

## NOTES

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### Growth rate peaks at intermediate cell size in marine photosynthetic picoeukaryotes

**Abstract**—We have performed an in situ test of Raven's prediction that there is a reversal of the relationship between cell size and maximum achievable growth rate in unicellular algae at the low end of size classes. In a natural population of marine phytoplankton, including the smallest picoeukaryote known to date, and under both nutrient sufficiency and deficiency, we find a maximum in growth rate (4.8 and 3.3 divisions  $d^{-1}$ , respectively) in the 2–3- $\mu m$  size class represented by coccoid *Chlorella*-like cells, with lower growth rates in both higher and lower size classes. This 2–3- $\mu m$  size class is also the most robust under nutrient deficiency, reducing its growth rate by 14% only relative to nutrient-sufficient conditions, versus 50–60% for the lowermost and uppermost size classes, respectively.

The relationship between cell size and maximum achievable growth rate in unicellular algae has been shown to be generally inverse (Chan 1978; Banse 1982; Tang 1995). This is due to both a thinner diffusion boundary layer and a greater surface to volume ratio which confer to small cells a greater capacity to acquire nutrients and efficiency in their use for growth and maintenance (Raven 1998). Such properties constitute a competitive advantage for picophytoplankton (<2  $\mu m$  in size) in oligotrophic waters because they are able to use scarce resources more efficiently than larger cells (Chisholm 1992). The size of the prokaryotic and eukaryotic picophytoplankton are close to the minimum possible size estimated from the occurrence of non-scalable essential cell components, i.e., their genome, the plasmalemma, and other membranes (Raven 1986, 1994, 1998). Thus, the cell sizes of two major components of autotrophic picoplankton in oceans, the coccoid cyanobacteria of the genera *Prochlorococcus* (0.6  $\mu m$ ) and *Synechococcus* (0.9  $\mu m$ ), are significantly smaller than the arbitrary upper limit of picoplanktonic cell size. Among the photosynthetic picoeukaryote community, *Ostreococcus tauri* is the smallest free-living eukaryote known to date (0.95  $\mu m$ , Courties et al. 1994). It has a minimal cellular organization with a single chloroplast and mitochondrion, and the smallest genome described among free-living eukaryotic cells (Derelle et al. 2002). Its small cellular and genome sizes unveiled the “bare limits” of life as a free-living photosynthetic eukaryote (Derelle et al. 2006). The extensive size reduction of picoplanktonic cells and the necessary occupation of a larger fraction of the biomass by non-scalable components put such constraints on maximum achievable growth rate that it has been suggested (Raven 1986, 1994) that, below a certain size, growth decreases with decreasing size of unicellular algae

both when resources are saturating and when they are limiting.

The different level of the structural organization between prokaryotic and eukaryotic algae has important physiological consequences (Raven 1986; Weisse 1993). Compared with cyanobacteria, eukaryotes must devote a larger fraction of their internal metabolism to maintenance processes because of the presence of internal membranes. Consequently, at a given size, eukaryotes have a higher specific metabolism than prokaryotes. On the other hand, the genome and the thickness of membranes impose higher limits on the smallest size of eukaryotic than of prokaryotic cells (Raven 1986, 1994, 1998).

Among the eukaryotic algae, the reversal of allometric relationship is supported by observations from laboratory cultures of chlorophytes highlighting a reduction of the growth rate for spherical cells whose diameter was less than 4  $\mu m$  (Raven 1994). Despite its attractiveness, the prediction that non-scalable components can reduce the maximum specific growth rate of very small cells has never been tested with field data. In the natural environment, the first estimates of growth rates of autotrophic picoeukaryotes by size class (Dupuy et al. 2000) were in agreement with Raven's theory, with an inversion of the allometric relationship for picoeukaryotes smaller than 2  $\mu m$  and a maximum growth rate (3.3 or 4.7 divisions  $d^{-1}$ ) for autotrophic eukaryotes belonging to the 2–4- $\mu m$  equivalent sphere diameter size class. However, this was a single experiment and without rigorous statistical treatment. Here we test this reversal of allometric relationship on a more extensive data set in natural populations of marine phytoplankton less than 10  $\mu m$  in cell diameter, including the smallest autotrophic eukaryote, *O. tauri* (Courties et al. 1994).

