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Assessing phytoplankton lysis in Lake Kinneret

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Abstract

We determined lysis rates of phytoplankton cells in Lake Kinneret and in lake-water microcosms by measuring the activities of particle-associated and dissolved esterases and the decay rate of the latter. Over a 2-yr period from May 2002, dissolved esterase activity (DEA) in epilimnetic lake water, corrected for nonenzymatic hydrolysis of the assay substrate, fluorescein diacetate (FDA) averaged 39 nmol FDA L^{-1} h^{-1}, (range 6–120 nmol FDA L^{-1} h^{-1}) and particulate esterase activity (PEA) averaged 93 nmol FDA L^{-1} h^{-1} (range 20–244 nmol FDA L^{-1} h^{-1}). Algal cell esterase content normalized to chlorophyll (Chl), PEA Chl^{-1}, averaged 6.9 nmol FDA (μg Chl)$^{-1}$ h^{-1}, range 0.4–18.7 nmol FDA (μg Chl)$^{-1}$ h^{-1}. In monoalgal cultures, levels of PEA Chl^{-1} also varied widely both with algal species and growth phase. Most (>90%) of the PEA in the lake was associated with algal cells, thus bacteria, protozoa, and zooplankton were insignificant sources of DEA. Decay rates of DEA in lake water averaged 0.16 (SD $\pm$ 0.13) h^{-1} and were much faster than those previously reported for marine waters. Based on these data, calculated phytoplankton lysis rates (LR) in Lake Kinneret averaged 0.91 (SD $\pm$ 0.59) d^{-1}, with more rapid rates roughly corresponding to seasons of lower Chl concentrations. Because of the high variability in the measured key method parameters (DEA, PEA Chl^{-1}, DEA decay rates) and uncertainty of extrapolation to daily values, we prefer to regard these rates of phytoplankton lysis as apparent and suggest that they may be overestimates. Nevertheless, our results indicate a sizable flux of PEA from phytoplankton to DEA in this lake and emphasize the potential importance of phytoplankton cell lysis as a dynamic process for transferring material from the particulate phase of the primary producers to the soluble phase in aquatic environments.

In aquatic environments, losses of phytoplankton biomass occur through respiration, exudation of organic matter, grazing, sedimentation, and cell lysis. With the exception of cell lysis, these processes have been extensively quantified in many natural water bodies. Phytoplankton lysis may be caused by the action of algal viruses (Suttle 1994; Brussaard 2004), by cell senescence and death (Berges and Falkowski 1998; Berman-Frank et al. 2004; Bidle and Falkowski 2004), or by environmental stress, such as exposure to ultraviolet or high irradiance.

The assessment of actual rates of phytoplankton lysis in aquatic environments has been problematic. Van Boekel et al. (1992) introduced a method based on the measurement of dissolved esterase activities to obtain a semiquantitative estimate of cell lysis rates in a *Phaeocystis* bloom in the North Sea. The dissolved esterases were assumed to result from cell breakage or membrane damage (predominantly of algal cells) and could therefore serve as tracers of cell lysis. This approach was subsequently used by Brussard et al. (1995) to compare the loss of carbon through algal lysis to other phytoplankton-mediated carbon fluxes in coastal North Sea waters. Agusti et al. (1998) modified the esterase method by assuming that ambient levels of dissolved esterase and the rates of this activity remained at steady state over a 24-h period. These workers also showed that, by far, the major source of dissolved esterase activity (DEA) indeed derived from phytoplankton with only a minor contribution from heterotrophic plankton. Agusti et al. (1998) reported high lysis rates (LR) in summer in the northwestern Mediterranean (maximum 1.47 d^{-1}), with much lower rates in winter. A similar seasonal pattern was also found in the Blanes Bay, northeastern Spain (Agusti and Duarte 2000). In the unproductive areas of the eastern subtropical Atlantic, Agusti et al. (2001) estimated that much of the dissolved organic carbon pool was derived from algal lysis and postulated that the relative importance of this carbon-flux pathway increased with increasing oligotrophy of the environment. Although the published quantitative results for phytoplankton lysis obtained by variations of the esterase method have been subject to some debate (Riegman et al. 2002; Agusti and Duarte 2002), nevertheless, they have raised awareness of the potential importance of algal lysis as a process leading to the release of dissolved organic matter (DOM) in aquatic ecosystems (Kirchman 1999).

As yet, estimates of phytoplankton LR using the esterase or other methods have been made only for marine waters. In this paper, we report on a study of phytoplankton lysis in a freshwater environment, mesotrophic–eutrophic Lake Kinneret, Israel. In order to optimize this method for freshwater, we were obliged to modify the esterase assay. In addition, we checked the applicability of some of the key assumptions of this method made by Van Boekel et al. (1992) and Agusti et al. (1998) as applied to the lake environment.

Methods

Measurement of particulate and dissolved esterases—Samples of phytoplankton in lake water (250 ml) or algal culture (5–50 ml) were collected on glass fiber filters (GF/

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C). Three methods were tested for extraction of intracellular particulate esterase activity (PEA): (1) Glass–glass homogenization. In this method, the filter was ground in 4 ml of 0.1 mol L^{-1} phosphate buffer (pH 7.8) in a power-driven, glass–glass homogenizer (Ace Glass). (2) Sonication. The filter with the cells was cut into small pieces, placed in 20 ml of 0.1 mol L^{-1} phosphate buffer (pH 7.8), and sonicated in a MSI Sonicator three times for 30 s at full power. (3) Bead Beater (mixer mill). The filter with the cells was cut into small pieces, placed in 20 ml of 0.1 mol L^{-1} phosphate buffer (pH 7.8) with 1 g glass beads, and homogenized four times for 30 s in a Bead Beater Cell Disrupter (Biospec Products). The cell slurries were cooled with ice during the above treatments. After extraction, nonspecific esterase activity was determined using fluorescein diacetate (FDA; Van Boekel et al. 1992; Chrost et al. 1999) as follows.

Duplicate samples of the homogenate (4 ml) were mixed with 950 μl of 0.5 mol L^{-1} phosphate buffer (pH 7.8) and 50 μl of 2.5 mmol L^{-1} FDA in acetone, freshly prepared from a stock solution, giving a final FDA concentration of 25 μmol L^{-1}. After incubation at 30°C for 1 h in the dark, the sample was centrifuged, and triplicate aliquots (200 μl) of the supernatant were placed in the wells of a 96-place microwell plate. Fluorescence was measured in a Biotek FL500 microplate reader set at excitation wavelength, 485 $\pm$ 10 nm; emission wavelength, 530 $\pm$ 12.5 nm. For controls, we ran the assay with 4 ml of distilled water instead of homogenate. Blanks obtained from these controls were subtracted from the fluorescent readings given by experimental samples. A standard curve of fluorescein (0–500 nmol L^{-1}) was measured at the same time as the enzyme assay to convert the fluorescence to fluorescein concentration. Esterase activities were calculated as nmol FDA L^{-1} h^{-1}. PEA was normalized to chlorophyll (Chl), PEA Chl^{-1}, as nmol FDA (μg Chl)$^{-1}$ h^{-1}.

