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An analysis of metabolic characteristics of planktonic heterotrophic protozoans

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ABSTRACT

Respiration rate data on 61 heterotrophic planktonic protozoans were compiled and analyzed as a function of body mass [wet mass (WM), dry mass (DM), carbon (C) or nitrogen (N)], temperature, measuring method (Cartesian divers, Warburg, and electrodes, among others), physiological condition (growing, starved or unspecified), and taxon (amoebae, flagellates or ciliates). Stepwise multiple-regression analyses revealed that body mass was the most important parameter, followed by the physiological condition and temperature. The taxon specific effects of body mass were detected for ciliates. Overall, the regression models explained 84% of the variance of the respiration data. Similar analyses could not be performed for the rates of ammonia excretion because of insufficient data. The comparison of the regression line of the respiration rates on WM and that of the ammonia excretion rates on WM, both of growing heterotrophic protozoans, yielded an O:N ratio of 7.2, which indicates protein-oriented metabolism. A comparison of the results of this study with the routine respiration rates of marine pelagic metazoans showed that the rates of growing protozoans were lower (0.71-fold) than those of crustaceans and fishes based on WM but were equivalent to those based on C mass.

INTRODUCTION

Planktonic protozoans, including ciliates, flagellates, and sarcodines (radiolarians, foraminiferans and phaeodarians), are key components of the microbial loop through which picophytoplankton and dissolved organic matter are incorporated into bacteria and transported to higher trophic level animals in marine food chains (Pomeroy, 1974; Azam *et al*., 1983; Legendre and Rassouzladegan, 1995). Because of their ubiquitous distribution, high abundance, trophic importance and extreme thermal tolerance, physiological rates of protozooplankton are of particular relevance to understanding oceanic biogeochemical cycles of carbon and other elements in the present and future oceans (Dolan, 1997; Caron and Hutchins, 2013; Storch *et al*., 2014).

Of various physiological processes, respiration (oxygen consumption) is of prime importance in supplying the energy required for the lives of aerobic animals. Hemmingsen (1960) plotted "standard" ("basal") respiration rates of diverse organisms with widely different body masses (bacteria to large mammals) on a log-log graph and found that the data fell on three distinct lines of similar slope (0.75) but with different intercepts, i.e., a low "unicellular" line and a high "homeotherm" line, with a "poikilotherm" line intermediate. The intercept of the "unicellular" line (which included bacteria, yeasts, protozoans, and fertilized marine eggs) is 1/8 of that of the "poikilotherm" line. Based on later analyses, although the slopes are approximately the same, the difference in the intercepts between unicellular and poikilotherm (invertebrates) lines was not significant (Gillooly *et al*., 2001), the slope was near unity instead of 0.75 (Makarieva *et al*., 2008), and changes in the slope with life-form specific body mass were detected, i.e., 1.72 for prokaryotes, 0.97 for protists, and 0.76 for metazoans (DeLong *et al*., 2010). In these studies, protozoans are treated as a

component of a broad group of unicellular organisms that includes autotrophs, mixotrophs and heterotrophs with different nutritional requirements and the different taxon of prokaryote and eukaryote.

For heterotrophic, free-living protozoans, Fenchel and Finlay (1983) compiled comprehensive respiration rate data. These authors noted that the rates varied greatly depending on the physiological conditions of animals (growing or being starved) and that the rates of growing protozoans were near comparable with those of the "poikilotherm" line established by Hemmingsen (1960). As part of an analysis of scaling properties of vital rates of 9 marine pelagic taxa that included protozoans, Kiørboe and Hirst (2014) show respiration rates of heterotrophic planktonic protozoans [a mixture of various physiological states, from Fenchel and Finlay (1983)] are lower than routine respiration rates of the marine planktonic metazoan taxa. Since the comprehensive review of Fenchel and Finlay, respiration rates have been reported for six planktonic ciliates (Kawakami *et al*., 1985; Verity, 1985) and two planktonic flagellates (Caron et al., 1986; Schmoker *et al*., 2011). Fenchel and Finlay overlooked respiration data on two ciliates (Klekowski and Tumantseva, 1981). Dolan (1997) reviewed phosphorus and ammonia excretion rate data for flagellates and ciliates, and expressed the rates as a function of the body mass. The atomic ratio of respiration rate to ammonia excretion (O:N ratio), which is used as an index of protein utilization as a metabolic substrate for metazooplankton (Mayzaud and Conover, 1988; Ikeda *et al*., 2000), has been studied for some planktonic protozoans (Verity, 1985).

The aim of the present study was (1) to assemble the respiration data from all new and old literature and then explore important independent variables and construct empirical models, (2) to gain broad insight into metabolic substrates by combining

respiration data and ammonia excretion data, and (3) to compare with the latest data on routine respiration rates of marine pelagic metazoans such as crustaceans, fishes and cephalopods to highlight any unique features the protozoans as a pelagic taxon.

METHOD

Protozoan data

The respiration data on 54 free-living heterotrophic protozoans compiled by Fenchel and Finlay (1983) include both freshwater and marine species. The respiration rates of freshwater and marine metazooplankton are evaluated to be similar under equivalent body mass and temperature (Hernández-León and Ikeda, 2005), and therefore the two habitats were not separated in the present analyses. An isolated datum of dinoflagellates (*Gyrodinium dominance*, Table I) was included in those of flagellates in the present analyses. The respiration rate data, which were adjusted to the rates at 20^{o}C by using a Q_{10} value of 2 in Fenchel and Finlay, were calculated back to the rates at original temperatures. These datasets (excluding data on cysts) and those for 8 planktonic heterotrophic protozoan species, which were not cited or were published since Fenchel and Finlay, were pooled in the present analyses [a total of 61 species, Table I, Supplemental material (S1-1)]. By analogy with metazooplankton metabolism (Ikeda et al., 2000), these respiration rates derived from uncontrolled or normal activity of protozooplankton in the laboratory can be classified to “routine” metabolism. Ammonia excretion data for 7 flagellates (all heterotrophic) and 6 ciliates (heterotrophic) were selected from Dolan (1997) [Table II, Supplemental material (S1-2)]. The data on the same species, but determined at different temperatures or by different workers, were treated as independent data. Almost all the protozoan data represent those from

115 laboratory cultures, which were necessary to obtain sufficient numbers of specimens for
116 accurate measurements of respiration or ammonia excretion. Because protozoan body
117 sizes are too small to determine body mass directly even with the microbalances
118 available on the market (accurate to 1 μg or 0.1 μg in contrast to the ng or pg wet mass
119 of individual protozoans), body volume (V) is computed by measuring linear
120 dimensions of body parts under the microscope followed by best geometric
121 approximation. These V data were converted to wet mass (WM), dry mass (DM),
122 carbon (C) or nitrogen (N) by using the conversion factors, 1 μm^3 V = 1 pgWM = 0.15
123 pgDM = 0.071 pgC = 0.0185 pgN for respiration data (Fenchel and Finlay, 1983) and, 1
124 pgWM = 0.20 pgDM for ammonia excretion data (Dolan, 1997). The same conversion
125 factors were also used to estimate WM when body mass was given by DM, C or N units
126 only. To facilitate intuitive understanding of the size of animals, V data were converted
127 to the Equivalent Spherical Diameter (ESD, μm) as; $ESD = 2 \times (V \times 3/4\pi)^{1/3}$.
128
129 *Metazoan data*:
130 Respiration data for marine pelagic crustaceans (including 109 copepod species, 24
131 euphausiid species, 32 amphipod species, 32 mysid species and 43 decapod species)
132 were from Supporting materials in Ikeda (2014), and those for 90 marine pelagic fish
133 and 41 cephalopod species were from Ikeda (2016). All these data are characterized by
134 routine metabolism of wild specimens from diverse habitat temperatures
135 (subzero-temperatures in polar seas to warm tropical seas) and various depth horizons
136 (shallow to deep-seas). Among these data, O:N ratios are also reported for124
137 crustaceans, 41 fishes and 6 cephalopods (Ikeda, 2016). In addition to DM, C and N
138 body mass data, WM data are available for fishes and cephalopods (Ikeda, 2016), but

not for crustaceans. For the comparison of respiration data of crustaceans with the data of protozoans in the present analyses, WM of crustaceans was estimated based on the mean water content (% of WM) of individual taxa [copepods, 81.4 % (± 5.1, SD); euphausiids, 76.9 % (± 3.7); amphipods, 78.7 % (± 8.3); mysids, 77.6 % (± 5.4), and decapods, 73.9 % (± 5.2), all in Ikeda (2014)].