To examine growth rates in various size classes of natural eukaryotic phytoplankton, we sampled in the marine Mediterranean Thau lagoon where *O. tauri* was initially described. Cell-based gross growth rates were determined by the dilution technique (Landry and Hassett 1982) under both nutrient-sufficient and nutrient-deficient conditions. Flow cytometry was used to measure cell densities in different size classes, which were determined by both standard beads and cultures of unicellular algae of known cell diameters determined by optical microscopy.

**Methods**—Experimental procedure: Samples from the Thau lagoon (Southern France, 43°22'N, 3°35'E) were obtained on a monthly basis between May and September 1999, and from June to August 2001. Data from other

periods were not used because phytoplankton growth was found to be limited by irradiance, and therefore could not reach maximum growth rates under nutrient enrichment (Bec et al. 2005). Water temperature ranged between 20°C and 25°C, and salinity between 35.8 and 38.2. Water was sampled by immersing 20-liter polycarbonate (PC) carboys under the surface. Growth measurements were carried out according to a modified version of the dilution technique (Landry and Hassett 1982). An aliquot was gravity filtered on Suporcap (Pall-Gelman) 0.2- $\mu\text{m}$  cartridges that had been rinsed with 1 liter of deionized water and 1 liter of seawater. Filtered and raw water were mixed in order to obtain the following dilution series in duplicates: 0.09, 0.17, 0.43, 0.74, and 1.0 ( $x$ , unfiltered fraction) in 1-liter PC bottles. The enrichment was prepared on the basis of  $f/2$  medium (Guillard and Ryther 1962) where nitrate was replaced by ammonium at a final concentration of 20  $\mu\text{mol N L}^{-1}$ , which was sufficient to satisfy the nutrient demand over 24 h. All other nutrients were in stoichiometric proportions. Two control bottles were filled with unfiltered water without nutrient additions. All bottles were incubated 24 h in situ at 0.5-m depth, starting around 1000 h local time.

Estimation of growth rates: Temporal changes in cell densities were used to compute the apparent growth rate  $k(x)$  for each dilution ( $x = 1$  for the undiluted sample, and  $x = 0$  at infinite dilution),

$$k(x, t) = 1/t \ln(C_{x,t}/C_{x,0}) \quad (1)$$

where  $C(x, 0)$  and  $C(x, t)$  are initial and final cell densities. Linear regressions between apparent growth rate and dilution factor (Landry and Hassett 1982) allow simultaneous determination of the instantaneous growth rate  $\mu_{\text{max}}$  ( $Y$ -axis intercept) and the phytoplankton mortality rate due to microzooplankton grazing  $g$  (slope of the line):

$$k(x) = \mu_{\text{max}} - gx \quad (2)$$

where  $\mu_{\text{max}}$  is determined in incubations with complete nutrient enrichment and is assumed to be independent of dilution effect on population density (Landry and Hassett 1982). In order to estimate the phytoplankton growth without nutrient addition, the mortality rate ( $g$ ) was then added to the apparent phytoplankton growth rate ( $k_0$ ) in unenriched controls (Landry et al. 1998),

$$\mu_0 = k_0 + g \quad (3)$$

Determination of size classes: Density and cell size of phytoplankton were estimated by flow cytometry (FACS-Calibur, Becton-Dickinson). Eukaryotic phytoplankton populations were discriminated on the basis of light diffraction (forward scatter [FSC], which is related to cell size) and red fluorescence emissions (chlorophyll pigments, wavelength > 650 nm). All samples were analyzed with a mixture of fluorescent beads ("Fluoresbrite," Polysciences) of various nominal sizes (1, 2, 3, 6, and 10  $\mu\text{m}$ ). Cell size classes were estimated from the FSC data of natural populations in comparison with FSC of fluorescent beads. The size classes were also calibrated with different