DEA was determined in the same way, except that 4 ml of GF/C filtrate was used instead of the homogenate. In addition, to correct for nonenzymatic breakdown of FDA (Chrost et al. 1999; Riegman et al. 2002), we passed 10 ml of GF/C filtrate from lake water through a <10-kDa centrifuge filter device (CFD; Millipore) by centrifuging for 20 min at 4,000 rpm. The measured rates of FDA hydrolysis in the filtrate (~9.8 ml) were taken as deriving from nonenzymatic hydrolysis. The esterase activity in the supernatant (~0.2 ml) retained in the upper part of the CFD, containing ~50-fold concentrated DEA, was also measured. The corrected ambient DEA was then calculated by subtracting the nonenzymatic hydrolytic activity from that of the DEA activity in the supernatant fraction. Preliminary trials with commercial porcine esterase had shown that >99% of the enzyme protein was retained in the supernatant of the CFDs.

Esterase die-off experiments—In order to determine the decay constant of DEA in Lake Kinneret water, we added 250 μl of 100-fold diluted porcine esterase (Sigma #2884) or appropriate amounts of DEA from algal cultures to 100 ml of lake water and incubated in the dark at ambient lake temperature for 4–6 h. Aliquots to determine DEA were taken hourly and the decay constant was calculated from a best-fit exponential curve.

Algal cultures—Monoalgal cultures (250 ml) of some important lake algal species (*Peridinium gatunense*, *Pediastrum* sp., *Scenedesmus* sp., *Chlamydemonas* sp., *Tetraedron* sp., *Synedra* ssp., *Aulacoseira* spp., *Cyclotella* spp., *Aphanizomenon ovalisporum*, *Anabaena* sp., and *Microcystis* sp.) were grown at 20–22°C at ~80 μmol quanta m^{-2} s^{-1} illumination and a 12 : 12 dark : light (DL) regime. Levels of PEA and chlorophyll (Chl) were determined at late exponential or early stationary growth phase in these cultures.

Several experiments were also made with algal monocultures in larger volumes (2–8 liter), which permitted measurements of Chl, PEA, and DEA at different phases in the growth cycle.

Lake microcosm experiments—On four occasions (experiment A, 22 December 2002; experiment B, 19 January 2003; experiment C, 28 October 2003; experiment D, 22 March 2004) 10–24 liters of near-surface water were collected from a central lake site (Sta. A). The samples were incubated in glass carboys, with or without nutrient addition (100 μmol L^{-1} NO_3^- and 10 μmol L^{-1} PO_4^{3-}) for up to 21 d in the laboratory at close to ambient lake temperatures with a 12 : 12 DL regime at ~100 μmol quanta m^{-2} s^{-1} illumination. The carboys were sampled initially and during the course of the incubation for chlorophyll, PEA, and DEA. During experiments C and D, the DEA decay rate was also measured initially, after 7 and 14 d (experiment C), and after 4 d (experiment D).

Phytoplankton LR in lake water—Starting from May 2002 until August 2004, samples were taken biweekly from 3 depths (1, 5, and 10 m) at Sta. A together with an additional sample (1-m depth) from a shallow littoral station (Amnon). The levels of PEA, DEA, and Chl concentrations were measured in each of these samples. Because of logistic difficulties, we could not determine DEA decay rates on the same days but used measurements made in lake water within several days of the sampling for calculations of phytoplankton lysis rates.

LR in lake water and in the lake microcosm experiments were calculated from measured PEA, DEA, and DEA decay rates as in Agusti et al. (1998), except that PEA was determined directly and DEA was corrected for nonenzymatic hydrolysis of FDA in each sample.

PEA in microzooplankton, protozoa, and bacteria—We determined the PEA and biovolume of microzooplankton (predominantly copepods and cladocerans) taken from the lake on four occasions. Fifty to 100 individual zooplankton, taken by 63-μm net hauls, were transferred to ~5 ml of GF/C-filtered lake water. The animals were counted in the microscope and their biovolume calculated. They were then macerated in a glass–glass homogenizer and filtered through a GF/C filter. The extracted esterase activity in the filtrate was measured as above and the PEA per biovolume of zooplankton calculated with corrections made for initial DEA in the sample. The potential contribution of microzooplankton PEA to the total measured lake PEA was estimated by multiplying the zooplankton PEA per zooplankton biovolume (measured as described previously) by the total biovol-

ume of microzooplankton in the upper 15 m of the lake water as determined during the routine biweekly lake monitoring program.

Similar experiments were carried out to estimate the PEA of protozoa and bacteria. In this case, however, we grew monocultures of ciliates, flagellates, or lake bacteria that were concentrated in 5–10 ml of GF/C-filtered lake water prior to being homogenized with a glass–glass grinder. The PEA content of these organisms was calculated on a per cell basis. Because there were no current measurements of protozoan abundance in the lake, we used the greatest abundance reported by Hadas and Berman (1998) for ciliate and flagellate populations in Lake Kinneret to estimate the maximum potential PEA contribution by protozoa. For the potential PEA contribution of bacteria, we used an average abundance of 3.3×10^9 bacteria L^{-1} in epilimnetic lake water (Berman et al. 2004).

Chl—Chl was measured fluorometrically using acetone extraction (Holm-Hansen et al. 1965).

Results

Optimizing PEA extraction from algal cells—Preliminary tests to extract PEA from algal cells collected on GF/C filters gave the best results with the glass–glass homogenizer. We therefore used this method throughout most of this study. However, at a later stage, when these experiments were repeated more extensively, we found that usually, but not always, sonication gave somewhat higher extraction efficiencies of PEA from lake phytoplankton than the glass–glass homogenizer, while the bead beater was the least effective. On average, for 10 separate experiments, extraction of PEA by glass–glass homogenizer and bead beater was 91.2% (SD $\pm$ 27.1%) and 67.4% (SD $\pm$ 37.2%), respectively, of that given by sonication. There was, however, considerable variability between the extraction efficiencies measured on different dates.

We attempted to release cytoplasmic esterases by pretreating samples with lysozyme (Agusti et al. 1998). Lysozyme addition raised fluorescence levels not only in experimental samples but also in controls without homogenate, presumably because of some intrinsic esterase activity associated with the lysozyme (Riegman et al. 2002). Variability between replicates was also much higher with lysozyme and, therefore, we did not use this method.

pH and temperature optima for esterase assays—PEA extracted from algal cultures or from lake phytoplankton was measured over a pH range from 3 to 9. Acetate, phosphate, and Tris buffers were used for pH ranges from 3 to 6, 5 to 8, and 7 to 9, respectively. As a control, FDA, in the absence of homogenate, was also incubated with each buffer to follow abiotic hydrolysis of the substrate. In addition, a fluorescein standard curve was determined at each pH value to eliminate the effects of pH on fluorescence. Lake-water samples showed optimum esterase activity at pH 7.8.