Regression models

In addition to body mass (BM) and temperature (TEMP), measuring methods, physiological conditions and taxa are factors that potentially affect protozoan respiration (Fenchel and Finley, 1983; Laybourn-Parry, 1987) and therefore were treated as independent variables in the present analyses. Measuring methods were grouped arbitrarily into 3 categories of Cartesian or gradient diver (DIV), Warburg/Gilson differential respirometer (WG), or oxygen-electrode, Winkler titration, oxygen optode and ^{14}C-labelling (MISC); physiological conditions into 3categories of growing (GR), starved (ST) or unspecified (UNS); and taxa into 3 categories of amoebae (AM), ciliates (CIL) or flagellates(FLG). All protozoans studied by Klekowski and Tumantseva (1981), Kawakami *et al*. (1985), Verity (1985), Caron *et al*. (1986) and Schmoker *et al*. (2011) in S1-1 and Caron *et al*. (1986) in S1-2 were assumed to be in a physiological state of growing. The regression model adopted is a version of the “global-bathymetric model” for the analyses of metabolism of marine metazooplankton (Ikeda, 2014), which is described as follows:

$$\ln R = a_0 + a_1 \times \ln BM + a_2 \times 1000/TEMP + a_3 \times FLG + a_4 \times CIL + a_5 \times (FLAG \times \ln BM) + a_6 \times (CIL \times \ln BM) + a_7 \times (FLAG \times 1000/TEMP) + a_8 \times (CIL \times 1000/TEMP) + a_9 \times DIV + a_{10} \times MISC + a_{11} \times GR + a_{12} \times UNS$$

where lnR is the logarithm (base e) of respiration rate (R: μLO_2 individual^{-1} h^{-1}), lnBM is the logarithm (base e) of body mass (WM, DM, C or N, all mg), Temp is temperature (K), and DIV, MISC, GR, UNS, FLG and CIL are dummy (binary) variables which take a value 1 or 0 otherwise. Dummy variables WG, AM and ST, which do not appear in the regression equation (= reference category), take values of 0 in either case (for details, see S1-1). FLAG × lnBM, CIL × lnBM, FLAG × 1000/TEMP and CIL × 1000/TEMP are interaction terms to detect differential effects of BM and TEMP on lnR of the three protozoan taxa. The repetition of the analysis using WM, DM, C or N was anticipated yielding similar regression coefficients but different intercepts because almost the same mass conversion factors were used (see above). However, the analysis was repeated to make direct comparison possible with the results of marine zooplankton taxa (Ikeda, 2014; Kiørboe and Hirst, 2014).

In comparing respiration rates of planktonic protozoans with routine respiration rates of marine pelagic crustacean (CRU), fish (FIS) and cephalopod (CEPH) metazoans, experimental temperatures (TEMP) associated with protozoan respiration data are assumed to be equivalent to habitat temperature (see “Body mass/temperature effects” in DISCUSSION below), and possible differences in the effects of BM, TEMP and

habitat depth (DEPTH) among taxa (BM range: 0.5×10^{-7}–1.0×10^{7} mgWM, 10^{14} –fold difference) were assumed to be unimportant and neglected (if not, the results would vary depending on the choice of the value of these independent variables):

$$\ln R = a_0 + a_1 \times \ln BM + a_2 \times (1000/TEMP) + a_3 \times \ln DEPTH + a_4 \times PRO1 + a_5 \times PRO2 + a_6 \times PRO3 + a_7 \times FIS + a_8 \times CEPH$$

where, the data for amoebae, flagellates and ciliates were pooled and re-grouped as those growing (PRO1), starved (PRO2) or unspecified (PRO3), and their BM represented by WM and C. WM and C were chosen because WM is the direct measure of BM from body volume V in most protozoan data, and C is the second best estimate of BM from using C:V ratios (Note: N:V ratio data are scarce). Dummy variables PRO1, PRO2, PRO3, FIS and CEPH had a value 1 or 0 otherwise. CRUS (reference category) did not appear in the regression equation and had values of 0 in either case. The same regression model was used for the analyses of O:N ratios among protozoans (PRO1 only), crustaceans, fishes and cephalopods. DEPH for the protozoans was designated as 1 m.

As an index of temperature effects, the activation energy (E_a) is calculated from the coefficient a_2 [= $-E_a$/k, where k is Bolzmann's constant (8.62×10^{-5} eV/K); E_a = $a_2 \times 1000 \times 8.62 \times 10^{-5}$]. Temperature effects on physiological rates are usually expressed by Q_{10} instead of E_a. By defining a temperature range (t_1 and t_2, both in ^{o}C), E_a can be converted to Q_{10} as follows:

$$Q_{10} = \exp[10 \times E_a/(k \times (273 + t_1) \times (273 + t_2)] \text{ (Ivleva, 1980).}$$

198 The attributes of the variables were analyzed simultaneously by using a stepwise
199 multiple-regression (forward selection) method (Sokal and Rohlf, 1995). Independent
200 variables were added and removed at the p = 0.05 level; therefore, partial regression
201 coefficients from the resultant equations were all significant ($p \leq 0.05$), unless otherwise
202 noted. The calculation was conducted using a linear regression program in SYSTAT
203 version 10.2 statistical software package. When the program detected outliers (based on
204 Studentized residuals), they were excluded from the regression calculation.
205
206 **RESULTS**
207 **Protozoan metabolism**
208 *Respiration*
209 Across the three protozoan taxa considered in the present analyses, the temperature
210 ranged from 5°C to 35°C; body mass (WM) from 0.025×10^{-6} to 0.125 mg; and
211 respiration rate from 0.053×10^{-6} to 0.083 µL O_2 individual^{-1} h^{-1} (S1-1).
212 The overall results of the stepwise multiple regressions showed that body mass,
213 temperature, physiological condition (growing) and interactions between the variables
214 (CIL × lnBM; Table II) combined explained 84% (adjusted R^2 = 0.840–0.842) of the
215 variance in respiration rates, regardless the choice of body mass units (Table III). As
216 judged by the standardized coefficients of these variables, the most important variable
217 was body mass, followed by physiological condition (growing), the interaction CIL ×
218 lnBM and temperature. Respiration rates increase with the increases in body mass and
219 temperature. Multicollinearity between these variables is likely small because the
220 variation inflation factors (VIF) of these variables (1.022–1.048, not shown in Table III)

were < 5 (cf. Kutner et al., 2004). Irrespective of the choice of body mass units, the coefficient a_1 (the scaling exponent of the body mass) was significantly less than unity (0.696–0.703, all $p < 0.0001$). With regard to the effect of temperature, the E_a (eV) calculated from the coefficient a_2 varied somewhat depending on the choice of body mass units from 0.601 to 0.616 (equivalent to 2.34–2.39 in terms of Q_{10} between –2°C and 30°C).

By the definition of dummy variables, respiration rates determined by Cartesian/gradient diver or miscellaneous methods for which regression coefficients were not significant ($p > 0.05$, blanks in Table III) were equivalent to the rates measured by Warburg/Gilson differential respirometer (reference category). Respiration rates of animals with an unspecified condition were not significantly different from those of starved animals (reference category), but the rates of growing animals were significantly greater (2.7-fold) than both of those categories. For the interaction terms of dummy variables and quantitative variables, the effect of BM on R was significant for ciliates only (Table III).

The differences in respiration-body mass relations between ciliates and amoebae + flagellates were better extracted from the regression statistics (Table III) by calculating the standardized respiration rate (R_{std}), which is free from the effects of the other independent variables (i.e., temperature, physiological conditions):

$$\ln R_{std} = a_0 + a_1 \times \ln WM \text{ or } \ln N$$

where $\ln R_{std} = \ln R - a_2 \times 1000/Temp - a_{11} \times GR$.

To clearly delineate intercept differences of regression lines of ciliates and amoebae + flagellates, BM was represented by WM, and WM-specific R_{std} (SR_{std}) was plotted

against WM on a log-log graph (Fig. 1).

