

This is an electronic appendix to the paper by Atkinson *et al.* 2003 Protists decrease in size linearly with temperature: *ca.* 2.5% °C<sup>-1</sup>. *Proc. R. Soc. Lond. B* **270**, 2605–2611. (DOI 10.1098/rspb.2003.2538.)

Electronic appendices are refereed with the text. However, no attempt is made to impose a uniform editorial style on the electronic appendices.

#### Electronic appendix A

1. Data on rates of cell division were converted to natural logarithm-based specific growth rates (d<sup>-1</sup>).
2. A single outlying datum (*Rhizamoeba* sp. at 5°C, Butler & Rogerson 1996) was removed from the analysis, as its deviation from the cell volume at 15°C, expressed as a fraction of the cell volume at 15°C (Fig. 2a) was 2.5 times greater than that of the point with the next largest value (co-ordinate: 2.5, -10, not included in Fig. 2).
3. To test for biases due to differing sizes of dataset, we compared results derived from unweighted data (see paper) with those in which the relative thermal sensitivity of cell volume ( $a/V_{15}$ ) was weighted for each dataset by the number of points used to calculate the regression coefficient. The weighting factor = (number of datasets \* number of values in dataset)/total number of values from all datasets combined. As indicated in the paper, weighting made little difference to the estimates of mean relative thermal sensitivity of cell volume ( $a/V_{15}$ ).
4. The weighted mean ( $\pm$ se) relative thermal sensitivity of cell volume for the 65 datasets containing the 427 data points was  $-0.026^{\circ}\text{C}^{-1}$  ( $\pm$  0.003). Inclusion of the outlier mentioned in 2, above, changed the weighted mean ( $\pm$ se) to  $-0.027^{\circ}\text{C}^{-1}$  ( $\pm$  0.004). In either case, the relationship did not deviate from linearity ( $p > 0.4$ ).
5. Since there was rarely sufficient information to distinguish mixotrophs from other modes of nutrition (autotrophy, heterotrophy), ‘trophy’ was assigned according to the energy source provided in the experiment. Thus, an organism that may have been capable of mixotrophy in nature was coded as a heterotroph if it was cultured on an organic energy source.
6. Salinities indicated in the experiments were assigned to categories, thus: 0‰ – freshwater; 12 - 28‰ - brackish; 34 - 35‰ – marine.
7. **Table 1** below lists the datasets used in this study, and individual relationships between cell volume and temperature, ( $V_{15}$  is cell volume at 15°C, estimated from fitted linear regression equation; asterisk indicates estimate derived by extrapolation and therefore not used in meta-analysis; otherwise, estimates are from interpolations). Probability values are only given for significant ( $p < 0.05$ ) regressions.

