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Potential respiration is a better respiratory predictor than biomass in young *Artemia salina*

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ABSTRACT

These experiments test whether respiration can be predicted better from biomass or from potential respiration, a measurement of the mitochondrial and microsomal respiratory electron transport systems. For nearly a century Kleiber's law or a similar precursor have argued the importance of biomass in predicting respiration. In the last decade, a version of the Metabolic Theory of Ecology has elaborated on Kleiber's Law adding emphasis to the importance of biomass in predicting respiration. We argue that Kleiber's law works because biomass packages mitochondria and microsomal electron transport complexes. On a scale of five orders of magnitude we have shown previously that potential respiration predicts respiration as well as biomass in marine zooplankton. Here, using cultures of the branchiopod, *Artemia salina* and on a scale of less than 2 orders of magnitude, we investigated the power of biomass and potential respiration in predicting respiration. We measured biomass, respiration and potential respiration in Artemia grown in different ways and found that potential respiration (Φ) could predict respiration (R), both in $\mu lO_2\,h^{-1}$ ($R = 0.924\Phi + 0.062$, $r^2 = 0.976$), but biomass (as mg dry mass) could not ($R = 27.02DM + 8.857$, $r^2 = 0.128$). Furthermore the R/Φ ratio appeared independent of age and differences in the food source.

1. Introduction

The Metabolic Theory of Ecology (MTE) (Brown et al, 2004; Allan and Gillooly, 2007; Kolokotrones et al., 2010) and Kleiber's law (Kleiber, 1932, 1961; White, 2010) argue the importance of biomass in controlling respiration. Modern biochemistry since the time of David Keilin argues that the respiratory electron transport system controls respiration (Keilin, 1966; Nelson and Cox, 2005). We have shown before that in marine zooplankton the respiratory electron transport system (ETS) activity is a better predictor of respiration than biomass (King and Packard, 1975; Packard and Gómez, 2008). Furthermore, Findlay et al (1983) have shown that over 11 orders of magnitude ETS and respiration follow a linear relationship. Here, we examine ETS activity as a predictor of respiration in young brine shrimp of *Artemia salina*.

Our investigation builds on the results of Ikeda et al. (2001) working with *Acartia tonsa*; Ikeda et al. (2004) with *Neocalanus cristatus*; Varó et al. (1993, 2000) with different species of *Artemia*; Irwin et al. (2007), with *Artemia franciscana,* and many others. Using ETS activity as a measure of potential respiration minimizes problems of stress, starvation as well as bacterial growth that confound incubation-based respiration measures. It measures the flux of electrons after they are stripped from tricarboxylic acids and other organic compounds and transferred to the ATP-generating and the O_2-consuming machinery of the respiratory ETS. It is quantitative, and, in theory, measures the maximal rate at which reducing equivalents are transferred from organic oxidation reactions to the oxygen consuming ones. *Artemia salina* was chosen for these experiments, because it has a history of being a model organism in both ecological and physiological studies as well as being commercially important to aquaculture.

In this research, respiration, biomass, and ETS activity are purposely measured in two independently grown cultures of *A. salina* to investigate whether ETS activity or biomass is a better index of respiration and to determine the strength of this relationship.

2. Materials and methods

Respiration, ETS activity, dry mass, and protein, were measured in triplicate in two independent sets of experiments with *A. salina* cultures maintained under controlled temperature, light, and food conditions (Table 1). Our main objective was to study the respiration, ETS activity and the coherence of *R*/ETS ratio under some different growth conditions.

2.1. Culturing

Artemia cysts, obtained from Metaframe (San Francisco Bay Division Network, California) were hatched in filtered aerated seawater at constant temperature and after 24 h the nauplii were placed in new aquaria. The first set of rate measurements were performed at 25 °C for 15 days with nauplii fed on *Nanoclorosis* sp. at a cell density of

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Table 1
Parameters in the two sets of experiments with *Artemia salina*. The rate data in set 2 were measured at 22 °C and corrected via the Arrhenius equation to 25 °C. The rate data in set 1 were measured at 25 °C. Mean, standard deviations and ranges are given.