To determine the optimum temperature for the esterase assay described above, 1-h incubations were carried out over a range of 15–50°C. Maximum PEA for mixed populations of lake phytoplankton occurred at ~30°C, and this temperature was used for the experiments described here.

Contribution of nonenzymatic hydrolysis of FDA to measurements of DEA—As noted by Chrost et al. (1999) and Riegman et al. (2002), the measurement of DEA may be compromised by nonenzymatic hydrolysis of the FDA substrate used in the esterase assay. We determined the extent of this nonenzymatic breakdown by removing the enzyme protein from GF/C-filtered lake water (or algal culture medium) with microfilter centrifugation as described in the Methods. Nonenzymatic hydrolysis in the <10-kDa filtrate from 29 different lake samples averaged 46.1 nmol (SD $\pm$ 15.2 nmol) FDA L^{-1} h^{-1}, or 39.7% (SD $\pm$ 13.8%) of the total (i.e., uncorrected) DEA measured in lake water.

In addition to quantifying the nonenzymatic hydrolytic activity in the filtrate, measuring the concentrated esterase activity retained in the supernatant of the CFDs also proved to be an effective means of improving the accuracy of DEA determinations.

Decay rates of DEA—Decay rates of added porcine esterase in near surface Lake Kinneret water were measured in 35 samples taken at various seasons with temperatures ranging from 15°C to 30°C. We observed enzymatic deactivation rates ranging from 0.03 h^{-1} to 0.49 h^{-1}, with an average of 0.16 h^{-1} (SD $\pm$ 0.13 h^{-1}). There was no significant difference between decay rates in lake water between porcine esterases and esterases extracted from algal cultures (data not shown).

Experiments with monoalgal cultures—In a series of small-volume (250 ml) monoalgal cultures in late exponential or early stationary growth phase, the levels of PEA Chl^{-1} ranged widely, from 0.3 nmol to 29.5 nmol FDA (μg Chl) $^{-1}$ h^{-1}, with an average of 3.9 nmol FDA (μg Chl) $^{-1}$ h^{-1} (SD $\pm$ 5.5) nmol FDA (μg Chl) $^{-1}$ h^{-1} (Table 1).

We followed the changes in total PEA, DEA, and PEA Chl^{-1} during the growth over 2–3 weeks of common Lake Kinneret algae (*Tetraedron* sp., *Microcystis* sp., *Anabaena* sp., *Synechococcus* sp., *Pediastrum* sp., *Scenedesmus* sp., and *Peridinium gatunense*) in 1–2 liter monoalgal, batch cultures. In all cultures, there was an increase of biomass (as Chl) but different patterns of growth were observed (Fig. 1). In some cases (*Tetraedron Pediastrum, Microcystis*) , Chl concentrations peaked and then fell; in others (*Anabaena, Scenedesmus, Peridinium*), growth continued throughout the experimental period. Cultures of *Synechococcus* increased and then remained in a stationary phase until the end of the experiment. A summary showing the ranges of Chl, PEA, DEA, and PEA Chl^{-1} in this experimental series is shown in Table 2.

Various patterns of PEA Chl^{-1}, with no clear relationship to growth phase, were observed (Fig. 1). For example, in the *Tetraedron* culture, PEA Chl^{-1} remained very low (<1.0 nmol FDA (μg Chl) $^{-1}$ h^{-1}) for the initial 10 d and then increased to ~17.0 nmol FDA (μg Chl) $^{-1}$ h^{-1} by the end of the experiment, whereas in *Anabaena, Scenedesmus* and *Peridinium* (not shown), the values of PEA Chl^{-1} declined steadily throughout. PEA Chl^{-1} remained relatively constant

Table 1. Particulate esterase activity (PEA) Chl^{-1} in algal monocultures.

	PEA Chl^{-1} (nmol FDA μg Chl^{-1} h^{-1})*
Chlorophyta	
Tetraedron sp.	1.0, 2.0
Chlorella sp.	0.8, 1.8
Scenedesmus sp.	0.7, 4.4
Pediastrum duplex	2.2, 4.3, 5.2, 6.9, 8.2
Chlamydemonas sp.	0.3, 0.4
Cyanobacteria	
Aphanizomenon	0.3, 1.0, 1.4, 2.6, 5.0
Microcystis sp.	1.8, 2.5, 3.3
Diatoms	
Synedra sp.	4.6
Aulocoseira sp.	7.5
Cyclotella sp.	29.5
Dinoflagellates	
Peridinium gatunense	0.8, 7.3
Overall average (±SD)	3.9±5.5

* Each number represents a separate batch culture, sampled in late exponential or early stationary phase.

in *Pediastrum,* with the exception of two time points. During the growth of *Synechococcus,* this parameter fluctuated from 9.1 to 20.1 nmol FDA (μg Chl)$^{-1}$ h^{-1}. *Microcystis* showed an initial rise in PEA Chl^{-1}, followed by a rapid drop to constant low values.

Time-course experiments with Lake Kinneret water—Four time-course experiments with near-surface water from Lake Kinneret were made at different seasons when the initial phytoplankton assemblages also differed. The addition of nutrients clearly stimulated growth only in experiments A and C. Experiments B and D, sampled when there were initially high N and P concentrations in the lake water, showed similar increases of Chl concentration in both supplemented and unsupplemented samples. The ranges of Chl, PEA, DEA, PEA Chl^{-1}, and lysis rates that were measured in this experimental series are given in Table 3.

PEA levels generally followed the pattern of Chl development except in experiment D. In this experiment, run with a sample taken from a dense *Peridinium* bloom, the initial Chl concentration increased from ~275 to 360 μg Chl L^{-1}, while PEA declined from 225 to 150 nmol FDA L^{-1} h^{-1} in both supplemented and unsupplemented carboys.

PEA Chl^{-1} did not show a consistent pattern. In experiment A, this parameter remained constant for 2 weeks, then declined; in experiments B and D, there was a twofold to threefold drop over the entire incubation period. PEA Chl^{-1} was highest in experiment C, range 4.9–9.9 nmol FDA (μg Chl)$^{-1}$ h^{-1}, and lowest in experiment D, 0.4–0.6 nmol FDA (μg Chl)$^{-1}$ h^{-1}. There was a slight tendency for lower PEA Chl^{-1} in nutrient-supplemented samples of experiments A, B, and C (Table 3).