Ammonia excretion and O:N ratio

For the 7 flagellates and 6 ciliates, the temperature ranged from 10°C to 30°C; body mass (WM) from 8.7×10^{-9} to 0.13×10^{-3} mg; and ammonia excretion rates from 1.1×10^{-7} to 8.2×10^{-5} µg N individual^{-1} h^{-1} (S1-2). The same multiple-regression model used for respiration data analyses could not be applied to ammonia excretion data because the number of data sets was small (N = 21). As an alternative, standardized, WM-specific ammonia excretion rates were computed by applying the same regression coefficients of temperature (–4.719) obtained for the respiration rates, and the results were compared with standardized, WM-specific respiration rates on a log-log graph (Fig. 2). Because the ammonia excretion data represent maximum rates of growing protozoan cultures (Dolan, 1997), the respiration data compared in Fig. 2 are those of growing protozoans only. Since the slopes of the two regression lines did not differ significantly (ANCOVA, $F = 0.5410$, df = 1, 90; $p > 0.25$), a common slope (–0.357) was computed. From the intercepts of these two parallel regression lines, an atomic O:N ratio was derived as 7.2 $[(14/11.2) \times (e^{13.920})/(e^{12.171})]$.

Protozoan metabolism vs. metazoan metabolism

Preliminary analyses detected five outliers in the protozoan data [one of the 10 data for *Chaos carolinense* and of the 16 data for *Tetrahymena pyriformis*, the single data of *Strombidium* sp., and two of the 3 data of *Stentor coeruleus*, all in Fenchel and Finlay (1983)], which were omitted before the analyses.

Based on the present analyses, for the respiration rates of the specimens of the

same body mass (WM or C) living at identical temperatures and from similar depths, the respiration rates of growing, starved and unspecified protozoans were all less than those of the three metazoan groups on a WM basis, and only growing protozoans had respiration rates equivalent to those of the crustaceans and fishes on a C basis (Table IV). On a C basis, the respiration rates of the cephalopods were greater than the rates of the protozoans, crustaceans and fishes by 1.45-fold. To show these differences in the regression lines among protozoans, crustaceans, fishes and cephalopods more clearly, C-specific, standardized respiration rates (SR_{std}), which are free from the effects of the other independent variables (i.e., temperature, depth), were calculated and plotted against C on a log-log graph (Fig. 3).

Mean O:N ratios were 10.2 (± 3.2, SD) for protozoans (S1-2, this study), 24.8 (± 16.5, N =125) for crustaceans (Ikeda, 2016), 29.7 (± 15.1, N = 41) for fishes (Ikeda, 2016) and 27.5 (± 34.8, N = 6) for cephalopods (Ikeda, 2016), with a grand mean of 25.2 (± 17.0, N = 182). The regression analyses of log-transformed O:N ratio data revealed that body mass (represented by C) was the only significant variable (Table IV). The effects of C were positive and explained 12.5% (adjusted R^2 = 0.125) of the variance of O:N ratios.

DISCUSSION

Methodological constraints

Routine metabolic rates of pelagic metazoans can be determined on one or a few specimens captured in the field by using conventional sealed-chamber methods for zooplankton (Ikeda et al., 2000) or swim-tunnel respirometers for large, active pelagic fishes and cephalopods (Webber and O'Dor, 1985; Steffensen, 2005). However, the

same methods cannot be applied directly to protozoans because their small size requires that hundreds or even thousands of specimens (“protozoan soup”, cf. Laybourn-Parry, 1987) must be prepared in laboratory culture before the measurements of metabolic rates. As exceptions, Cartesian/gradient diver methods (Zeuthen, 1947; Hamburger, 1981) are capable of measurements with only one or a few protozoans and therefore are an apparent advantage over the other methods for the study of protozoan metabolism (Laybourn-Parry, 1987). However, the diver methods require skill, are labor intensive, cannot be used at sea, and most importantly, over several hours of incubation under food-deprived conditions are required for specimens. Because of high mass-specific metabolic rates, protozoans are highly sensitive to food deprivation, with immediate effects on their growth and metabolic rates (Fenchel and Finlay, 1983; Fenchel, 2005). For these reasons, Warburg respirometry, O_2-electrodes or similar methods using a “protozoan soup” remain preferable because both methods reliably determine respiration rates with incubation times as short as 10–15 min (Fenchel and Finlay, 1983). The present analyses showed no significant differences in protozoan respiration rates derived from diver methods and other methods (Table III). “Crowding” effects of animals during experiments have been considered one likely factor affecting the resultant metabolic rates; however, no consistent positive/negative effects have been found for metazooplankton (mostly crustaceans) (Ikeda et al., 2000).

Body mass/temperature effects

In contrast to the mass scaling exponent of 0.75 (Hemmingsen, 1960; Fenchel and Finlay, 1983), Fenchel (2014) proposed a biphasic model; the exponent (equivalent to the regression coefficient a_1 of the present model, Table III) is 1.0 for small and

316 medium-sized (50–10^4 μm^3 V or 4.6–26.7 μm ESD, cf. Fig. 1) protozoans which are
317 characterized by a spherical body and distribution of mitochondria throughout the cell,
318 and the exponent is 0.75 for large (> 10^4 μm^3 V or > 26.7 μm ESD) protozoans with a
319 flattened or very oblong body and mitochondria clustered close to the outer cell
320 membrane. According to the "metabolic-level boundaries (MLB)" hypothesis (Glazier,
321 2009), the exponent = 1.0 is indicative of low maintenance cost which is amply
322 accommodated by surface dependent processes, and the exponent = 2/3 or 0.67 when
323 maintenance costs are high and under the constraint of body surface-mediated fluxes of
324 materials. The broad mass scaling analyses of endogenous (standard) metabolism,
325 including not only heterotrophic protozoans but also bacteria, yeasts and other
326 autotrophic and mixotrophic unicellular organisms, yielded an exponent near 1.0
327 (Makarieva *et al*., 2008, Johnson *et al*., 2009, Delong *et al*., 2010).
328 At present, the verification of the biphasic model of Fenchel (2014) is difficult
329 because the data for the two size categories (small-medium vs. large protozoans) are
330 limited in number and widely scattered (Fig. 1). In this study, the regression analyses of
331 respiration rates on a WM basis yielded the exponent of 0.70 for amoebae and
332 flagellates and 0.61 for ciliates, which were both significantly different from 1.0 (Table
333 III). A similar exponent (0.622) was reported from the regression analyses of ammonia
334 excretion rates on DM for a mixture of mixotrophic and heterotrophic protozoans
335 (Dolan, 1997). Until the bi-phasic model is verified by data, empirical or statistical
336 models would be the method of choice to describe the relations between metabolic rate
337 and body mass taking into account several other independent variables as in the present
338 study.
339 Among the three protozoan taxa considered in the present study, ciliates had

higher respiration rates than those of flagellates or amoebae (Fenchel and Finlay, 1983; Table III, Fig. 1 of this study). Excluding amoebae which are characterized by very low motility, ciliates have higher body length (L)-specific swimming speeds (up to 200 L s^{-1}) than those of flagellates (approximately 10 L s^{-1}) (Crawford, 1992; Vogel, 2008). Fenchel and Finlay (1983) noted that the theoretical fraction of energy required for motility of planktonic ciliates is <1% of total energy expenditure. However, Crawford (1992) calculated the cost to vary from <1% to >100% depending on the size of protozoans. Metabolic costs of protozoan motility remain unclear and are likely environment and species-specific (Guasto *et al*., 2012). Motility is of special importance in the feeding behavior of planktonic protozoa; high motile ciliates and flagellates are broadly classified to feeding-current and cruise feeders, while low motile amoebae and small flagellates to diffusion feeders and ambush feeders, respectively (Kiørboe, 2010). In this respect, ciliates have also higher clearance, ingestion and growth rates than flagellates (Hansen and Bjørnsen, 1997; Kiørboe and Hirst, 2014) and although the gross growth efficiencies [growth × 100/(growth + metabolism)] of these two taxa are similar, 20–30% (Straile, 1997).