Measurements	Set 1 25 °C	Set 2 25 °C	Set 2 25 °C
Incubation period (days)	0–15	0–15	15–30
Food	*Nanoclorosis sp.* (5000 cells·ind^{-1})	*Dunaliella salina* (1000 cells·ind^{-1})	*Dunaliella salina* (1000 cells·ind^{-1})
Biomass (μgC·ind^{-1})	2.06 ± 4.64 (0.51–20.04) $n=17$	1.04 ± 0.37 (0.54–2.18) $n=21$	10.76 ± 8.64 (1.58–26.42) $n=11$
Growth rate (d^{-1})	0.26 ± 0.43 (−0.52–1.16) $n=14$	0.12 ± 0.83 (−1.20–1.73) $n=14$	0.51 ± 0.64 (0.09–2.07) $n=8$
Respiration rate (μlO$_2$·mgDM^{-1}h^{-1})	55.79 ± 22.82 (14.90–94.92) $n=15$	9.48 ± 4.53 (3.87–19.72) $n=12$	20.21 ± 12.13 (8.93–33.22) $n=4$
Potential respiration rate (μlO$_2$·mgDM^{-1}h^{-1})	70.45 ± 31.71 (35.13–134.89) $n=15$	9.72 ± 7.49 (2.33–32.81) $n=20$	10.67 ± 12.25 (1.32–35.89) $n=9$

50,000 cells·ml^{-1}, 5000 cells·ind^{-1} (Set 1). The second set of measurements was carried out at 22 °C for 30 days with nauplii fed on *Dunaliella salina* (7155 cells·ml^{-1}, 1000 cells·ind^{-1}). The phytoplankton were fed to the nauplii every 24 h. The aquaria were illuminated under a 10/14 hour photoperiod with 36 W fluorescent tubes.

2.2. Biomass and growth

Dry mass was determined in triplicate by the method of Lovegrove (1966). We refer to dry mass instead of dry weight according to Postel et al (1995) and Meyer et al. (2010). Biomass (M) in carbon units was calculated assuming that carbon represents 40% of dry mass (Omori and Ikeda, 1984; Parsons et al., 1984; Båmstedt, 1986). Protein was measured by the Lowry et al. (1951) method as modified by Rutter (1967) in order to be able to measure low levels of protein. Dry mass (DM) and protein (P) (Fig. 1) were related to each other by the Eq. (1):

$$DM = 1.649\,P + 1.03\left(r^2 = 0.97\right) \quad (1)$$

which is consistent with the average nitrogen content of zooplankton dry mass being 10% (Parsons et al., 1983) and the average nitrogen content of protein being 16% (Wolff, 1876; Postel et al., 2000; Nelson and Cox, 2005). Thus, when not measured directly, dry mass was calculated from protein content.

Growth was measured according to Kimmerer and McKinnon (1987). The growth rate (μ) in units of d^{-1} was related to the change in protein concentration by Eq. (2),

$$\mu = [(\mathrm{Lnprot}_2 - \mathrm{Lnprot}_1)/\Delta t] \quad (2)$$

where t refers to time in days.

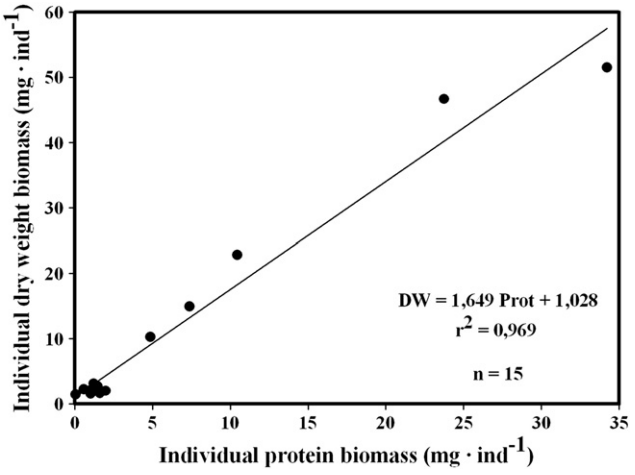

Fig. 1. Dry-mass versus protein content per individual for *Artemia salina*. Duplicate samples of Artemia were taken daily through the life of the culture for the protein and dry-mass measurements.