The levels of DEA remained relatively constant over several days in some cases, e.g., experiment A (unsupplemented), experiment B (supplemented), but in others, wide variations of DEA, up to fourfold, were observed (Fig. 2A,B). The highest levels of DEA, 154 nmol FDA L^{-1} h^{-1}, were measured in experiment D, sampled at the height of the *Peridinium* bloom in 2004.

Esterase decay rates were measured only in experiment C (0.10 h^{-1} and 0.18 h^{-1} in unsupplemented and supplemented samples, respectively) and experiment D (0.13 h^{-1} and 0.25 h^{-1} in unsupplemented and supplemented samples, respectively). For LR rate calculations in experiments A and B, we used the averaged decay rate values from experiments C and D. In three of four experiments, estimated LR was markedly higher in the nutrient supplemented than in the unsupplemented carboys, averaging 1.7 d^{-1} and 1.2 d^{-1}, respectively (Table 3).

PEA, PEA Chl^{-1}, DEA, and LR in Lake Kinneret—We followed the variations of PEA, PEA Chl^{-1}, DEA, and LR biweekly over a 2-yr period at a pelagic and a shallow coastal location in Lake Kinneret. During this period, water temperatures in the epilimnion ranged from 14.8°C to 30.3°C; and Chl concentrations at 1 m varied from 4.0 to 410 μg L-1 (Fig. 3). The peaks of Chl in 2003 and 2004 reflected the annual blooms of *Peridinium,* a regular characteristic of this lake.

PEA concentrations reached a maximum at the peak of the *Peridinium* blooms in April–May 2003 and March–April 2004 (Fig. 4). There were significant correlations between PEA and Chl concentrations at depths of 1 m ($r^2 = 0.35$, $p < 0.0001$), 5 m ($r^2 = 0.18$, $p < 0.0026$), and at the near-shore station ($r^2 = 0.15$, $p < 0.0073$), but not at 10-m depth. Only during the period of the dinoflagellate blooms were PEA values distinctly higher in near-surface waters (1 m) than at 10-m depth. At the shallow station, PEA values had a similar temporal pattern to those at 1-m depth at the pelagic site but tended to be somewhat higher (Table 4).

The DEA measured in lake water, corrected for nonenzymatic FDA hydrolysis, averaged 38.8 nmol FDA L^{-1} h^{-1} and ranged from 6.1 to 120 nmol FDA L^{-1} h^{-1}, with high variability and no clear seasonal pattern (Fig. 4). DEA usually decreased with depth (Table 4; Fig. 4). At the shallow station, Amnon, DEA levels were intermediate between those from 1- and 5-m depths at the pelagic site. On five separate dates, DEA taken every hour for 5–6 h directly from the lake varied from 1.3- to 1.7-fold for each sampling period (e.g., Fig. 2C,D). We found similar DEA variability over 2 consecutive d (16–17 June 2004 and 26–27 July 2004).

PEA Chl^{-1} values in lake water (Fig. 4) ranged from a low of 0.4 (Amnon, March 2004) to a maximum of 18.7 nmol FDA (μg Chl)$^{-1}$ h^{-1} (Sta. A, 5-m depth, November 2003). Highest values were observed during the summer months with minima occurring during the *Peridinium* blooms in 2003 and 2004. There was a tendency for PEA Chl^{-1} to be higher at the deepest sampling depth and lowest at the shallow, near-shore station (Table 4).

In Fig. 5, we show the variations in LR over the sampling period from May 2002 through May 2004. The overall average LR in lake water was 0.91 (SD ± 0.58) d^{-1}. LR tended

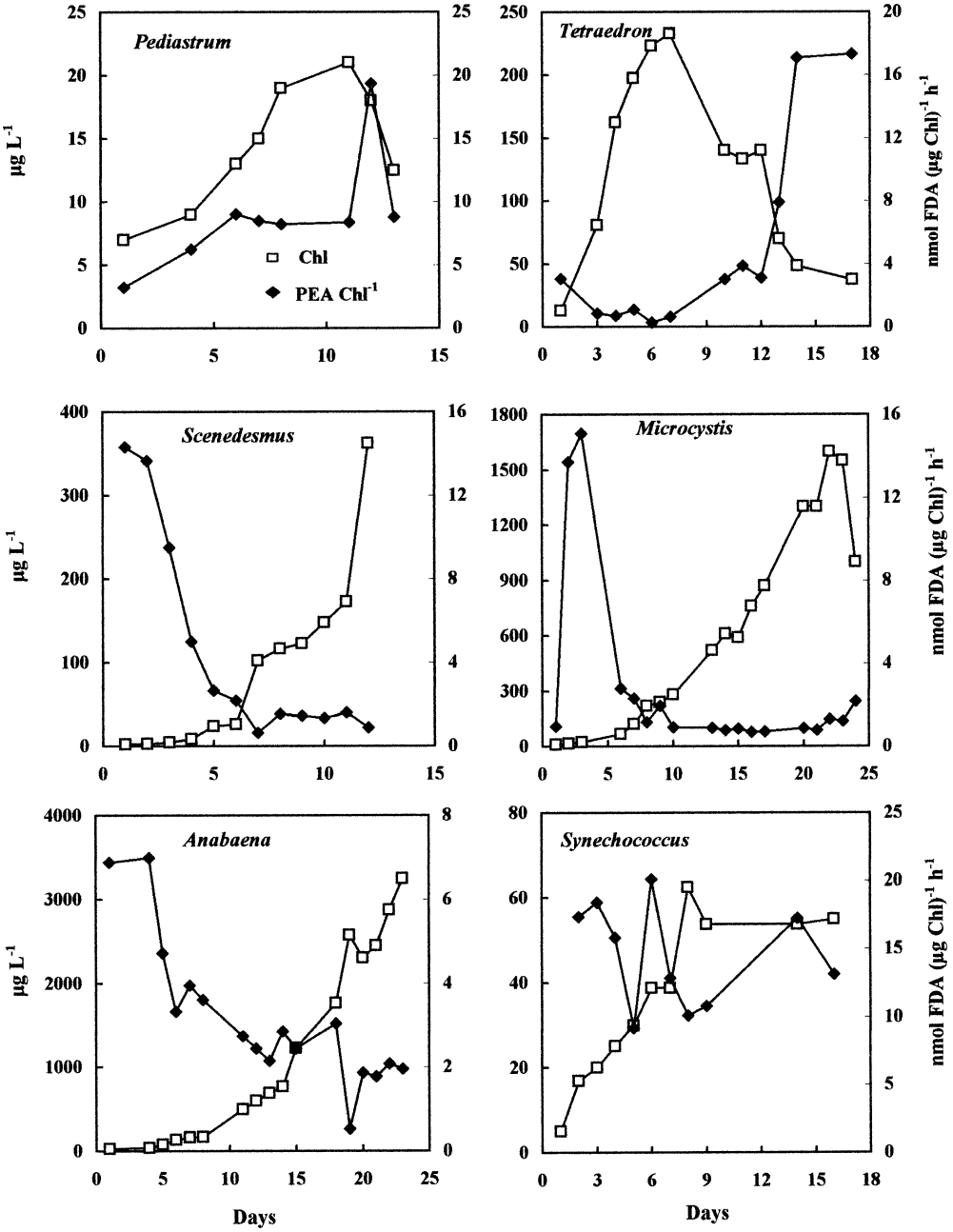

Fig. 1. Chlorophyll (Chl; μg L^{-1}) and particulate esterase activity (PEA) Chl^{-1}, (nmol FDA (μg Chl)$^{-1}$ h^{-1}) in monoalgal cultures of some Lake Kinneret algae.

to be highest in 1-m pelagic samples and decreased with depth (Table 4; Fig. 5).