**Table 1**

Species	Ref	CV <sub>15</sub>	Regression coefficient / CV <sub>15</sub>	Number of points	Sig. of regression p
AMOEBAE:					
<i>Clydonella rosenfieldi</i>	Butler & Rogerson 1996	410	-0.0539	4	
<i>Dactylamoeba</i> sp.	Butler & Rogerson 1996	2592	-0.06070	3	
<i>Paraflabellula reniformis</i>	Butler & Rogerson 1996	453	0.00199	4	
<i>Platyamoeba</i> sp.	Butler & Rogerson 1996	32	-0.056	3	
<i>Rhizamoeba</i> sp.	Butler & Rogerson 1996	123	-0.184	4	
<i>Stereomyxa ramosa</i>	Butler & Rogerson 1996	664	0.00166	3	
<i>Vahlkampfia baltica</i>	Butler & Rogerson 1996	334	-0.0523	4	
<i>Vannella caledonica</i>	Butler & Rogerson 1996	364	-0.0414	4	0.0078
<i>Vannella</i> sp.	Butler & Rogerson 1996	40	-0.010	4	
<i>Acanthamoeba polyphaga</i> Puschkarew	Baldock <i>et al.</i> 1980	1230	0.000815	3	
<i>Cochliopodium minus</i> Page	Baldock <i>et al.</i> 1980	3450	-0.0233	4	
<i>Glaeseria mira</i> Glässer	Baldock <i>et al.</i> 1980	396	0.0211	4	
<i>Saccamoeba limax</i> Page	Baldock <i>et al.</i> 1980	5970	-0.0131	3	
<i>Vannella</i> sp.	Baldock <i>et al.</i> 1980	7840	0.00329	4	
<i>Vexillifera bacillipedes</i> Page	Baldock <i>et al.</i> 1980	263	0.0211	3	
CILIATES:					
<i>Vorticella microstoma</i> Ehrenberg	Finlay 1977	21335	-0.019919	3	
<i>Cyclidium glaucoma</i> Müller	Finlay 1977	2430	-0.009953	3	
<i>Tetrahymena pyriformis</i> Ehrenberg	Finlay 1977	8524	-0.01764	3	
<i>Chilodonella uncinata</i> Ehrenberg	Finlay 1977	6200	0.01064	3	
<i>Colpidium campylum</i> Stokes	Finlay 1977	48700	-0.038472	3	
<i>Loxocephalus plagiatus</i> Stokes	Finlay 1977	32766	-0.019154	3	
<i>Paramecium aurelia</i> Ehrenberg	Finlay 1977	236205	0.0293266	3	
<i>Paramecium bursaria</i> Ehrenberg	Finlay 1977	213705	-0.0413323	3	
<i>Stentor polymorphus</i> Müller	Finlay 1977	1893000	0.04927	3	
<i>Spirostomum teres</i> Claparede and Lachmann	Finlay 1977	215476	0.002	3	
<i>Euplotes balteatus</i> (Dujardin)	Lee & Fenchel 1972	16100	-0.0163	4	
<i>Strombidinopsis multiauris</i>	Montagnes & Lessard 1999	166565	-0.0448429	6	0.0431
<i>Urotricha farcata</i> (Lake Schöhsee)	Weisse & Montagnes 1998	2313.35	-0.0339389	15	<0.0001
<i>Urotricha furcata</i> (mixed clone, Lake Schöhsee)	Weisse & Montagnes 1998	976.10	-0.016246	11	0.0004
<i>Urotricha furcata</i> (mixed clone, Lake Constance)	Weisse & Montagnes 1998	1533.25	-0.0622546	8	0.001
<i>Urotricha furcata</i> (clone A, Lake Constance)	Weisse & Montagnes 1998	1821.35	-0.0391547	8	0.0005
<i>Urotricha furcata</i> (clone B, Lake Constance)	Weisse & Montagnes 1998	1428.85	-0.0277248	12	0.0001
<i>Urotricha furcata</i> (clone C, Lake Constance)	Weisse & Montagnes 1998	1519.00	-0.0317290	12	0.0001
<i>Tetrahymena pyriformis</i> (HS)	James & Read 1957	14373	-0.023916	3	
DIATOMS:					
<i>Plagiotropis</i> sp.	Mühlig - Hofmann (unpubl.)	1688.25	-0.0106920	22	
<i>Cylindrotheca closterium</i>	Mühlig - Hofmann (unpubl.)	247.85	-0.017015	25	0.0099
<i>Skeletonema costatum</i> (Grev.) Cl.	Jørgensen 1968	169	-0.0435	4	0.0207
<i>Phaeodactylum tricornerutum</i> Bohlin	Morris & Glover 1974	330	-0.0556	3	
<i>Nitzschia closterium</i> (Ehrenberg)	Morris & Glover 1974	906	-0.108	2	
<i>Cylindrotheca closterium</i>	Waring (unpubl.)	227.0	-0.05972	17	<0.0001
<i>Achnanthes</i> sp.	Waring (unpubl.)	354.6	-0.03302	17	<0.0001
<i>Phaeodactylum tricornerutum</i>	Montagnes & Franklin 2001	77	-0.021	15	0.0172
<i>Ditylum brightwellii</i>	Montagnes & Franklin 2001	17440	-0.082229	12	0.0003
<i>Skeletonema costatum</i>	Montagnes & Franklin 2001	218	0.0275	12	