2.3. ETS activity and potential respiration

Potential respiration (Φ) and ETS activity were determined by the tetrazolium reduction method of Gómez et al. (1996) using an electron accepting tetrazolium salt. ETS is measured by saturating the mitochondrial and the microsomal ETS with their natural substrates, NADH and succinate, for the mitochondrial ETS, and NADPH, for the microsomal ETS (Borgmann, 1977) and then detecting the resulting electron flux along the ETS via the electron acceptor, INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyl-tetrazolium chloride). Of the many tetrazolium salts, the ETS assay here employs INT because of its sensitivity to dehydrogenase activity (Fonseca et al., 2001). ETS activity is calculated in moles of electrons (μmol e^{-1}h^{-1}DM^{-1}), but then converted stoichiometrically into volumetric oxygen units (μlO$_2$h^{-1}DM^{-1}) and reported as potential respiration. Equivalent cell-free homogenates were prepared using tissue grinding in the first group of experiments (set 1) and sonication in the second group of experiments (set 2). In our laboratory sonication and tissue grinding yield equivalent results. The tissue grinding was done in 3 ml of buffer, at 0–4 °C, for 2 min using a teflon-glass tissue grinder rotating at 1500 rpm. Sonication was done in 1 ml of water (Milli-Q double-distilled) for 45 s with an ultrasonic probe (Cole Parmer). After centrifugation (0 °C, 10 min, 4000 rpm), an aliquot of the homogenate was assayed for ETS activity. Homogenate dilution was necessary at high biomass levels. In set 2, an automatic sample pretreatment injection line system with a robotic arm (ASPEC-Gilson 221/222) was used to carry out the essays. ETS activity was calculated from the absorbance of the INT-formazan production rate in the ETS assay and from the formazan molar extinction coefficient (Kenner and Ahmed, 1975; Oxtoby et al., 1999) according to Gómez et al. (1996). ETS activity measured in this manner is consistent with the cytochrome c reduction ETS assay of Borgmann (1977).

2.4. Respiratory oxygen consumption

Respiration in set 1 was measured by the Winkler method of Bryan et al. (1976) as described in Parsons et al. (1984). This method measures the end point photometrically, with a sample incubation period between 6 and 24 h. Equivalent respiration rates were measured in set 2 using Clark-type oxygen electrodes (Strathkelvin 928 6-Channel Oxygen System) that measured oxygen concentration continuously over a period of an hour and a half. Respiration rates made at 22 °C were corrected to 25 °C by the Arrhenius Equation (Arrhenius, 1889, 1915),

$$R_2 = R_1 \cdot e^{\left[Ea(1/T_1 - 1/T_2)/R_g\right]}, \quad (3)$$

where R is the respiration rate in units of μl O$_2$h^{-1}·animal^{-1}, Ea is the molar-based activation energy (≈62.7 KJ·mol-K^{-1} or 15 Kcal·mol-K^{-1} (Packard et al., 1971, 1975), R_g is the molar-based gas constant (8.31 J·mol-K^{-1} or 1.987 cal·mol-K^{-1}) and T the absolute temperature (°K). ETS activities, growth rates, and potential respiration rates, when not measured at 25 °C, were corrected for the temperature difference in the same manner. This procedure is consistent with Arrhenius's application of Boltzmann's and Van't Hoff's theories to chemical and biochemical reactions as well as to physiological processes (Arrhenius,

Table 2
Artemia growth rates (mean, standard deviation and range) here and in the literature corrected by the Arrhenius equation to 25 °C. T is the incubation temperature.

Experiments	T (°C)	Samples	*n*	Growth rate at 25 °C (d^{-1})	Type of Food
Set 1	25	*A. salina*	15	0.26 ± 0.43 (−0.52 –1.16)	*Nanoclorosis sp.* (50,000 cells ml^{-1})
Set 2	22	*A. salina*	21	0.33 ± 0.72 (−1.20–2.07)	*Dunaliella salina* (7155 cells ml^{-1})
Sapienza and Mague (1979)	15	*A. salina*	25	1.54 ± 2.09 (0.06–3.01)	*Dunaliella tertiolecta* (0–100,000 cells ml^{-1})
Berges et al. (1990)	25	*A. franciscana*	8	0.28 ± 0.2 (0.077–0.482)	*Dunaliella salina* (2000 cells ml^{-1})
			8	0.48 ± 0.16 (0.363–0.595)	*Dunaliella salina* (10,000 cells ml^{-1})
			8	0.83 ± 0.04 (0.802–0.850)	*Dunaliella salina* (50,000 cells·ml^{-1})
Evjemo and Olsen (1999)	27	*A. franciscana*	12	0.20 ± 0.23 (0.03–0.36)	*Isochrysis galbana* (130,000 cell·ml^{-1})
Lora-Vilchis et al. (2004)	27	*A. franciscana*	60	0.49 ± 0.31 (0.27–0.71)	*Isochrysis sp.* (130,000 cells ml^{-1})

1915). It is at odds with the MTE because of the MTE's choice of the atomic-based Boltzmann constant. Nevertheless, in this way, all data here were comparable. Values from the literature were also corrected in this manner to be comparable with our results. As a result, all the rate data presented here are for 25 °C.

