggest that the MTE would improve if it differentiated between photosynthetic and non-photosynthetic organisms, and should include radiation in the case of the former.

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