Protozoans can tolerate wider temperature ranges and higher temperatures than those of metazoans, with the increase in tolerance attributed to the lower level of organization of the protozoans (Storch *et al*., 2014). Species diversities of marine protozoans are lower than those of metazoans at a global scale (Hillebrand *et al*., 2001; Fenchel and Finlay, 2004). Despite these differences, the relationship between body-mass normalized resting or standard metabolic rates (the rates adjusted to 1 g WM) and temperature for a broad suite of organisms including unicellular organisms (including protozoans), plants, invertebrates and vertebrates is relatively constant and

364 expressed by the activation energy (E_a) of 0.6–0.7 eV (Gillooly *et al*., 2001), which is
365 equivalent to Q_{10} = 2.3–2.7 for the temperature range of –0.2°C to 30°C [universal
366 temperature dependence (UTD) hypothesis]. Alternatively, Clarke and Johnston (1999)
367 and Clarke and Fraser (2004), working on fish metabolism over a global range of
368 temperatures spanning 40°C, distinguished intraspecific Q_{10} (= 2.40) from interspecific
369 Q_{10} (= 1.83); the intraspecific Q_{10} represents the adjustment of an organism to a new
370 temperature in the laboratory (acclimation), and the interspecific Q_{10} represents the
371 evolutionary adjustment of the physiology of an organism to the environment
372 (adaptation) [evolutionary trade-off (ET) hypothesis]. Support for the ET hypothesis
373 (acclimated Q_{10} > adapted Q_{10}; in contrast to acclimated Q_{10} = adapted Q_{10} of the UTD
374 hypothesis) is provided by some marine pelagic metazoan taxa (Ikeda, 2014, 2016).
375 However, evaluation of the ET hypothesis is not possible for planktonic protozoans
376 because intraspecific Q_{10} values vary greatly (0.12–7.36; Laybourn-Parry, 1987), and
377 the interspecific Q_{10} values (2.34–2.39, Table III) in the present study represent culture
378 temperatures and not necessarily environmental temperatures of the species studied.
379 Nevertheless, the interspecific Q_{10} = 2.34–2.39 obtained from the present analyses is
380 close to the low value of the Q_{10} = 2.3–2.7 predicted from the UTD hypothesis.
381 Hypothetical Q_{10} values used to adjust protozoan respiration rates to the rate at a
382 standard temperature are 2.0 (Fenchel and Finlay, 1983; Makarieva *et al*., 2008), 2.4
383 (DeLong et al., 2010) and 2.8 (Kiørboe and Hirst, 2014).
384
385 **O:N ratio and ammonia excretion**
386 For marine pelagic metazoan taxa, global synthesis of the O:N ratio indicates that the
387 ratio is nearly stable at approximately 23 (Ikeda, 1985) or 19–20 (Ikeda, 2016) with

388 minor effects of body mass, habitat temperature and/or taxon. In the present analyses,
389 pooling the O:N ratios determined for the three protozoan species (see S1-2) and for
390 those of marine pelagic metazoans revealed that the ratio was stable across protozoans
391 and metazoans with a small but significant effect of body mass that contributed to
392 12.5% of the variance of the ratios (Table IV). These results suggest strongly that
393 ammonia excretion rates change largely in parallel with respiration rates. As underlying
394 mechanisms for this parallel change, broadly similar body chemical compositions
395 among these animals (primarily C:N ratios) (Ikeda, 1974; Brey *et al*., 2010; Kiørboe,
396 2013) and ammonia as a common end-product of protein catabolism in aquatic animals
397 (Prosser 1962) may be possibilities. Specifically, the O:N ratio anticipated from
398 zooplankton of which average C:N:P composition is 106:16:1 by atom (i.e., Redfield
399 ratio) is 17 (cf. Ikeda et al., 2000). In this respect, Richards's (1965) model for a
400 sequence of decomposition of plankton organic matter (C:N:P = 106:16:1) leads to the
401 O:N ratio = 13 because the end-product of protein catabolism is nitrate, not ammonia, in
402 his model.

403 Based on the parallel changes in respiration and ammonia excretion in planktonic
404 protozoans (Fig. 2), comparison of respiration-WM regression line with ammonia
405 excretion-WM regression line, both derived from independent studies on different
406 protozoans, yielded the O:N ratio of 7.2, which is fairly consistent with the ratio of 10.2
407 (95% CI: 2.9–17.5, S1-2) that was directly determined. Theoretically, the O:N ratio is 7
408 when only protein is metabolized and is calculated, respectively, as 21 or 13 when
409 protein-and-lipid or protein-and-carbohydrate are catabolized in equal quantities
410 simultaneously [Table 10.3 in Ikeda et al. (2000)]. On this theoretical ground, these O:N
411 ratios derived directly (10.2) or indirectly (7.2) for protozoans suggest protein-oriented

412 metabolism. With the present lack of empirical models for protozoan ammonia
413 excretion rates comparable to those of their respiration rates, an indirect estimation from
414 the respiration rate coupled with the mean O:N ratio (7.2) derived from the comparison
415 of the relationships between respiration rates, ammonia excretion rates and WM (Fig. 2)
416 may be an acceptable alternative.
417
418 **Protozoans vs. metazoans**
419 The present analyses show that the respiration rates of growing protozoans are slightly
420 less (0.71-fold) than the rates of pelagic metazoans when compared on a WM basis but
421 similar to the rates of pelagic crustaceans and fishes on a C basis (Table IV). Thus, the
422 present results are somewhat inconsistent with those of previous workers (Hemmingsen,
423 1960; Fenchel and Finlay, 1983; Makarieva *et al*., 2008; DeLong *et al*., 2010; Kiørboe
424 and Hirst, 2014; and see "INTRODUCTION"). However, the parameters of the present
425 study were different from those of the other studies. The poikilotherm metazoan data
426 compiled by previous workers [except Kiørboe and Hirst (2014)] are "standard"
427 metabolism (or "basal" metabolism) in contrast to the "routine" metabolism of this
428 study, and the taxa included are a broad mixture of both aquatic and terrestrial species
429 (mollusks, crustaceans, insects, spiders, fishes, amphibians and reptiles); whereas
430 strictly marine pelagic taxa were used in this study. Despite the similar definition of
431 metabolism (routine metabolism) and poikilotherm metazoans (pelagic marine taxa), the
432 differences between the results of Kiørboe and Hirst (2014) and this study were due to
433 the use of the protozoan data of mixed physiological states in Kiørboe and Hirst and the
434 separation of the growing phase from the other physiological conditions in this study.
435 Additionally, the hypothetical Q_{10} value (2.8) adopted by Kiørboe and Hirst to

standardize the rates at 15°C is higher than the 2.4 obtained in the present analyses. Considering that high culture temperatures (20–35°C, with a mean of 23.3°C) prevailed in the protozoan data compiled by Fenchel and Finlay, their use of a high Q_{10} value would lead to underestimation of the rates. These differences in the definition of the metabolism, composition of poikilotherms, different physiological conditions of protozoans and the use of hypothetical Q_{10} values might partially or totally explain the dissimilar conclusions reached between these previous workers and this study.

The cephalopod respiration rates, which were similar to the rates of the crustaceans and fishes on a WM basis but were higher than the rates of these two metazoan taxa on a C basis (Table IV, Fig. 2), deserve attention. Pelagic cephalopods are characterized by greater water content yet lower C content than pelagic crustaceans and fishes [90% vs. 77–80% of WM, and 36% vs. 42–46% of DM, respectively, cf. Ikeda's (2014, 2016) Supplementary Data]. These taxon-specific body compositions of pelagic cephalopods explain the different results obtained with the choice of body mass units (WM or C) in metabolic comparisons among the three pelagic metazoans.

Conclusions/Future aspects

Because protozoan respiration rates vary widely depending on the physiological state of the cells by up to 50-fold on an individual basis, predicting protozoan respiration rates in nature is thought to be of little value other than to delimit a potential range (Fenchel and Finlay, 1983; Fenchel, 2005). However, the same data revealed that the factor reduces to 10-fold or less for body mass (or volume) specific respiration rates because the decline in respiration rates of protozoans under adverse physiological states is always accompanied by a decrease in body mass [Table 2 in Fenchel and Finlay (1983)].