3. Results

3.1. Growth

Growth rates in the first 15 days of the cultures varied from 0.12 to 0.26 d^{-1} (Table 1, first 15 days) and were not related to respiration ($R = 5.759\,\mu + 33.83$, $r^2 = 0.015$), ETS activity, or biomass. The relationships with ETS activity and biomass (not shown) were as weak ($r^2 < 0.1$) as the one with respiration. The variations in the initial growth rates were likely caused by the high cell density of the algal food in set 1. From the data of Berges et al. (1990) (Table 2), it is clear that Artemia exposed to high algal densities grow faster. The impact of the higher algal cell densities in set 1 can also be seen in the early onset of the exponential growth in this set of experiments (Fig. 2); it begins 5 days earlier than in set 2. In general, when compared to other growth rates of Artemia, our growth rates are in the same range (Table 2).

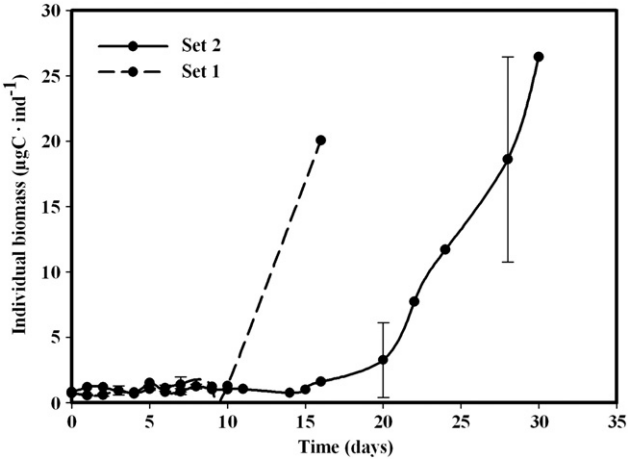

Fig. 2. Biomass time-course for the two sets of experiments (Table 1) with *A. salina*. Note that the algal concentration in set 1 is 7× higher than the algal concentration in set 2. The Artemia eggs hatched on day 0. Even in set 2 the Artemia did not reach sexual maturity.

3.2. Respiration

Respiration rates, normalized by dry-mass, vary between 12.74 ± 9.10 $\mu lO_2 \cdot mg\ DM^{-1} h^{-1}$ and 55.79 ± 22.82 $\mu lO_2 \cdot mg\ DM^{-1} h^{-1}$, well within the range of values found in literature for similar crustaceans (Table 3). However, the respiration rates are clearly lower in the cultures exposed to low concentrations of *D. salina* (Fig. 3A) than they are in the cultures with the higher concentrations of *Nanoclorosis* sp., but whether this is caused by the low cell density or by the algal species difference, cannot be determined without additional data. With biomass and with growth rate one might have expected a direct positive relationship with respiration. But in neither case was there a good relationship ($R = 27.02DM + 8.857$, $r^2 = 0.128$ and above).

3.3. Potential respiration and its relation to respiration

Rates of Φ, normalized by dry-mass, ranged from 10.02 $\mu lO_2 \cdot mg\ DM^{-1} h^{-1}$ to 70.45 $\mu lO_2\ mgDM^{-1} h^{-1}$ (Table 4). Φ, by itself, is not related to biomass and cannot be used for predictive purposes ($\Phi = 27.24DM + 10.98$, $r^2 = 0.113$). With respect to the food levels, the rates of Φ are lower in the cultures exposed to low concentrations of *D. salina* (Fig. 3B).

The ratio of respiration to potential respiration (R/Φ) did not vary with differences in the quantity and quality of the diet (Fig. 4A and Table 1). The R and Φ were higher in the cultures with the higher cell numbers (set 1), but because this offset was about the same in R and Φ, one would expect the ratio, R/Φ, to be unaffected. In fact, pooling the data from both sets of experiments (Fig. 4A), and plotting R vs Φ yielded an R/Φ ratio of 0.89 ($r^2 = 0.976$). This demonstrated that in spite of the differences in nutrition and species of the food, R/Φ stayed constant. In addition, the ratio did not change with the age of the nauplii (Fig. 4B).