Potential esterase contribution from zooplankton, protozoa, and bacteria—In Table 5, we compare the amounts of PEA measured in microzooplankton, ciliates, flagellates, and bacteria to total PEA directly measured in the epilimnion of Lake Kinneret. Our results indicate that, although all these organisms showed perceptible levels of intracellular esterase activity, the total amounts of PEA in these populations were usually only a small fraction ($\ll$10%) of PEA contained in the phytoplankton. Therefore, the input to lake DEA from heterotrophic microorganisms should be very low compared with input to DEA from photoautotrophs and should not affect the estimates of LR.

Discussion

As used here, the term *esterase* refers to a host of cytoplasmic enzymes capable of cleaving ester linkages. The esterase method as originally proposed and applied during the decline of a phytoplankton bloom in the North Sea by Van Bockel et al. (1992) only claimed to be a semiquantitative measure of lysis because of uncertainty about the relative

Table 2. Chlorophyll (Chl), particulate esterase activity (PEA), dissolved esterase activity (DEA), and PEA Chl^{-1} ranges in time-course experiments with monoalgal cultures.*

	Chl	PEA	DEA	PEA Chl^{-1}
Tetraedron sp.	13.0–140	40–827	22–511	0.3–17.3
Microcystis sp. (brown)	5.9–1650	13–2987	27–1987	0.8–10.7
Microcystis sp. (green)	9.6–1600	9–2182	23–523	0.7–15.1
Anabaena sp.	27.0–3250	186–2183	31–424	0.5–7.0
Synecococcus sp.	5.0–55	284–927	21–261	9.1–56.8
Pediastrum duplex	7.0–21	23–34	30–71	3.2–19.4
Scenedesmus sp.	2.3–362	32–320	13–140	0.7–14.3
Peridinium gatunense	5.0–800	2–26	98–379	<0.1–5.2

* Chl (μg L^{-1}); PEA, DEA (nmol FDA L^{-1} h^{-1}); PEA Chl^{-1} (nmol FDA μg Chl^{-1} h^{-1}).

contributions to ambient levels of DEA by planktonic photoautotrophs and heterotrophs. Agusti et al. (1998) found that >90% of the DEA in the northwestern Mediterranean derived from autotrophs. This, together with modifications of the method including the added assumption of constant ambient concentrations of DEA over a 24-h time scale, enabled Agusti et al. (1998) to determine phytoplankton LR in the northwestern Mediterranean, in Blanes Bay (Agusti and Duarte 2000), and in the eastern tropical North Atlantic (Agusti et al. 2001). Some reservations about these results were discussed by Riegman et al. (2002) and Agusti and Duarte (2002).

In applying the esterase method to the Lake Kinneret, we slightly changed the esterase assay as used by Agusti et al. (1998) to optimize pH and temperature conditions for our freshwater environment. Our assay incubation temperature was 5°C and 10°C higher than that used by Agusti et al. (1998) and Riegman et al. (2002), respectively. Esterase activity assays were run in the laboratory and our standard 1-h incubation temperature was not related to ambient lake temperatures. In contrast, for the determination of esterase decay rates in lake water, the samples were held at ambient lake temperatures in the dark for up to 6 h and sampled hourly for dissolved esterase activity.

We examined some of the key assumptions on which the esterase method is based. Similar to Agusti et al. (1998), we found that the bulk of PEA in the lake epilimnion was associated with phytoplankton, with a negligible contribution, probably much less than 10%, from microzooplankton, protozoa, or bacteria (Table 5). Thus, most of the input to the DEA pool would be expected to derive from phytoplankton rather than from heterotrophic organisms.

Except for samples from 10-m depth, PEA levels were significantly correlated with Chl concentrations, as might be expected if phytoplankton was the principal source of esterase activity released into the water upon cell lysis. The patterns of PEA concentration in lake samples generally reflected those of the phytoplankton standing stocks, with two peaks of PEA corresponding to the *Peridinium* blooms in 2003 and 2004, respectively, and with 10-m samples consistently lower than those from 1- and 5-m depths (Fig. 4).

Both Van Boekel (1992) and Agusti et al. (1998) used a constant value of the parameter PEA Chl^{-1} (averaged from several representative algal cultures) together with measured in situ Chl concentrations to derive the PEA concentrations of the ambient phytoplankton population. We found that PEA Chl^{-1} varied widely, not only between different algal species in culture (Tables 1, 2), but also at different stages in the growth of the same organism (Fig. 1). Furthermore, there appeared to be no general pattern of PEA Chl^{-1} change with growth phase. PEA Chl^{-1} also varied by as much as 40-fold in lake-water samples (Table 4; Fig. 4) with very low values during the *Peridinium* bloom. Because of the high variability of this parameter, we used direct measurements of PEA rather than estimating PEA from Chl measurements for the calculation of LR in our time-course experiments and routine lake-sampling series.

The values of PEA Chl^{-1} that we observed, both in mon-

Table 3. Chlorophyll (Chl), particulate esterase activity (PEA), dissolved esterase activity (DEA), and PEA Chl^{-1} ranges and lysis rate (LR) in time-course experiments in unsupplemented (LKW) or 100 μmol $NO3^-$ and 10 μmol $PO4^{3-}$ supplemented (LKW+) Lake Kinneret water microcosms.*

Experiment	Date	Chl	PEA	DEA	PEA Chl^{-1}	LR	*n*
A	(Dec 2002) LKW	8–37	38–76	18–46	2.0–5.2	0.9	7
A	(Dec 2002) LKW+	11–79	49–122	15–40	1.4–4.9	1.7	7
B	(Jan 2003) LKW	14–70	47–105	19–88	1.1–3.7	1.9	6
B	(Jan 2003) LKW+	14–80	47–109	19–80	0.9–3.3	1.8	6
C	(Oct 2003) LKW	7–10	45–89	15–43	6.1–12.3	0.9	6
C	(Oct 2003) LKW+	11–23	60–116	17–92	4.6–9.2	1.2	6
D	(Mar 2004) LKW	275–360	147–228	52–125	0.4–0.8	1.0	4
D	(Mar 2004) LKW+	275–350	141–228	52–154	0.4–0.8	2.1	4

* Chl (μg L^{-1}); PEA, DEA (nmol FDA L^{-1} h^{-1}); PEA Chl^{-1} (nmol FDA μg Chl^{-1} h^{-1}), and LR (d^{-1}). *n* is the number of samples measured in each experiment.