460 The empirical regression model (Table III) built in the present study accommodates
461 such variations by designating the body mass and physiological condition as
462 independent variables. According to the model, the difference in respiration rates due to
463 the physiological condition of the animal is 2.7-fold (growing/starved or unspecified),
464 which is far less than 50-fold. Perhaps, the physiological conditions of planktonic
465 protozoans in the field are somewhere between the two extremes (growing and starved).
466 Uncertainties of this magnitude are inherent with predicted respiration rates of
467 planktonic protozoans and are of the same magnitude as the “specific dynamic action
468 (SDA)” (cf. Secor, 2009) in marine pelagic metazoans associated with their feeding
469 conditions in the field and not considered in the empirical models for estimating routine
470 metabolic rates of them (Ikeda, 2014, 2016).
471 Based on an examination of the data coverage of protozooplankton taxa from a
472 variety of habitats across the oceans of the world, dinoflagellates, foraminiferans,
473 radiolarians and phaeodarians are clearly underrepresented in the model (Table III). In
474 addition, the collection of data on planktonic protozoans living at various depth
475 horizons in the oceans is urgently required to judge whether or not habitat depth is a
476 significant variable [similar to the global-bathymetric model for marine
477 metazooplankton taxa cf. Ikeda (2014)]. To this end, the model could permit the
478 assessment of the carbon or nitrogen flows mediated by complex protozooplankton
479 communities in the field.
480
481 **SUPPLEMENTARY DATA**
482 Supplementary data can be found online at http://plankt.oxfordjournals.org
483

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Table I: A list of planktonic heterotrophic protozoans of which respiration rates were used for the present analyses. For detals, see **S1-1**.

Protozoa	Genus and species
Amoebae	*Acanthamoeba* sp.
	A. castellanii
	A. palestinensis
	A. rhysodes
	Actinosphaerium eichhorni
	Allogromia laticollaris
	A. proteus
	Chaos carolinense
	Difflugia sp.
	Rosalina leei
	Spiroloculina hyalini
Flagellates	*Astasia klebsii*
	A. longa
	Chilomonas paramecium
	Euglena gracilis var. *bacillaris*
	Gyrodinium dominans
	Ochromonas sp.
	Paraphysomonas imperforata
	Pleuromonas jaculans
Ciliates	*Blepharisma undulans*
	Bresslaua insidiatrix
	Carchesium polypinum
	Coleps hirtus
	Colpidiurn campylum
	Colpoda cucullus
	C. steinii
	Didinium nasutum
	Dileptus cygnus
	Diophrys sp.
	EupIotes vannus
	Favella ehrenbergii
	Favella taraikaensis
	Frontonia leucas
	lntranstylum steini
	Opercularia nulans
	Paramecium aurelia
	P. calkinsi
	P. caudatum
	Placus sp.
	Podophrya fixa
	Spirostomum ambiguum
	S. intermedium
	S. minus
	S. teres
	Stentor coeruleus
	Strombidium sp. 1
	Strombidium sp. 2
	Stylonychia mytilus
	Tetrahymena geleii
	T. pyriformis
	Tiarina fusus
	Tintinnopsis acuminata
	Tintinnopsis vasculum
	Tracheloraphis sp.
	Uronema marinum
	Urostyla grandis
	Vorticella convallaria
	V. ephemerae
	V. microstoma
	V. similis
	Zoothamnium carinogammari

Table II: A list of planktonic heterotrophic protozoans of which ammonia-N excretion data were used for the present analyses. For details, see **S1-2.**

Protozoa	Genus and species
Flagellates	Chrysomonad
	"flagellate"
	Monas sp.
	Oxyrrhis sp.
	Paraphysomonas sp.
	Paraphysomonas imperforata
	Pseudobodo sp.
	Spumella sp.
Ciliates	*Cyclidium* sp.
	Euplotes vannus
	Paramecium aurelia
	Strombidium sulcatum
	Tintinnopsis acuminata
	Tintinnopsis vasculum

Table III: Multiple stepwise (forward selection, $P_{in} = P_{out} = 0.05$) regression statistics on protozoan respiration rates (R: μLO_2 individual $^{-1}$ h^{-1}) of body mass [BM, in terms of wet mass (WM), dry mass (DM), carbon (C) or nitrogen (N), all mg], temperature (TEMP, K), measuring methods [Cartesian diver (DIV), Warburg/Gilson differential respirometer (WG) or other methods (MISC)], physiological conditions [growing (GR), starved (STA) or unknown conditions (UNK)] or taxa [amoebae (AM), flagellates (FLA) or ciliates (CIL)]: $\ln R = a_0 + a_1 \times \ln BM + a_2 \times 1000/TEMP + a_3 \times FLG + a_4 \times CIL + a_5 \times (FLAG \times \ln BM) + a_6 \times (CIL \times \ln BM) + a_7 \times (FLAG \times 1000/TEMP) + a_8 \times (CIL \times 1000/TEMP) + a_9 \times DIV + a_{10} \times MISC + a_{11} \times GR + a_{12} \times UNK$, where DIV, MISC, GR, UNK, FLAG and CIL are dummy variables which take a value 1or 0 otherwise. Dummy variables WG, STA and AM which do not appear in the regression equation (reference category) take values of 0 in either case. FLAG × lnBM, CIL × lnBM, FLAG × 1000/TEMP and CIL × 1000/TEMP are interaction terms to detect differential effects of BM and TEMP on R among the three protozoan taxa.

Regression	Body Mass (BM) unit							
statistics	WM		DM		C		N	
N	135		135		135		135	
Adjusted R^2	0.842		0.841		0.841		0.840	
Coefficient (std error; std coeff)								
a_0	20.909	(6.347; 0.000)	22.468	(6.370; 0.000)	23.058	(6.379; 0.000)	23.372	(6.397; 0.000)
a_1	0.703	(0.026; 0.927)	0.699	(0.026; 0.922)	0.698	(0.026; 0.921)	0.696	(0.027; 0.919)
a_2	−7.042	(1.872; −0.131)	−7.120	(1.877; −0.132)	−7.144	(1.879; −0.133)	−6.968	(1.884; −0.129)
a_3								
a_4								
a_5								
a_6	−0.093	(0.018; −0.180)	−0.078	(0.015; −0.178)	−0.073	(0.014; −0.178)	−0.067	(0.013; −0.179)
a_7								
a_8								
a_9								
a_{10}								
a_{11}	1.004	(0.179; 0.198)	1.000	(0.179; 0.197)	0.998	(0.179; 0.196)	1.006	(0.180; 0.198)
a_{12}								

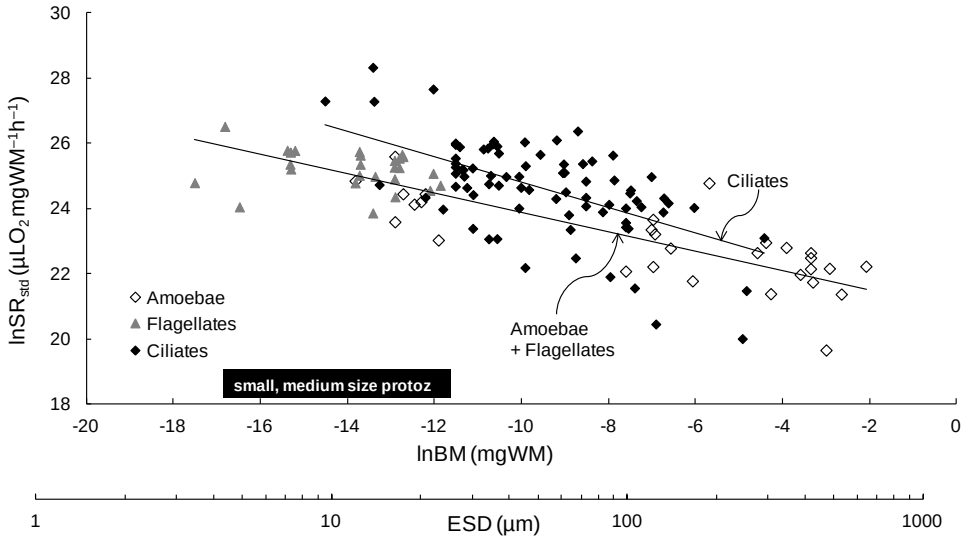
Note: For estimating R of growing protozoans from above results (in case of WM)

Ciliates: $\ln R = 20.909 + (0.703 - 0.093) \times \ln WM - 7.042 \times (1000/TEMP) + 1.004$