Table 3
Dry mass-specific respiration rates here and in the literature, measured at or corrected to 25 °C. T is the incubation temperature. Means, standard deviations and ranges given.

References	T (°C)	Sample	Respiration rate at 25 °C ($\mu lO_2 \cdot mgDM^{-1} h^{-1}$)	Type of food
This work Set 1	25	*Artemia salina*	55.79 ± 22.82 (14.90–94.92)	*Nanoclorosis sp.*
This work Set 2	22	*Artemia salina*	12.74 ± 9.10 (3.87–45.87)	*Dunaliella salina*
King and Packard (1975)	15	*Calanus pacificus*	32.1 ± 11.35 (24.07–40.12)	Environmental Phytoplankton
Varó et al. (1993)	25	*Artemia sp.*	7.05 ± 6.81 (2.24–11.87)	Yolk sac
Simčič and Brancelj (1997)	20	*Daphnia sp.*	23.11 ± 10.89 (15.41–30.81)	*Scenedesmus sp.* Yeast
Varó et al. (2000)	24	*Artemia parthenogenetica*	3.87 ± 3.16 (1.63–6.09)	*Dunaliella sp.*
Ikeda et al. (2001)	22	*Acartia tonsa*	10.35 ± 2.35 (8.69–12.01)	Environmental Phytoplankton
Ikeda et al. (2004)	2	*Neocalanus cristatus*	3.16 ± 0.47 (2.83–3.49)	Environmental Phytoplankton
Simčič and Brancelj (2004)	20	*Daphnia sp.*	12.71 ± 14.71 (2.31–23.11)	*Scenedesmus quadricauda* Yeast
Irwin et al. (2007)	25	*Artemia franciscana*	10.41 ± 4.28 (7.39–13.44)	Yolk sac

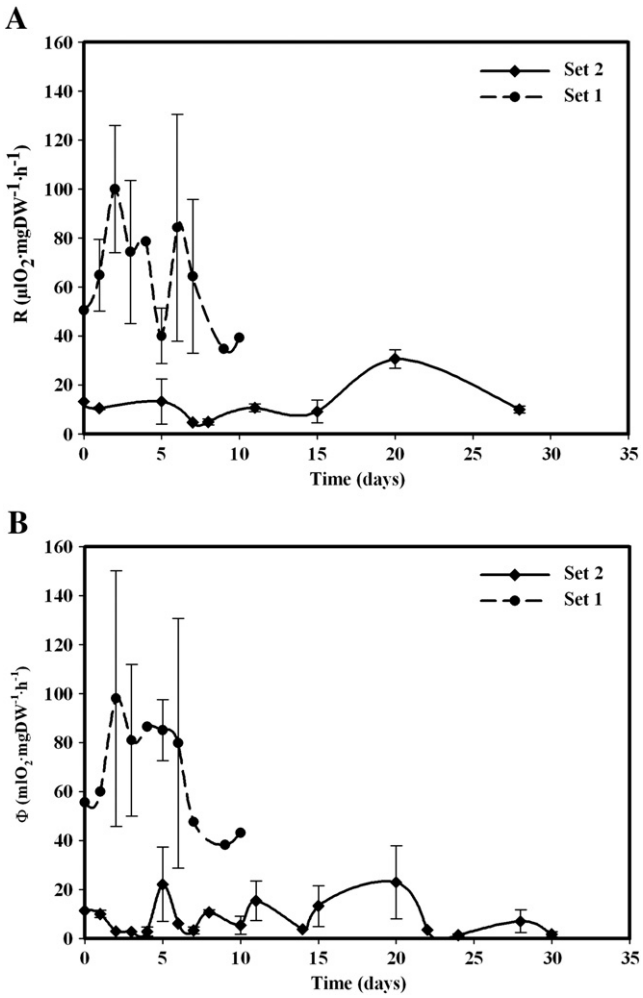

Fig. 3. Respiration (A) and potential respiration (B) time courses for both sets of experiments with *A. salina*. Both measurements are normalized by biomass (dry-mass).