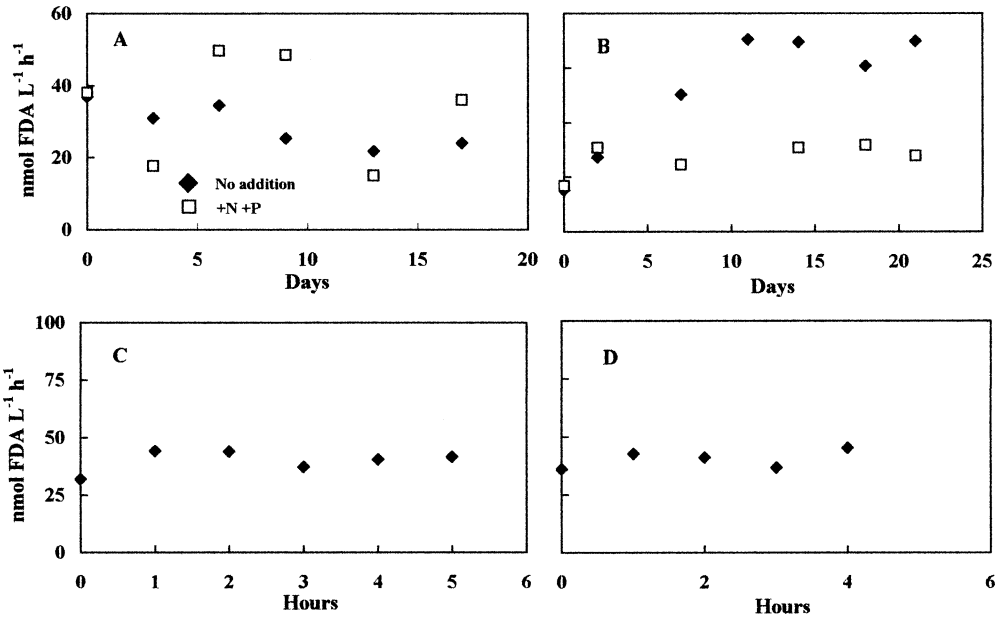

Fig. 2. Dissolved esterase activity (DEA) (nmol FDA L^{-1} h^{-1}) in lake-water microcosms with and without added 100 μmol L^{-1} NO_3^- and 10 μmol L^{-1} PO_4^{3-}. (A) Experiment A and (B) Experiment B in lake water (1-m depth) sampled at 1-h intervals, (C) on 16 June 2004 and (D) 17 June 2004.

oalgal cultures and in lake water, were generally lower than those reported by Agusti and Duarte (2000, 2002) and Agusti et al. (2001), 45.7–250 nmol FDA (μg Chl)$^{-1}$ h^{-1}, but were in a similar range to that given by Riegman et al. (2002), <0.1–71.9 nmol FDA (μg Chl)$^{-1}$ h^{-1}. The latter also showed that this parameter was affected by ambient light conditions.

Although large cells of *Peridinium* (~50–70 μm) had the lowest, 0.1–5.2 nmol FDA (μgChl)$^{-1}$ h^{-1}, and the small cells of *Synechococcus* the highest values, 9.1–56.8 nmol FDA (μgChl)$^{-1}h^{-1}$, of PEA Chl^{-1}, there was no clear relationship between cell size and PEA Chl^{-1}. (See, for example, the extremely low values on some of the time points of the *Scenedesmus* and *Tetraedron* cultures in Fig. 1). The high variability of PEA Chl^{-1} is perhaps not surprising because intracellular esterases (a very mixed bag of enzymes) would not be expected to remain constant at all growth phases or under varying environmental conditions.

As noted by both Riegman et al. (2002) and Agusti and Duarte (2002), the efficiency of esterase extraction from algal cells is a critical factor in this method directly affecting the levels of PEA Chl^{-1}. Initially, we found that solubilization of esterases from algae and lake phytoplankton with a power-driven glass–glass homogenizer gave higher extraction efficiencies than sonication (Riegman 2002), disruption in a bead homogenizer (Agusti and Duarte 2002), or pretreatment with lysozyme (Agusti et al. 1998). Previously, the glass–glass homogenizer had been shown to be effective for extracting intracellular enzymes from especially recalcitrant

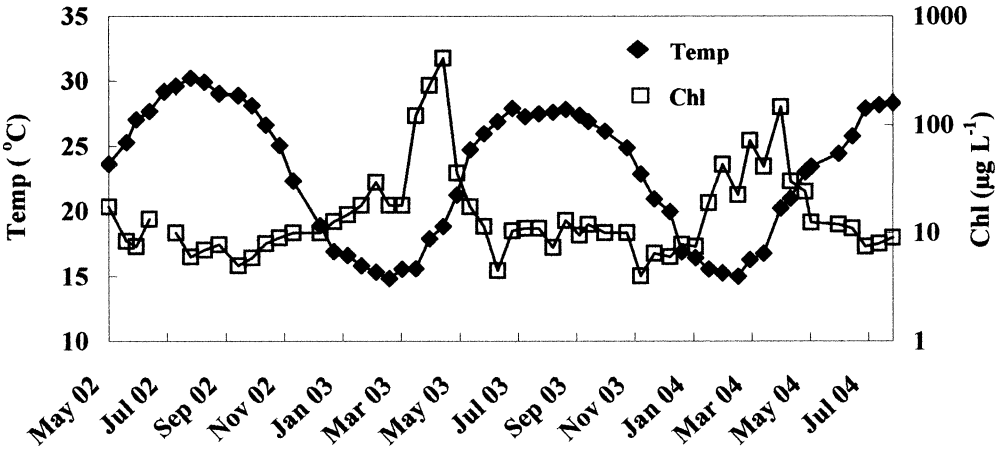

Fig. 3. Temperature (°C) and chlorophyll (μg L^{-1}) at 1-m depth in Lake Kinneret from May 2002 to August 2004.