Amoebae, Flagellates: $\ln R = 20.909 + 0.703 \times \ln WM - 7.042 \times (1000/TEMP) + 1.004$

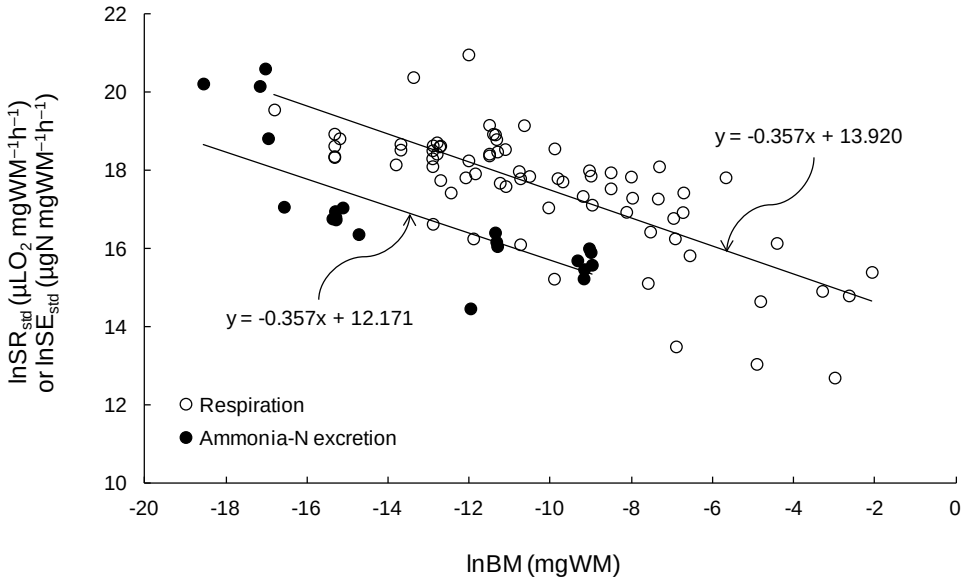
Table IV: Multiple stepwise (forward selection, $P_{in} = P_{out} = 0.05$) regression statistics on respiration rates (R: μLO_2 $individual^{-1}$ h^{-1}) or O:N atomic ratios of body mass [BM, representedy by wet mass (WM) and/or carbon (C), mg], temperature (TEMP, K), habitat depth (DEPTH), protozoans [PRO1(growing), PRO2 (starved), PRO3 (unspecified)], crustaceans (CRUS), fishes (FIS) and cephalopods (CEPH): lnR or ln(O:N) = $a_0 + a_1 \times \ln BM + a_2 \times 1000/TEMP + a_3 \times DEPTH + a_4 \times PRO1 + a_5 \times PRO2 + a_6 \times PRO3 + a_7 \times FIS + a_8 \times CEPH$, where PRO1, PRO2, PRO3, FIS and CEPH are dummy variables which take a value 1or 0 otherwise. Dummy variable CRUS which does not appear in the regression equation (reference category) takes values of 0 in either case. Note: R represents routine metabolism for CRUS, FIS and CEPH. *NA:* not applicable.

Dependent variable	Regression statistics	Body Mass (BM) unit			
		WM		C	
Respiration	*N*	719		719	
	Adjusted R^2	0.982		0.983	
	Coefficient (std error; std coeff)				
	a_0	19.882	(1.226; 0.000)	22.737	(1.168; 0.000)
	a_1	0.825	(0.008; 1.020)	0.831	(0.005; 1.038)
	a_2	–5.780	(0.358; –0.141)	–6.058	(0.343; –0.148)
	a_3	–0.194	(0.014; –0.120)	–0.177	(0.014; –0.110)
	a_4	–0.421	(0.151; –0.024)		
	a_5	–1.792	(0.184; –0.067)	–1.449	(0.144; –0.054)
	a_6	–1.389	(0.160; –0.059)	–1.055	(0.125; –0.045)
	a_7				
	a_8			0.371	(0.104; 0.018)
O:N	*N*			182	
	Adjusted R^2			0.125	
	Coefficient (std error; std coeff)				
	a_0			3.072	(0.059; 0.000)
	a_1			0.063	(0.012; 0.360)
	a_2				
	a_3				
	a_4				
	a_5 *(NA)*				
	a_6 *(NA)*				
	a_7				
	a_8				



Ikeda Fig. 1

Fig. 1. Scatter diagram of standardized WM-specific respiration rates versus WM for planktonic heterotrophic amoebae, flagellates and ciliates. Respiration data are from Fenchel and Finlay (1983) plus those published ever since (Table I). Based on the results in Table III, regression lines are superimposed for ciliates, and amoebae plus flagellates. Black belt on upper abscissa is the range of "small and medium" sized protozoans (V: 50 to 104 µm^3) defined by Fenchel (Fenchel, 2014). Equivalent Sphere Diameter (ESD) corresponding WM is superimposed on abscissa.



Ikeda Fig. 2

Fig. 2. Scatter diagram of standardized WM-specific respiration rates and ammonia excretion rates plotted against WM for planktonic heterotrophic protozoans both in growth phase. Ammonia excretion data are from Dolan (1997), and the same temperature coefficient (a_2) being used for respiration data was adopted. See text for details.

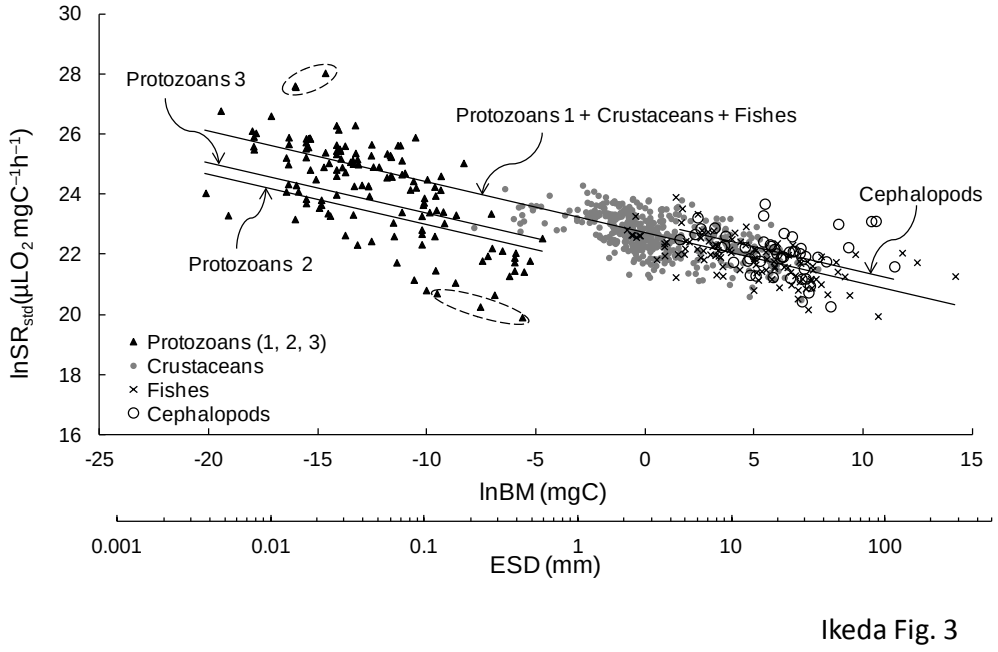


Fig. 3. Scatter diagram of standardized C-specific respiration rates versus C for planktonic protozoans (1: growing, 2: starved, 3: unspecified), and marine pelagic metazoans including crustaceans, fishes and cephalopods. Marine pelagic metazoan data are from Ikeda (2014, 2016). Four regression lines are superimposed based on the results in Table III. The data encircled by hatched line are outliers. Equivalent Sphere Diameter (ESD) corresponding C mass is superimposed on abscissa.

S1-1: Species name, temperature (Temp), respiration rate (R), body volume (V), body mass (WM, DW, C or N), dummy variables (method, physiological state, and taxon) and data source of planktonic heterotrophic protozoans in Table I. As dummy variables, WG (Warburg/Gilson differential respirometer) for method, ST (starved) for physiological state, and AM (amoebae) for taxon are reference categories, and do not appear in the table. DIV: Cartesian or gradient diver, MISC: oxygen-electrode, Winkler titration, oxygen optode, GR: growing, UNS: unspecified, FLG: flagellates, CIL: ciliates.