Species	Ref	CV <sub>15</sub>	Regression coefficient / CV <sub>15</sub>	Number of points	Sig. of regression p
<i>Thalassiosira weissflogii</i>	Montagnes & Franklin 2001	1362*	-0.1709*	6	
<i>Chaetoceros simplex</i> var. <i>calcitrans</i>	Montagnes & Franklin 2001	166	-0.0113	12	
<i>Cyclotella cryptica</i>	Montagnes & Franklin 2001	728	-0.0177	15	<0.0001
<i>Coscinodiscus</i> sp.	Montagnes & Franklin 2001	1700000	0.0221	9	
<i>Chaetoceros</i> sp.	Lomas & Glibert 1999	67	0.017	3	
<i>Thalassiosira weissflogii</i> (Grunow) Fryxell et Hasle	Lomas & Glibert 1999	1533	-0.01925	2	
<i>Phaeodactylum tricornutum</i> Bohlin	Kudo <i>et al.</i> 2000	137	0.0125	2	
<i>Phaeodactylum tricornutum</i> Bohlin	Thompson <i>et al.</i> 1992	42	0.0704	4	<0.05
<i>Chaetoceros calcitrans</i>	Thompson <i>et al.</i> 1992	40	0.0109	4	
<i>Thalassiosira pseudonana</i>	Thompson <i>et al.</i> 1992	48	0.0289	4	
<i>Chaetoceros simplex</i>	Thompson <i>et al.</i> 1992	59	0.0282	4	
DINOFLAGELLATES:					
<i>Alexandrium ostenfeldii</i>	Jensen & Moestrup 1997	22450	-0.070367	6	0.0003
<i>Gonyaulax tamarensis</i> Lebour	Watras & Chisholm 1982	9610	-0.0347	25	<0.0001
<i>Gonyaulax polyedra</i> Stein	Meeson & Sweeney 1982	57000	-0.056	2	
<i>Ceratium furca</i>	Meeson & Sweeney 1982	93000	-0.037	2	
<i>Prorocentrum minimum</i> (Pavillard) Schiller	Lomas & Glibert 1999	1468	-0.06397	3	
<i>Gyrodinium uncatenum</i> Hulburt	Lomas & Glibert 1999	17944	-0.038698	2	
<i>Heterocapsa triquetra</i>	Aelion & Chisholm 1985	1572	-0.02867	5	0.0105
<i>Thoracosphaera heimii</i> (Lohmann)	Karwath <i>et al.</i> 2000	1646.4*	0.011151*	7	
<i>Gymnodinium galatheanum</i> Braarud	Nielsen 1996	982	-0.0257	4	
FLAGELLATES:					
<i>Dunaliella tertiolecta</i>	Eppley & Sloan 1966	363	-0.0446	6	0.002
<i>Dunaliella tertiolecta</i> Butcher	Morris & Glover 1974	391	-0.0801	3	
<i>Rhodomonas salina</i>	Montagnes & Franklin 2001	353	-0.000651	9	
<i>Isochrysis galbana</i>	Montagnes & Franklin 2001	98	-0.053	12	0.0017
<i>Paraphysomonas imperforata</i> (Arctic)	Choi & Peters 1992	258	-0.00705	3	
<i>Paraphysomonas imperforata</i> (Newfoundland)	Choi & Peters 1992	184	-0.0126	3	
<i>Pavlova lutheri</i>	Thompson <i>et al.</i> 1992	38	0.0214	4	
<i>Dunaliella tertiolecta</i>	Thompson <i>et al.</i> 1992	204	-0.0060	4	
<i>Isochrysis galbana</i>	Thompson <i>et al.</i> 1992	41	0.0080	4	
<i>Dunaliella tertiolecta</i> Butcher	Sosik & Mitchell 1994	185	-0.0111	8	

## REFERENCES

- Aelion, C. M. & Chisholm, S. W. 1985 Effect of temperature on growth and ingestion rates of *Favella* sp.. *J. Plankton Res.* **7**, 821–831.
- Baldock, B. M., Baker, J. H., Sleigh, M. A. 1980 Laboratory growth rates of six species of freshwater *Gymnamoebia*. *Oecologia* **47**, 156–159.
- Butler, H. & Rogerson, A. 1996 Growth potential, production efficiency and annual production of marine benthic naked amoebae (gymnamoebae) inhabiting sediments of the Clyde Sea area, Scotland. *Aquat. Microb. Ecol.* **10**, 123–129.
- Choi, J. W. & Peters, F. 1992 Effects of temperature on two psychrophilic ecotypes of a heterotrophic nanoflagellate, *Paraphysomonas imperforata*. *Appl. Environ. Microbiol.* **58**, 593–599.
- Eppley, R. W. & Sloan, P. R. 1966 Growth rates of marine phytoplankton: correlation with light absorption by cell chlorophyll *a*. *Physiol. Plant* **19**, 47–59.
- Finlay, B. J. 1977 The dependence of reproductive rate on cell size and temperature in freshwater ciliated protozoa. *Oecologia* **30**, 75–81.