species of phytoplankton cultures, *O. tauri* (1  $\mu\text{m}$ ), *Nannochloropsis* sp. (3.6  $\mu\text{m}$ ), *Porphyridium cruentum* (6.6  $\mu\text{m}$ ), *Thalassiosira pseudonana* (7.9  $\mu\text{m}$ ), *Dunaliella salina* (10.3  $\mu\text{m}$ ), *Tetraselmis suecica* (12.1  $\mu\text{m}$ ), and *Alexandrium catenella* (25  $\mu\text{m}$ ), whose cell diameter had been determined by optical microscopy and analyzed by Optilab Pro software. The smallest cell diameter of photosynthetic picoeukaryotes observed in this study (0.6  $\mu\text{m}$ ) was determined by electron microscopy.

Statistical analysis: For the relationship between growth rate ( $\mu$ ) and size ( $S$ ) (assumed to be the center of size classes defined above) of picoplankton, we first fitted a quadratic linear regression to the data:  $\mu = \alpha + \beta(S) + \gamma(S^2)$ . If the two coefficients of the regression ( $\beta$  and  $\gamma$ ) are not significantly different from zero, picoplankton growth rate is supposed not to vary with size class. If only the quadratic term ( $\gamma$ ) does not differ from zero, the relation is monotonic linear, and the slope defines the direction of the relation. Regressions in which the quadratic term ( $\gamma$ ) explains a significant amount of variance, after fitting the linear term, have a curvilinear shape. In such cases we also tested for a hump-shaped or peaked curve with the Mitchell-Olds test (Mitchell-Olds and Shaw 1987). This method tests whether an unconstrained least-squares model with a particular intermediate maximum provides a significantly better fit to the data than a model with a maximum at either the higher or lower end of the range of values. In other words, it tests whether there is evidence of a hump as opposed to simple monotonic change.

**Results**—Under nutrient-sufficient conditions (Fig. 1A and Table 1), individual values of maximum growth rates ( $\mu_{\text{max}}$ ) ranged between 0.3 and 3.3  $\text{d}^{-1}$  (corresponding to 0.4 and 4.8 divisions  $\text{d}^{-1}$ ). These minimum and maximum values were measured for autotrophic eukaryotes whose diameter was inferior to 1  $\mu\text{m}$  and in the 2–3- $\mu\text{m}$  equivalent sphere diameter size class, respectively. There was no significant relationship between water temperature and maximum growth rates ( $n = 21$ ,  $r^2 = 0.003$ ,  $p > 0.05$ ).

Mean values per size class ranged between 0.75 and 2.25  $\text{d}^{-1}$  (corresponding to 1.1 and 3.25 divisions  $\text{d}^{-1}$ ) and were observed in the 6–10- $\mu\text{m}$  and 2–3- $\mu\text{m}$  equivalent sphere diameter size class, respectively (Table 1). The statistical analysis of the relationship between maximum growth rate ( $\mu_{\text{max}}$ ) and eukaryotic algae size indicated that the quadratic term ( $\gamma$ ) differs significantly from zero, signifying a curvilinear shape of the relationship between cell size and growth rate (Fig. 1A). The Mitchell-Olds test also indicates that the change is not monotonic and an intermediate maximum exists in this relationship. This maximum growth rate (2.25  $\text{d}^{-1}$ ) was observed in the 2–3- $\mu\text{m}$  equivalent sphere diameter size class (Table 1). Smaller and larger algae had lower growth rates.