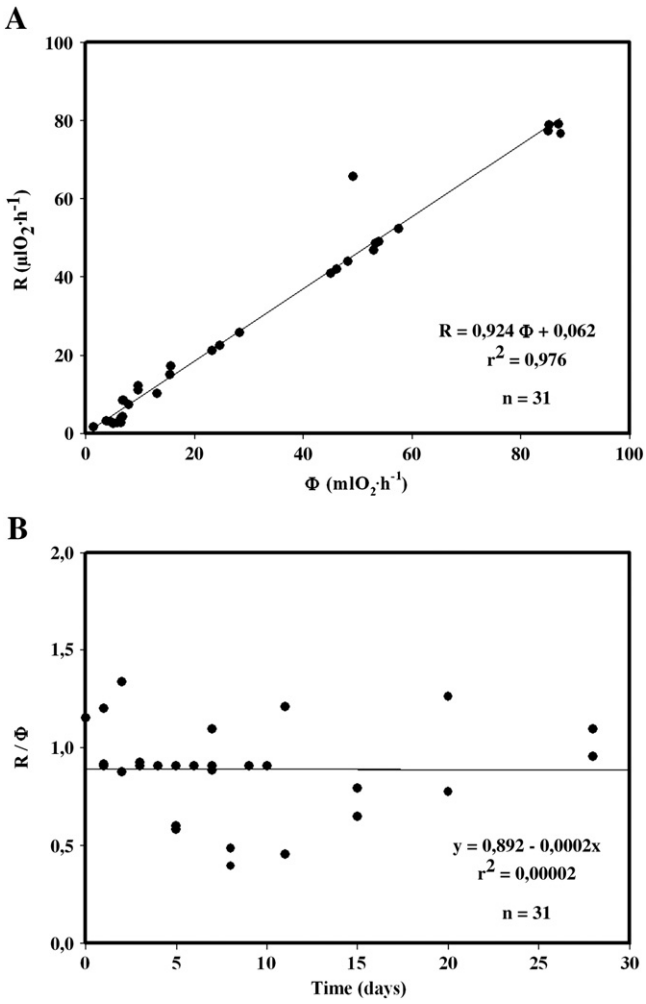

Fig. 4. (A) Respiration versus potential respiration (Φ) in the two cultures of *A. salina*. Measurements were taken on the same sample of Artemia. Immediately after the respiration rate was recorded, the Artemia were filtered from their media and frozen in liquid nitrogen for ETS analysis according to Gómez et al. (1996). (B) The time-course of the ratio of respiration to Φ in the pooled data from both *A. salina* cultures.

4. Discussion

4.1. Growth

From the literature (Table 2), the impact of the different prey species and the quantity (cells concentration) on the growth rate is not clear. In one case, when the food species remains the same, but the cell concentration increases, the growth rate increases in parallel with the food quantity (Berges et al., 1990). In another case, where the cell concentration does not change, but the food species may be different, the growth rate more than doubles (Evjemo and Olsen, 1999; Lora-Vilchis et al., 2004). In our case, both the food species and the cell concentration changes, but the growth rate remains the same suggesting that, as in the experiments of (Evjemo and Olsen, 1999; Lora-Vilchis et al., 2004), the food quality is likely an important factor. Accordingly, future experiments on the relationship between growth rate and food quality (protein, fatty acids, etc.) are needed to resolve this topic.

4.2. Respiration

Respiration in Artemia should be close to the rate at which its cytochrome oxidase reduces oxygen to water. This rate should equal stoichiometrically, the rate at which cytochrome oxidase receives electrons from Artemia's mitochondrial ETS. According to our experience with bacteria (Packard et al., 1996), the Krebs Cycle should rapidly oxidize the products of carbohydrate, lipid, and protein catabolism and produce NADH and succinate to drive the respiratory ETS at a high rate in well-nourished Artemia. Under these conditions both oxygen consumption

Table 4
Potential respiration here and from the literature. Means, standard deviations and ranges corrected to 25 °C. T is the incubation temperature.

References	T (°C)	Sample	Potential respiration rate at 25 °C ($\mu lO_2 \cdot mgDM^{-1} h^{-1}$)	Type of food
This work set 1	25	*Artemia salina*	70.45 ± 31.71 (35.13–134.89)	*Nanoclorosis sp.*
This work set 2	22	*Artemia salina*	10.02 ± 9.00 (1.32–35.90)	*Dunaliella salina*
King and Packard (1975)	15	*Calanus pacificus*	24.07 ± 3.41 (21.66–26.48)	Environmental Phytoplankton
Båmstedt (1980)	23	*Acartia tonsa*	14.08 ± 7.23 (8.9–19.26)	Phytoplankton samples (laboratory)
Simčič and Brancelj (1997)	20	*Daphnia sp.*	34.67 ± 27.23 (15.41–53.93)	*Scenedesmus sp.* Yeast
Simčič and Brancelj (2004)	20	*Daphnia sp.*	16.56 ± 20.16 (2.31–30.81)	*Scenedesmus quadricauda* Yeast

and ATP production should be high. On the contrary, when the Artemia are poorly nourished the opposite should occur. Again, building on our observations with bacteria, the mass-specific respiration rates should be higher in the well-fed culture (set 1) than in the poorly fed culture (set 2). Tables 1 and 3 show this to be the case.