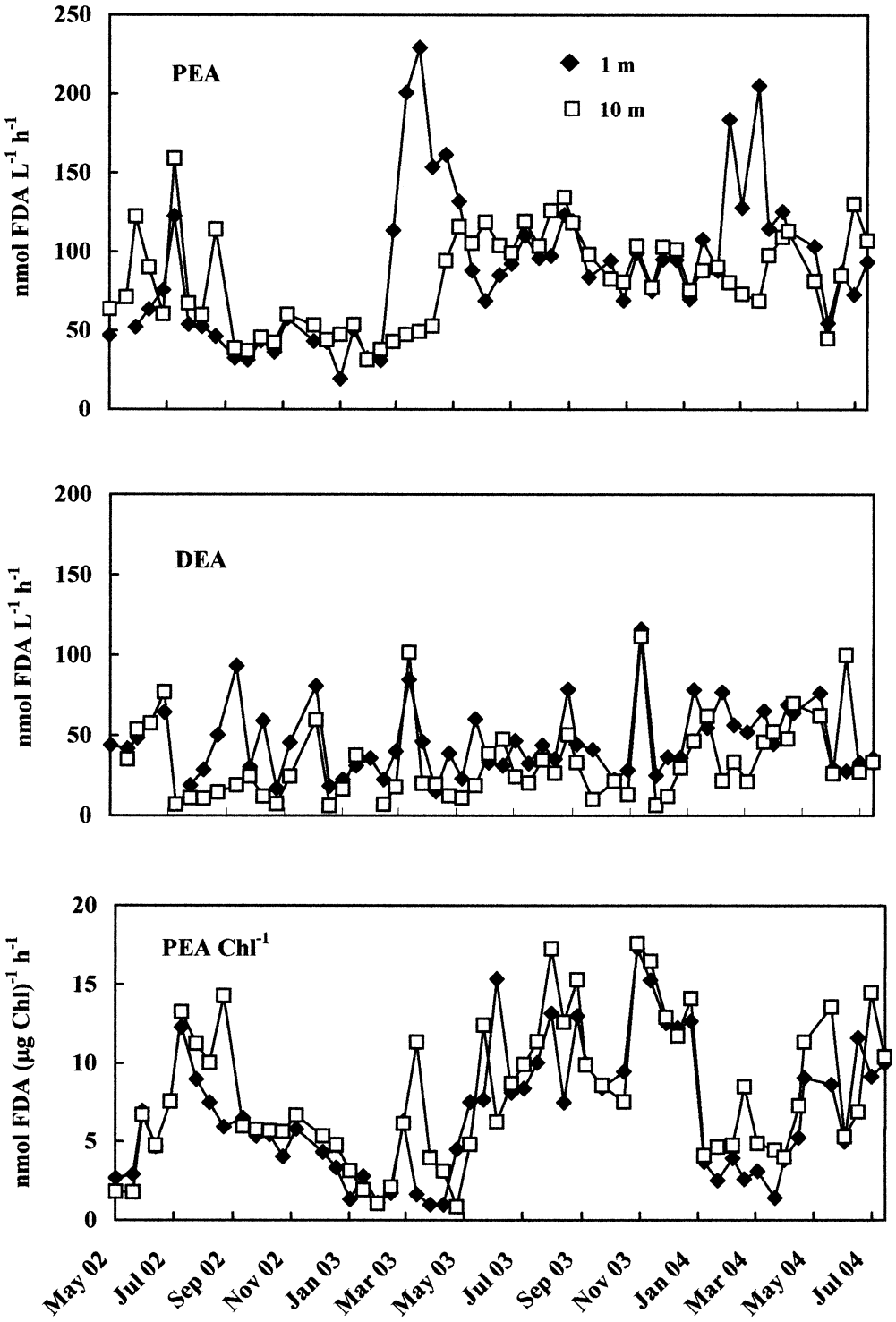

Fig. 4. Particulate esterase activity (PEA) and dissolved esterase activity (DEA) (nmol fluorescein diacetate [FDA] L^{-1} h^{-1}) and PEA Chl^{-1} (nmol FDA (μg Chl)$^{-1}$ h^{-1}) at 1- and 10-m depths in Lake Kinneret, May 2002 to August 2004.

algal cells such as *Peridinium* (Hochman et al. 1986). Nevertheless, later, more-detailed experiments with lake phytoplankton samples indicated that sonication most often (7 out of 10 trials) gave higher PEA extraction efficiencies, on average about 10% greater than the glass–glass homogenizer. In two trials, glass–glass homogenization was slightly more efficient. The high variability that we found in these experiments may have been due to different phytoplankton populations occurring at different dates. However, if sonication usually gives higher PEA extraction efficiencies, our values

Table 4. Chlorophyll (Chl), particulate esterase activity (PEA), PEA Chl^{-1}, dissolved esterase activity (DEA), and lysis rate (LR) in Lake Kinneret. Average, standard deviation (SD), minimum and maximum values from May 2002 until August 2004, measured at pelagic Sta. A (three depths) and at a shallow coastal station, Amnon.

Station/depth	Chlorophyll (μg Chl L^{-1})	PEA (nmol FDA L^{-1} h^{-1})	PEA:Chl (nmol FDA μg Chl^{-1} h^{-1})	DEA (nmol FDA L^{-1} h^{-1})	LR (d^{-1})
A/1 m	30.0±66.3 (4.0–410.0)	90±47 (20–230)	6.8±4.2 (1.0–17.3)	46±22 (15–116)	1.14±0.63 (0.20–3.14)
A/5 m	18.3±14.7 (4.0–73.0)	91±33 (36–156)	7.2±4.1 (1.3–18.7)	37±21 (8–120)	0.84±0.51 (0.05–2.21)
A/10 m	14.7±15.0 (4.2–112.0)	82±31 (31–159)	8.0±4.4 (0.8–17.6)	33±26 (6–112)	0.82±0.55 (0.10–2.27)
Amnon	55.6±111.3 (4.0–640.0)	109±56 (36–244)	5.7±3.7 (0.4–15.3)	38±25 (4–111)	0.83±0.65 (0.03–2.89)
Average all samples	29.3 (SD±65.9)	93 (SD±43)	6.9 (SD±4.2)	39 (SD±23)	0.91 (SD±0.59)

for PEA and PEA Chl^{-1} are likely to be underestimated by ~10%.

As suggested by Agusti et al. (1998) and Agusti and Duarte (2002), PEA may also be underestimated if extraction efficiencies decrease with increasing levels of algal biomass. We checked this by measuring PEA extracted from phytoplankton in 25, 50, 100, 150, 200, and 250 ml of lake water collected on GF/C filters. In five experiments, the extraction efficiency of PEA (measured as PEA Chl^{-1}) did not change significantly with rising phytoplankton biomass. These results imply that PEA extraction efficiency was not impaired by our routine procedure using 250-ml lake samples.