Under nutrient-deficient conditions (Fig. 1B and Table 1), growth rates ( $\mu_0$ ) were significantly ( $p < 0.0001$ ) lower than under nutrient-sufficient conditions and represented between 41% and 86% of growth rates (in the 2–3- $\mu\text{m}$  and in the 6–10- $\mu\text{m}$  equivalent sphere diameter size class, respectively) under nutrient sufficiency. Individual values of growth rates ( $\mu_0$ ) ranged between 0.1 and 3  $\text{d}^{-1}$ ,

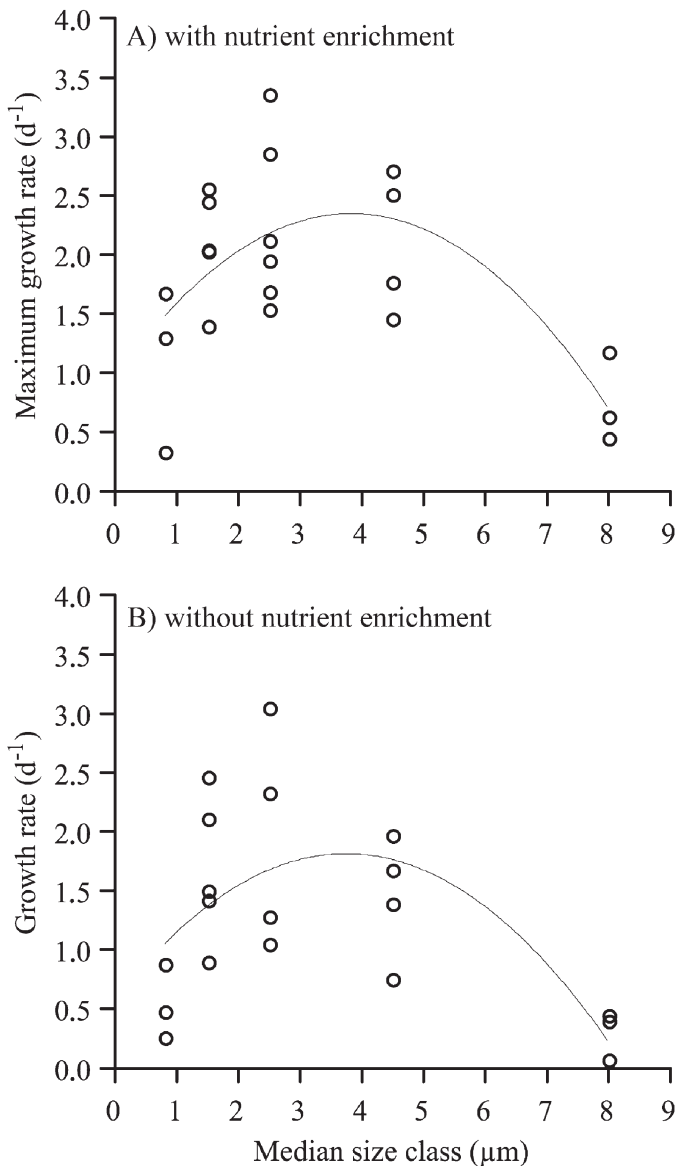


Fig. 1. Growth rates for several size classes of eukaryotes, incubated in situ for 24 h, (A) with nutrient enrichment ( $r^2 = 0.47$ ,  $n = 21$ ,  $p < 0.01$ ) and (B) without nutrient enrichment ( $r^2 = 0.42$ ,  $n = 19$ ,  $p < 0.05$ ). The relationships are significantly unimodal. See text for explanation of statistical methodology. For the 1–2- $\mu\text{m}$  size class ( $n = 5$ ), two maximum growth rates ( $\mu_{\text{max}}$ ) estimated with nutrient enrichment (A) are similar (2.03 and 2.04  $\text{d}^{-1}$  in May and September 1999, respectively) and overlap in the figure. See Table 1 for all data.