Respiration and biomass (protein, dry mass, etc.), at least over many orders of magnitude, are known to follow Kleiber's Law (Kleiber, 1932 and 1961; Whitfield, 2006), mainly because biomass packages the mitochondria and microsomes that control respiration (Packard and Gómez, 2008). However, on a smaller scale the relationship can be obscured by age, sex, physiological state (activity level, nutritional state, stress level, reproductive state, etc.) to the point where biomass is not useful for predictive purposes. Here, where the biomass and respiration range less than 2 orders of magnitude (Table 1 and 3), our data is an example of this situation. The respiration-biomass relationship is weak ($R = 27.02\text{DM} + 8.857$, $r^2 = 0.128$). Identifying the cause of this weak relationship awaits future experimentation.

4.3. Potential respiration

Φ, as measured by ETS activity, is a proxy for respiration in plankton (Ramírez et al, 2006) and accordingly we expected it to follow Kleiber's law. Φ in Artemia should be dominated by the maximum rate at which Artemia's ETS can transmit electrons from its beginning, at NADH dehydrogenase in Complex I, to its end at cytochrome oxidase in Complex IV. Φ is ETS activity expressed in terms of oxygen rather than electrons. Because it is analogous to an enzyme's V_{max}, Φ is a measure of the concentration of the complexes that constitute the ETS and also, since NADH dehydrogenase is the major driver of the mitochondrial ETS, Φ is a measure of the presence of mitochondria.

Should the Φ vary with nutritional state and biomass? With nutritional state, Φ should vary little if the ETS is constitutive to the mitochondria. However, if it is inducible by substrate abundance or ATP demand (as in growth), then it could vary with nutritional state. Addressing this question was not an objective here, nevertheless Table 4 shows Φ to be 7× larger in set 1 than in set 2. In set 1 the algal cell density was 5× the level that it was in set 2. This suggests that the ETS can be induced by substrate supply as suggested by the observations of Hernández-León and Gómez (1996). However, future experiments using the same algal species are needed to address this question thoroughly. With biomass, Φ should track closely if the ETS is constitutive and track loosely if the ETS or a key part of it is easily induced or repressed in an enzymatic sense. Here, as with biomass and respiration, biomass and Φ do not track each other particularly well, again suggesting that at any given biomass level the ETS can be induced (or repressed) by changing environmental factors.

4.4. R/Φ

Christensen et al. (1980) argue that the ratio of respiration to potential respiration (R/Φ) is an index of physiological state. In bacteria, R/Φ is low when the bacteria are nutrient-limited and high when they are well nourished (Christensen et al., 1980; Packard et al., 1996). In zooplankton Hernández-León and Gómez (1996) found that temperature, diet, physiological state, and age seemed important in determining the variability of this ratio. In Artemia (Tables 1, 5 and Fig. 4A and B) the ratio, R/Φ, is relatively constant even though the Artemia are grown under different conditions.

In theory, this ratio should not exceed 1.1 if the ETS assay is a true measure of Φ and if our biochemical understanding that the respiratory ETS is responsible for 90% of a cell's respiration (Nelson and Cox, 2005) is correct. In the older literature on R/Φ (R/ETS), ratios were reported with values higher than 1.1 (King and Packard, 1975; Packard et al., 1974) because the extraction-based ETS assays, used at that time, under-measured the V_{max} of the ETS (Christensen and Packard, 1979). These values have been recalculated according to Christensen and Packard (1979) in Table 5 (ETS activities measured by the newer methods are 3.3 times higher than the older ETS measurements) as well as in Packard and Gómez (2008). The remaining values above 1.1 may reflect an unknown type of metabolism in some marine organisms or some methodological difference in the respiration or ETS analyses. Regardless of the value of the ratio, it should be independent of biomass if, as we argue, biomass simply packages the mitochondria and their ETS complexes. In addition, the ratio should be independent if respiration and the ETS activity respond in parallel to changes in growth stage, physiological state, adult age, sex, food quality, and stress. In a single organism such parallelism is unlikely in the short term, but likely in the long term. In a mono-specific culture under controlled constant conditions Φ and R should track closely and be independent of biomass. Fig. 4B show this to be the case in our experiments.