Genus and species	Temp	R	Body volume	Body mass				Method		Physiological state		Taxon		Source*
				WM	DM	C	N							
	(°C)	($\mu LO_2\ ind^{-1}h^{-1}$, $\times10^{-3}$)	(μm^3)	(mg, $\times10^{-6}$)	(mg, $\times10^{-6}$)	(mg, $\times10^{-6}$)	(mg, $\times10^{-6}$)	DIV	MISC	GR	UNS	FLG	CIL	
Acanthamoeba sp.	28	0.010	4540	4540	681	322	84	0	0	0	0	0	0	*a*
	30	0.027	3000	3000	450	213	56	1	0	1	0	0	0	*a*
A. castellanii	30	0.025	3880	3880	582	275	72	0	0	1	0	0	0	*a*
	20	0.032	2490	2490	374	177	46	0	0	1	0	0	0	*a*
	20	0.004	2490	2490	374	177	46	1	0	1	0	0	0	*a*
	30	0.013	1000	1000	150	71	19	0	0	1	0	0	0	*a*
A. palestinensis	27	0.012	6760	6760	1014	480	125	0	0	1	0	0	0	*a*
A. rhysodes	30	0.017	5000	5000	750	355	93	0	0	0	0	0	0	*a*
Actinosphaerium eichhorni	21.8	1.13	14000000	14000000	2100000	994000	259000	0	1	0	1	0	0	*a*
Allogromia laticollaris	35	43.0	71000000	71000000	10650000	5041000	1313500	0	0	1	0	0	0	*a*
	21	8.89	54000000	54000000	8100000	3834000	999000	0	0	0	1	0	0	*a*
A. proteus	20	0.15	936000	936000	140400	66456	17316	0	0	0	1	0	0	*a*
	22.8	0.30	2320000	2320000	348000	164720	42920	1	0	0	0	0	0	*a*
	22.8	2.19	936000	936000	140400	66456	17316	0	1	1	0	0	0	*a*
	20	0.45	900000	900000	135000	63900	16650	0	1	0	0	0	0	*a*
	20	0.19	500000	500000	75000	35500	9250	1	0	1	0	0	0	*a*
	20	1.08	1400000	1400000	210000	99400	25900	1	0	1	0	0	0	*a*
Chaos carolinense	25	5.20	27600000	27600000	4140000	1959600	510600	0	1	0	0	0	0	*a*
	25	15.0	36800000	36800000	5520000	2612800	680800	0	0	1	0	0	0	*a*
	20	7.38	35100000	35100000	5265000	2492100	649350	0	0	0	1	0	0	*a*
	20	8.60	35100000	35100000	5265000	2492100	649350	1	0	0	1	0	0	*a*
	20	1.69	50000000	50000000	7500000	3550000	925000	1	0	1	0	0	0	*a*
	20	4.20	12500000	12500000	1875000	887500	231250	1	0	0	0	0	0	*a*
	26	9.33	20000000	20000000	3000000	1420000	370000	0	0	0	0	0	0	*a*
	25	7.85	35000000	35000000	5250000	2485000	647500	0	0	0	1	0	0	*a*
	25	3.76	10250000	10250000	1537500	727750	189625	1	0	0	1	0	0	*a*
Difflugia sp.	20	1.16	975000	975000	146250	69225	18038	1	0	1	0	0	0	*a*
Rosalina leei	25	82.6	125000000	125000000	18750000	8875000	2312500	0	0	1	0	0	0	*a*
Spiroloculina hyalini	20	19.3	3400000	3400000	510000	241400	62900	0	0	1	0	0	0	*a*
Astasia klebsii	25.2	0.070	6000	6000	900	426	111	0	0	1	0	1	0	*a*
	25.2	0.0019	1500	1500	225	107	28	0	0	0	0	1	0	*a*
A. longa	20	0.023	2460	2460	369	175	46	0	0	1	0	1	0	*a*
	26	0.026	2470	2470	371	175	46	0	0	1	0	1	0	*a*
	20	0.028	2470	2470	371	175	46	0	0	1	0	1	0	*a*
	28.5	0.050	5573	5573	836	396	103	0	0	1	0	1	0	*a*
	26	0.0047	1100	1100	165	78	20	0	0	0	0	1	0	*a*
	20	0.0060	1100	1100	165	78	20	0	0	0	0	1	0	*a*
	28.5	0.0067	2500	2500	375	178	46	0	0	0	0	1	0	*a*
Chilomonas paramecium	25	0.017	1124	1124	169	80	21	0	0	1	0	1	0	*a*
	25	0.037	2750	2750	413	195	51	0	0	1	0	1	0	*a*
	25	0.050	2750	2750	413	195	51	0	0	1	0	1	0	*a*
	20	0.038	3000	3000	450	213	56	0	0	1	0	1	0	*a*
	20	0.015	1124	1124	169	80	21	0	0	1	0	1	0	*a*
	24.5	0.003	1000	1000	150	71	19	0	0	0	0	1	0	*a*
Euglena gracilis var. *bacillaris*	26	0.0065	1580	1580	237	112	29	0	0	0	0	1	0	*a*
	26.5	0.063	7067	7067	1060	502	131	0	0	1	0	1	0	*a*
Gyrodinium dominans	17	0.031		2933	440	208	54	0	1	1	0	1	0	*b*
Ochromonas sp.	20	0.0039	250	250	38	18	5	0	0	1	0	1	0	*a*
	20	0.0001	70	70	11	5	1	0	0	0	0	1	0	*a*
Paraphysomonas imperforata	14	0.0020	226	226	34	16	4	0	1	1	0	1	0	*c*
	18	0.0019	224	224	34	16	4	0	1	1	0	1	0	*c*
	22	0.0023	228	228	34	16	4	0	1	1	0	1	0	*c*
	26	0.0053	210	210	32	15	4	0	1	1	0	1	0	*c*
Pleuromonas jaculans	20	0.0016	50	50	8	4	1	0	0	1	0	1	0	*a*
	20	0.000053	25	25	4	2	0	0	0	0	0	1	0	*a*
Blepharisma undulans	20	0.070	140000	140000	21000	9940	2590	0	0	0	1	0	1	*a*
Bresslaua insidiatrix	25	0.269	24000	24000	3600	1704	444	1	0	0	1	0	1	*a*
Carchesium polypinum	20	0.940	230000	230000	34500	16330	4255	1	0	0	1	0	1	*a*
Coleps hirtus	20	0.350	70000	70000	10500	4970	1295	1	0	0	1	0	1	*a*
Colpidiurn campylum	24	0.152	43000	43000	6450	3053	796	0	0	0	1	0	1	*a*
	20	0.113	43000	43000	6450	3053	796	0	0	1	0	0	1	*a*
Colpoda cucullus	24	0.113	45000	45000	6750	3195	833	1	0	0	1	0	1	*a*
C. steinii	30	0.169	10000	10000	1500	710	185	0	0	1	0	0	1	*a*
Didinium nasutum	20	0.690	294000	294000	44100	20874	5439	1	0	1	0	0	1	*a*
	20	2.75	1180000	1180000	177000	83780	21830	1	0	1	0	0	1	*a*
Dileptus cygnus	23	3.00	2400000	2400000	360000	170400	44400	1	0	0	1	0	1	*a*
Diophrys sp.	23	0.163	21000	21000	3150	1491	389	1	0	0	1	0	1	*a*
Euplotes vannus	25	0.010	7500	7500	1125	533	139	0	0	0	1	0	1	*a*
Favella ehrenbergii	20	4.96	660000	371831	55775	26400	6879	0	0	1	0	0	1	*d*
Favella taraikaensis	20	1.91	330000	185915	27887	13200	3439	0	0	1	0	0	1	*d*
Frontonia leucas	20	0.051	614000	614000	92100	43594	11359	1	0	0	1	0	1	*a*
Intranstylum steini	20	0.168	26000	26000	3900	1846	481	1	0	0	1	0	1	*a*
Opercularia nulans	20	0.447	120000	120000	18000	8520	2220	1	0	0	1	0	1	*a*
Paramecium aurelia	20	0.354	100500	100500	15075	7136	1859	0	0	1	0	0	1	*a*
	26	1.18	200000	200000	30000	14200	3700	1	0	1	0	0	1	*a*
	27	0.361	200000	200000	30000	14200	3700	1	0	0	1	0	1	*a*
	20	0.033	158000	158000	23700	11218	2923	1	0	0	1	0	1	*a*
P. calkinsi	20	0.106	135000	135000	20250	9585	2498	1	0	0	0	0	1	*a*
P. caudatum	21.2	0.533	500000	500000	75000	35500	9250	0	1	0	1	0	1	*a*
	20	2.11	640000	640000	96000	45440	11840	0	0	1	0	0	1	*a*
	22.7	1.50	200000	200000	30000	14200	3700	0	1	1	0	0	1	*a*
	22.7	5.35	1200000	1200000	180000	85200	22200	0	1	1	0	0	1	*a*
	20	0.75	530000	530000	79500	37630	9805	1	0	1	0	0	1	*a*
	25	1.41	560000	560000	84000	39760	10360	1	0	0	1	0	1	*a*
Placus sp.	28.8	1.76	383000	383000	57450	27193	7086	0	0	0	1	0	1	*a*
	28.8	1.44	712000	712000	106800	50552	13172	0	0	0	1	0	1	*a*
Podophrya fixa	20	0.021	49500	49500	7425	3515	916	1	0	1	0	0	1	*a*
	20	0.0077	14900	14900	2235	1058	276	1	0	0	0	0	1	*a*
Spirostomum ambiguum	25	2.50	8000000	8000000	1200000	568000	148000	0	0	1	0	0	1	*a*
	20	12.7	12000000	12000000	1800000	852000	222000	1	0	1	0	0	1	*a*
S. intermedium	20	0.270	500000	500000	75000	35500	9250	1	0	0	0	0	1	*a*
S. minus	20	0.310	500000	500000	75000	35500	9250	1	0	0	0	0	1	*a*
S. teres	20	0.041	349000	349000	52350	24779	6457	1	0	0	1	0	1	*a*