corresponding to 0.14 and 4.3 divisions  $\text{d}^{-1}$ , respectively (Fig. 1B). These minimum and maximum values were measured for microalgae whose diameter was in the 2–3- $\mu\text{m}$  and in the 6–10- $\mu\text{m}$  equivalent sphere diameter size class, respectively (Table 1). There was no significant relationship between water temperature and growth rates ( $n = 19$ ,  $r^2 = 0.001$ ,  $p > 0.05$ ). The Mitchell-Olds test (see above) also indicated a significant maximum in growth rate, likewise in the size class 2–3- $\mu\text{m}$  equivalent sphere diameter (Table 1). This size class was also the least sensitive to nutrient

deficiency, reducing its growth rate by 14% only. Classes at either end of the spectrum presented the greatest decreases in growth rate relative to nutrient sufficiency (50–60% for the lowermost and uppermost size classes, respectively).

*Discussion*—Our results confirm the prediction (Raven 1986, 1994) that picophytoplanktonic cells may deviate from the general allometric relationships (Chan 1978; Banse 1982; Tang 1995) with a reversal in the relationship between picoeukaryotic growth rate and cell size occurring below the 2–3- $\mu\text{m}$  equivalent sphere diameter size class. This size class is somewhat smaller than that found in laboratory cultures (4- $\mu\text{m}$  cell diameter) for chlorophytes (Raven 1986). Our data set does not appear to conform to the 3/4 and 1/1 scaling relations, so very small phytoplankton are an exception to this rule.

Theoretical decreases in  $\mu_{\text{max}}$  can be estimated from the decrease in catalytic machinery such as enzymes resulting from the increased fraction occupied by the genome and membranes as the cell size decreases (Raven 1998). Reducing the cell diameter from a median value of 2.5 to 1.5  $\mu\text{m}$  should lead to a 9% decrease in microalgal growth rate (vs. 7% observed here for  $\mu_{\text{max}}$  and 13% for  $\mu_0$ ), while a reduction from a median value of 1.5 to 0.8  $\mu\text{m}$  should lead to a 26% decrease (vs. 47% observed here for  $\mu_{\text{max}}$  and 68% for  $\mu_0$ ). The latter theoretical estimate is extremely sensitive to the lower limit of the size class under consideration, and this may explain the disagreement between theoretical and observed decreases in  $\mu_{\text{max}}$ . Under nutrient limitation, the actual decreases are greater than those predicted under nutrient-sufficient conditions in laboratory cultures.

Preliminary studies under similar field conditions have shown that  $\mu_{\text{max}}$  of picophytoplankton is unaffected over the first 7 h of incubation under nutrient limitation, but a decrease can be observed over 24 h of incubation. Such a duration of incubation (24 h) was chosen in order to estimate balanced growth, rather than transient phenomena. The “real world” situation is probably somewhere between the lines shown in Fig. 1A and 1B because they represent two extreme situations imposed by the techniques involved in the measurements. In spite of those limitations, the trends are rather well constrained.

We have thus shown that there exists an optimum size (2–3  $\mu\text{m}$ ) for achieving maximum growth under both nutrient-limited and nutrient-unlimited situations. This 2–3- $\mu\text{m}$  size class is also the most robust under adverse nutrient-limited conditions. In terms of interspecific competition, this size class seems well adapted to its environment, which is characterized by overall low nutrient concentrations (Collos et al. 1997), on which are superposed nutrient pulses from the watershed following rain events or from the sediment following anoxia events (Souchu et al. 1998). However, we have not observed a dominance of this size class during recent years (Vaquer et al. 1996; Bec et al. 2005), so that this growth capacity is not the overriding factor and other variables such as grazing must be taken into account to explain the seasonal succession of phytoplankton in those environments.