4.5. Significance

The significance of this research is twofold. It suggests an explanation to Kleiber's Law as well as an alternate way to predict respiration. The paper presents experimental evidence that Φ, as measured by the respiratory ETS, predicts respiration better than biomass and it argues that Kleiber's law works because biomass packages the mitochondria and the microsomes that drive respiration. Based not only on the relationship between Φ and respiration in Fig. 4A and in Fig. 4 of Packard and Gómez (2008), the predictive power of Φ goes back to Findlay et al. (1983)'s finding that the R-to-Φ relationship holds for bacteria, protozoans (ciliates), and metazoans (zooplankton) over 11 orders of magnitude. Corollary to this significance is the impact this R-to-Φ relationship can have on the Metabolic Theory of Ecology (Kolokotrones

Table 5
Variability of ratio R/Φ values found in literature. The ETS data from King and Packard (1975) were corrected to be consistent with the Owens and King (1975) ETS assay by the factor 3.3 (Packard and Gómez, 2008).

References	Sample	R/Φ	Location
Experiments (all dates)	*Artemia salina*	0.89 ± 0.23 ($n = 31$)	Laboratory Incubations
Set 1	*Artemia salina*	0.93 ± 0.11 ($n = 15$)	Laboratory Incubations
Set 2	*Artemia salina*	$0.85 \pm 0,30$ ($n = 16$)	Laboratory Incubations
King and Packard (1975)	*Calanus pacificus* (NI)	0.59 ± 0.19 ($n = 2$)	North Pacific
King and Packard (1975)	Copepods	0.71 ± 0.40 ($n = 6$)	North Atlantic (East) North Pacific (Tropical East)
King and Packard (1975)	Zooplankton	0.50 ± 0.17 ($n = 146$)	North Atlantic (East) North Pacific
Båmstedt (1979)	Copepods	1.39 ± 0.66 ($n = 84$)	Sweden (West Coast)
Båmstedt (1980)	*Acartia tonsa*	0.47 ± 0.08 ($n = 13$)	Florida (Miami)
Ikeda and Skjoldal (1980)	*Acartia australis*	0.16 ± 0.02 ($n = 4$)	Australia (Townsville)
Hernández-León and Gómez (1996)	Zooplankton (100–200 μm)	0.94 ± 0.47 ($n = 13$)	Baltic Sea (BALTEX)
		2.55 ± 0.86 ($n = 56$)	Canary Islands (EMIAC 9006)
		1.59 ± 0.88 ($n = 2$)	Canary Islands (EMIAC 9103)
		0.95 ± 0.98 ($n = 10$)	Gran Canaria (Onshore)

et al., 2010). It provides the foundation for an alternate model and equation for metabolism.

5. Summary

1. Respiration (R) of immature brine shrimp, *Artemia salina*, is more closely related to potential respiration (Φ), as measured by ETS activity than it is to biomass (DM). Linear regression yields a relationship between respiration and potential respiration ($R = 0.824\Phi + 0.062$, $r^2 = 0.976$) that can be used for predictive purposes and yields a much weaker relationship between respiration and dry mass ($R = 27.02\mathrm{DM} + 8.857$, $r^2 = 0.128$) that cannot.
2. Artemia respiration rates, normalized by dry-mass, were 12.74 ± 9.10 $\mu lO_2 \cdot mgDM^{-1} h^{-1}$ and 55.79 ± 22.82 $\mu lO_2 \cdot mgDM^{-1} h^{-1}$, independent of the age of the culture.
3. Potential respiration in Artemia, normalized by dry-mass, ranged from 10.02 $\mu lO_2 \cdot mgDM^{-1} h^{-1}$ DM to 70.45DM μlO_2 $mgDM^{-1} h^{-1}$.
4. Artemia growth rates for the first 15 days varied from 0.12 d^{-1}, when feeding on of the diatom, *Dunaliella salina* (cell density: 7155 cells ml^{-1}), to 0.26 d^{-1} when feeding on the *Nanoclorosis* sp. (cell density: 50000 cells ml^{-1}). They were not related to respiration, ETS activity, not biomass.

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