Stentor coeruleus	20	1.50	1330000	1330000	199500	94430	24605	1	0	0	0	0	1	*a*
	20	0.075	1000000	1000000	150000	71000	18500	1	0	1	0	0	1	*a*
	20	0.350	7300000	7300000	1095000	518300	135050	1	0	1	0	0	1	*a*
Strombidium sp. 1	22	0.128	1500	1500	225	107	28	1	0	0	1	0	1	*a*
Strombidium sp. 2	22.2	0.128		1540	231	109	28	0	0	1	0	0	1	*e*
Stylonychia mytilus	22	2.68	900000	900000	135000	63900	16650	1	0	0	1	0	1	*a*
Tetrahymena geleii	21	0.064	22449	22449	3367	1594	415	0	0	0	1	0	1	*a*
	26.8	0.433	54000	54000	8100	3834	999	0	0	1	0	0	1	*a*
	25	0.666	23600	23600	3540	1676	437	0	0	1	0	0	1	*a*
	26.8	0.140	32000	32000	4800	2272	592	0	0	0	0	0	1	*a*
T. pyriformis	22	0.052	10000	10000	1500	710	185	0	0	0	1	0	1	*a*
	25	0.028	10000	10000	1500	710	185	0	0	0	0	0	1	*a*
	28	0.912	50000	50000	7500	3550	925	0	0	1	0	0	1	*a*
	25	0.089	15000	15000	2250	1065	278	0	0	1	0	0	1	*a*
	26	0.300	10000	10000	1500	710	185	1	0	1	0	0	1	*a*
	25	0.010	5000	5000	750	355	93	0	0	0	0	0	1	*a*
	20	0.104	10000	10000	1500	710	185	0	0	1	0	0	1	*a*
	20	0.190	11000	11000	1650	781	204	1	0	1	0	0	1	*a*
	20	0.119	21500	21500	3225	1527	398	0	0	1	0	0	1	*a*
	20	0.022	21500	21500	3225	1527	398	1	0	1	0	0	1	*a*
	30	1.34	6000	6000	900	426	111	0	0	1	0	0	1	*a*
	20	0.064	13000	13000	1950	923	241	1	0	1	0	0	1	*a*
	30	0.294	14791	14791	2219	1050	274	0	0	1	0	0	1	*a*
	27	0.124	10000	10000	1500	710	185	0	0	0	1	0	1	*a*
	28	0.064	10000	10000	1500	710	185	0	0	0	0	0	1	*a*
	30	0.0076	1738	1738	261	123	32	0	0	0	0	0	1	*a*
Tiarina fusus	24	0.194	27000	27000	4050	1917	500	1	0	0	0	0	1	*a*
	23.8	0.194		26900	4035	1910	498	0	0	1	0	0	1	*e*
Tintinnopsis acuminata	15	0.056		12197	1830	866	188	0	1	1	0	0	1	*f*
	20	0.102		11930	1789	847	182	0	1	1	0	0	1	*f*
	25	0.149		11577	1737	822	175	0	1	1	0	0	1	*f*
Tintinnopsis vasculum	5	0.149		125915	18887	8940	1901	0	1	1	0	0	1	*f*
	10	0.410		122239	18336	8679	1875	0	1	1	0	0	1	*f*
	15	0.603		117423	17613	8337	1730	0	1	1	0	0	1	*f*
Tracheloraphis sp.	25	1.50	340000	340000	51000	24140	6290	1	0	1	0	0	1	*a*
Uronema marinum	25	0.019	500	500	75	36	9	0	0	0	1	0	1	*a*
Urostyla grandis	20	1.70	166000	166000	24900	11786	3071	1	0	0	0	0	1	*a*
Vorticella convallaria	20	0.840	550000	550000	82500	39050	10175	1	0	0	1	0	1	*a*
V. ephemerae	20	0.112	19000	19000	2850	1349	352	1	0	0	1	0	1	*a*
V. microstoma	20	0.010	26000	26000	3900	1846	481	1	0	0	1	0	1	*a*
V. similis	20	0.795	102000	102000	15300	7242	1887	1	0	0	1	0	1	*a*
Zoothamnium carinogammari	20	0.359	49000	49000	7350	3479	907	1	0	0	1	0	1	*a*

* [a] Fenchel and Finlay (1983); [b] Schmoker et al. (2011); [c] Caron et al. (1986); [d] Kawakami et al. (1985); [e] Klekowski and Tumantseva (1981); [f] Verity (1985).

S1-2: Species name, temperature (Temp), ammonia-N excretion (E), ammonia-N excretion:respiration (O:N), body mass (WM) and data source of planktonic herotrophic protozoans in Table II. Note: O:N ratio was calculated by the present author.

Genus and species	Temp (°C)	E (μgN $ind^{-1}h^{-1}$, $\times10^{-3}$)	O:N	WM (mg, $\times10^{-6}$)	Source*
Chrysomonad	22	0.0041		0.0400	*a*
"flagellate"	10	0.00031		0.0087	*a*
Monas sp.	30	0.0035		0.0350	*a*
Oxyrrhis sp.	20	0.0013		6.30	*a*
Paraphysomonas sp.	20	0.00071		0.270	*a*
Paraphysomonas imperforata	14	0.00033	7.5	0.226	*b*
	18	0.00048	5.0	0.224	*b*
	22	0.00049	5.9	0.228	*b*
	26	0.00058	11.5	0.210	*b*
Pseudobodo sp.	12	0.00011		0.0635	*a*
Spumella sp.	25	0.00087		0.0430	*a*
Cyclidium sp.	22	0.00059		0.400	*a*
Euplotes vannus	25	0.057		102.7	*a*
Paramecium aurelia	27	0.082		104.0	*a*
Strombidium sulcatum	12	0.038		88.00	*a*
Tintinnopsis acuminata	15	0.009	11.1	12.20	*c*
	20	0.013	14.0	11.93	*c*
	25	0.021	12.7	11.58	*c*
Tintinnopsis vasculum	5	0.032	8.3	125.9	*c*
	10	0.058	12.6	122.2	*c*
	15	0.082	13.1	117.4	*c*

* [a] Dolan (1997); [b] Caron et al. (1986); [c] Verity (1985).