Table 1. Growth rate ( $\pm$ SE, standard error) as a function of size class of phytoplankton. For each size class, mean growth rate is indicated in the first line followed by detailed data of water temperature and growth rates from dilution experiments conducted in 1999 and 2001.  $\mu_{\max}$ : maximum growth rate under nutrient sufficiency ( $n = 10$ ).  $\mu_0$ : growth rate without nutrient addition ( $n = 4$ ). The significance level of the linear regression, i.e. slope is significantly different from zero, is  $p < 0.05$ . NS, not significant.

Size class ( $\mu\text{m}$ )	Date	Temperature ( $^{\circ}\text{C}$ )	$\mu_{\max} \pm \text{SE}$ ( $\text{d}^{-1}$ )	$\mu_0 \pm \text{SE}$ ( $\text{d}^{-1}$ )
0.6–1	—	—	$1.10 \pm 0.40$	$0.54 \pm 0.18$
	May 1999	20	$1.68 \pm 0.06$	$0.88 \pm 0.00$
	Jun 2001	22.7	$0.33 \pm 0.04$	$0.26 \pm 0.03$
	Aug 2001	24.7	$1.30 \pm 0.07$	$0.48 \pm 0.04$
1–2	—	—	$2.10 \pm 0.20$	$1.68 \pm 0.27$
	May 1999	19.7	$2.03 \pm 0.04$	$1.50 \pm 0.06$
	Jun 1999	21.7	$2.45 \pm 0.03$	$0.90 \pm 0.07$
	Jul 1999	23.3	$2.56 \pm 0.06$	$2.46 \pm 0.11$
	Sep 1999	23.3	$2.04 \pm 0.02$	$2.11 \pm 0.00$
	Jul 2001	24.9	$1.40 \pm 0.06$	$1.42 \pm 0.04$
2–3	—	—	$2.25 \pm 0.29$	$1.93 \pm 0.47$
	Sep 1999	23.3	$2.12 \pm 0.05$	$2.33 \pm 0.06$
	Sep 1999	23.3	$2.86 \pm 0.11$	$3.05 \pm 0.10$
	Jun 2001	22.7	$1.69 \pm 0.04$	$1.54 \pm 0.49^{\text{NS}}$
	Aug 2001	24.7	$3.36 \pm 0.05$	$1.28 \pm 0.02$
	Jul 2001	24.9	$1.95 \pm 0.06$	$1.05 \pm 0.09$
	Jun 2001	22.7	$1.54 \pm 0.04$	$1.24 \pm 0.31^{\text{NS}}$
3–6	—	—	$2.11 \pm 0.30$	$1.45 \pm 0.26$
	May 1999	20	$1.77 \pm 0.06$	$1.38 \pm 0.08$
	Jun 1999	21.7	$1.46 \pm 0.09$	$0.75 \pm 0.06$
	Jul 1999	23.3	$2.71 \pm 0.05$	$1.68 \pm 0.10$
	Sep 1999	23.3	$2.51 \pm 0.12$	$1.97 \pm 0.08$
6–10	—	—	$0.75 \pm 0.22$	$0.31 \pm 0.12$
	Jun 1999	21.7	$1.18 \pm 0.15$	$0.45 \pm 0.08$
	Jun 2001	22.7	$0.45 \pm 0.08$	$0.40 \pm 0.20$
	Jul 2001	24.9	$0.63 \pm 0.04$	$0.07 \pm 0.02$