- James, T. W. & Read, C. P. 1957 The effect of incubation temperature on the cell size of *Tetrahymena pyriformis*. *Exp. Cell. Res.* **13**, 510–516.
- Jensen, M. Ø. & Moestrup, Ø. 1997 Autecology of the toxic dinoflagellate *Alexandrium ostenfeldii*: life history and growth at different temperatures and salinities. *Eur. J. Phycol.* **32**, 9–18.
- Jørgensen, E. G. 1968 The adaptation of plankton algae II. Aspects of the temperature adaptation of *Skeletonema costatum*. *Physiol. Plant.* **21**, 423–427.
- Karwath, B., Janofske, D., Tietjen, F., Willems, H. 2000 Temperature effects on growth and cell size in the marine calcareous dinoflagellate *Thoracospaera heimii*. *Mar. Micropal.* **39**, 43–51.
- Kudo, I., Miyamoto, M., Noiri, Y., Maita, Y. 2000 Combined effects of temperature and iron on the growth and physiology of the marine diatom *Phaeodactylum tricorutum* (Bacillariophyceae). *J. Phycol.* **36**, 1096–1102.
- Lee, C. C. & Fenchel, T. 1972 Studies on ciliates associated with sea ice from Antarctica II. Temperature responses and tolerances in ciliates from Antarctic, temperate and tropical habitats. *Arch. Protistenk. Bd.* **114**, 237–244.
- Lomas, M. W. & Glibert, P. M. 1999 Interactions between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. *Mar. Biol.* **133**, 541–551.
- Meeson, B. W. & Sweeney, B. M. 1982 Adaptation of *Ceratium furca* and *Gonyaulax polyedra* (Dinophyceae) to different temperatures and irradiances: growth rates and cell volumes. *J. Phycol.* **18**, 241–245.
- Montagnes, D. J. S. & Franklin, D. J. 2001 Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: reconsidering some paradigms. *Limnol. Oceanogr.* **46**, 2008–2018.
- Montagnes, D. J. S. & Lessard, E. J. 1999 Population dynamics of the marine planktonic ciliate *Strombidinopsis multiauris*: its potential to control phytoplankton blooms. *Aquat. Microb. Ecol.* **20**, 167–181.
- Morris, I. & Glover, H. E. 1974 Questions on the mechanism of temperature adaptation in marine phytoplankton. *Mar. Biol.* **24**, 147–154.
- Mühlig-Hofmann, A. (unpubl.) The effect of temperature on size and growth rate of marine benthic diatoms. B.Sc dissertation, 1999, University of Liverpool.
- Nielsen, M. V. 1996 Growth and chemical composition of the toxic dinoflagellate *Gymnodinium galatheanum* in relation to irradiance, temperature and salinity. *Mar. Ecol. Prog. Ser.* **136**, 205–211.
- Sosik, H. M. & Mitchell, B. G. 1994 Effects of temperature on growth, light absorption, and quantum yield in *Dunaliella tertiolecta* (Chlorophyceae). *J. Phycol.* **30**, 833–840.
- Thompson, P.A., Guo, M., Harrison, P.J. 1992 Effects of variation in temperature. I. On the biochemical composition of eight species of marine phytoplankton. *J. Phycol.* **28**, 481–488.
- Waring, J. (unpubl.) The effect of temperature on the growth rate and cell volume of marine benthic diatoms. B.Sc dissertation, 2000, University of Liverpool.
- Watras, C. J. & Chisholm, S. W. 1982 Regulation of growth in an estuarine clone of *Gonyaulax tamarensis* Lebour: salinity-dependent temperature responses. *J. Exp. Mar. Biol. Ecol.* **62**, 25–37.
- Weisse, T. & Montagnes, D. J. S. 1998 Effect of temperature on inter- and intraspecific isolates of *Urotricha* (Prostomatida, Ciliophora). *Aquat. Microb. Ecol.* **15**, 285–291.