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## References

- BANSE, K. 1982. Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. *Limnol. Oceanogr.* **27**: 1059–1071.
- BEAUCOURT, B., J. HUSSEINI-RATREMA, Y. COLLOS, P. SOUCHU, AND A. VAQUER. 2005. Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: Emphasis on the picoeukaryote community. *J. Plankton Res.* **27**: 881–894.
- CHAN, A. T. 1978. Comparative physiological study of marine diatoms and dinoflagellates in relation to irradiance and cell size. I. Growth under continuous light. *J. Phycol.* **14**: 396–402.
- CHISHOLM, S. W. 1992. Phytoplankton size, p. 213–237. *In* P. G. Falkowski and A. D. Woodhead [eds.], Primary productivity and biogeochemical cycles in the sea. Plenum.
- COLLOS, Y., A. VAQUER, B. BIBENT, G. SLAWYK, N. GARCIA, AND P. SOUCHU. 1997. Variability in nitrate uptake kinetics of phytoplankton communities in a Mediterranean coastal lagoon. *Estuar. Coast. Shelf Sci.* **44**: 369–375.
- COURTIES, C., A. VAQUER, M. J. CHRÉTIENNOT-DINET, M. TROUSSELLIER, J. NEVEUX, H. CLAUSTRE, J. LAUTIER, AND C. MACHADO. 1994. Smallest eukaryotic organism. *Nature* **370**: 255.

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- DERELLE, E., AND OTHERS. 2002. DNA libraries for sequencing the genome of *Ostreococcus tauri* (Chlorophyta, Prasinophyceae): The smallest free-living eukaryotic cell. *J. Phycol.* **38**: 1150–1156.
- , AND OTHERS. 2006. Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. *Proc. Natl. Acad. Sci.* **103**: 11647–11652.
- DUPUY, C., A. VAQUER, T. LAM-HÖAI, C. ROUGIER, N. MAZOUNI, J. LAUTIER, Y. COLLOS, AND S. LE GALL. 2000. Feeding rate of oyster *Crassostrea gigas* in a natural planktonic community of the Mediterranean Thau Lagoon. *Mar. Ecol. Prog. Ser.* **205**: 171–184.
- GUILLARD, R. R. L., AND J. H. RYTHER. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* **8**: 229–239.
- LANDRY, M. R., S. L. BROWN, L. CAMPBELL, J. CONSTANTINO, AND H. LIU. 1998. Spatial patterns in phytoplankton growth and microzooplankton grazing in the Arabian Sea during monsoon forcing. *Deep-Sea Res. II* **45**: 2353–2368.
- , AND R. P. HASSETT. 1982. Estimating the grazing impact of marine micro-zooplankton. *Mar. Biol.* **67**: 283–288.
- MITCHELL-OLDS, T., AND R. G. SHAW. 1987. Regression analysis of natural selection: Statistical inference and biological interpretation. *Evolution* **41**: 1149–1161.
- RAVEN, J. A. 1986. Physiological consequences of extremely small size for autotrophic organisms in the sea, p. 1–70. *In* T. Platt and W. K. W. Li [eds.], *Photosynthetic picoplankton*. Canadian Bulletin of Fisheries and Oceans.
- . 1994. Why are there no picoplanktonic O<sub>2</sub> evolvers with volumes less than 10<sup>-19</sup> m<sup>3</sup>? *J. Plankton Res.* **16**: 565–580.
- . 1998. Small is beautiful: The picophytoplankton. *Funct. Ecol.* **12**: 503–513.
- SOUCHU, P., A. GASC, Y. COLLOS, A. VAQUER, H. TOURNIER, B. BIBENT, AND J. M. DESLOUS-PAOLI. 1998. Biogeochemical aspects of bottom anoxia in a Mediterranean lagoon (Thau, France). *Mar. Ecol. Prog. Ser.* **164**: 135–146.
- TANG, E. P. Y. 1995. Why do dinoflagellates have lower growth rates? *J. Plankton Res.* **17**: 1325–1335.
- VAQUER, A., M. TROUSSELLIER, C. COURTIES, AND B. BIBENT. 1996. Standing stock and dynamics of picophytoplankton in the Thau Lagoon (northwest Mediterranean coast). *Limnol. Oceanogr.* **41**: 1821–1828.
- WEISSE, T. 1993. Dynamics of autotrophic picoplankton in marine and freshwater ecosystems, p. 327–370. *In* G. Jones [ed.], *Advances in microbial ecology*. Plenum Press.

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