The levels of DEA in lake water measured at any given time reflect a balance between incoming DEA, derived predominantly from phytoplankton lysis, and DEA lost due to exponential decay of esterase activity in the water. There is no intrinsic reason for the rates of DEA input or decay to be equal or to vary in such a manner as to maintain DEA at steady-state levels. We observed considerable daily fluctuations of DEA concentrations in some, but not all, of the time-course experiments with lake-water microcosms (e.g., Fig. 2A,B). On a time scale of 5–6 h, DEA sampled directly from the lake varied from 1.3- to 1.7-fold (e.g., Fig. 2C,D) with a similar DEA variability over 2 consecutive d. These results are from a limited number of experiments but emphasize a limitation of the esterase method, i.e., levels of DEA may not remain at steady state for periods as long as 24 h.

Riegman et al. (2002) emphasized the need to account for nonenzymatic hydrolysis of the assay substrate, FDA, when measuring low concentrations of DEA. We found that this was very important in DEA determinations in Lake Kinneret. Following Riegman et al. (2002), we measured nonenzymatic FDA hydrolysis in the <10-kDa filtrate fraction of CFD tubes. Additionally, we used CFD supernatant that contained a 50-fold concentration of esterase proteins to obtain a more precise measure of actual DEA. We recommend this procedure for all measurements of DEA in natural waters.

The decay rate of dissolved esterases in the natural environment is a critical parameter for determining LR with the esterase method. Riegman et al. (2002) observed that, in 0.2-μm-filtered North Sea water, decay rates of esterases extracted from natural algae had a half-life of 49 h compared with 31 h for commercially available porcine esterase. In contrast, Agusti and Duarte (2002) reported similar decay rates for porcine and esterases from algal cultures in unfiltered northwestern Mediterranean waters. Likewise, we found no significant differences in decay rates between porcine esterases and DEA from cultures in unfiltered lake water. Decay rates in unfiltered Lake Kinneret water (average half-life ~0.3 d) were more rapid than those reported by Agusti et al. (1998) (half-life 1.1 d), and Riegman et al. (2002) (half-life 1.3–2.0 d). Thus, the DEA decay rates in this lake (epilimnion temperature range ~15–30°C) were generally much higher than previously reported for colder and less eutrophic marine waters. There was also a tendency toward more rapid esterase decay in nutrient supplemented samples than unsupplemented samples in experiments with lake-water microcosms (Table 3). Intuitively, faster esterase decay rates would be expected in warmer, more eutrophic waters with greater

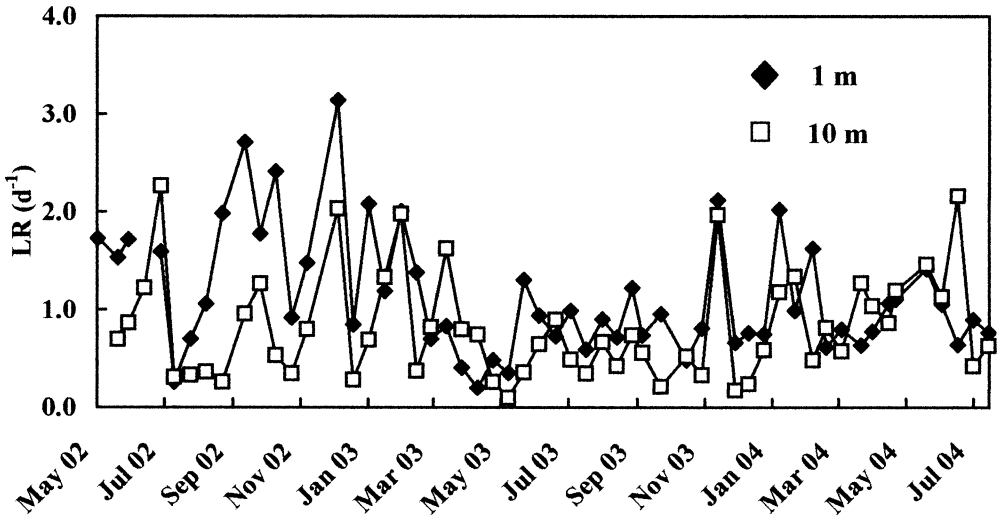

Fig. 5. Phytoplankton lysis rates (LR; d^{-1}) at 1-, 5-, and 10-m depths in Lake Kinneret, May 2002 to August 2004.

abundances and higher activity of bacteria and other microorganisms that degrade dissolved enzymes and other compounds in the DOM pool.

The LRs measured in Lake Kinneret from May 2002 until 2004 (Table 4; Fig. 5) are high compared with those reported by Agusti and Duarte (2000) for Blanes Bay (average 0.41 d^{-1} and 0.06 d^{-1} for summer and winter, respectively), 0.86 d^{-1} in the northwestern Mediterranean (Agusti et al. 1998) and 0.3 d^{-1} in the North Sea (Brussard et al. 1995). Lake Kinneret is a more eutrophic environment than any of the above, and thus the pattern of decreasing phytoplankton LR with increasing eutrophy suggested by Agusti and Duarte (2000) does not hold in this case. Note that higher LRs were also measured in the nutrient-supplemented samples than in untreated samples in three of four time-course experiments with lake water (Table 3). The LRs observed in the untreated samples were close to those estimated in the routine lakewater sampling series (Table 4).

Similar to Agusti et al. (1998), we observed that near-surface lake samples (0–1 m) usually had higher LR than those from deeper waters (Table 4; Fig. 5). Only during the dinoflagellate blooms was LR at 10-m depth higher than at 1-m depth, perhaps indicative of more rapid lysis in organisms sinking through the water column.

Measured LRs were more rapid from about September 2002 to February 2003 and again from November 2003 to February 2004 (with the exception of December 2003), roughly corresponding to relatively lower Chl concentrations during these periods. Conversely, lower LRs were observed when Chl biomass peaked, *Peridinium* blooms in March–June 2003 and 2004, respectively. Although an inverse relationship might be expected between phytoplankton bio-

Table 5. Particulate esterase activity (PEA) of microzooplankton, protozoa (ciliates, flagellates), and bacteria total measured PEA and percentage of total PEA attributed to heterotrophic plankton in Lake Kinneret.

		PEA* (mol FDA L^{-1} h^{-1})	Total PEA (nmol FDA L^{-1} h^{-1})	Heterotrophic PEA* (%)
Lake zooplankton				
Jul 02		0.2; 0.3	91.0	0.3
Oct 02		2.6; 2.1	36.3	7.2; 5.8
Jul 03		1.3; 0.8	94.2	1.3; 0.9
Protozoa				
Jun 02	Flagellates	4.9; 2.5	86	5.7; 2.9
Jun 02	Flagellates	3.5; 2.7	86	4.0; 3.1
Jul 02	Ciliates	0.6	106	0.6
Dec 02	Flagellates	0.6; 0.9	54	1.0; 0.7
Jun 03	Ciliates	1.4	140	1.0
Sep 03	Ciliates	0.34	67	0.5
Bacteria				
Apr 03	Isolate T_{10}	0.7	120	0.6
Jun 03	Isolate T_{10}	1.6	109	1.5
Sep 03	Isolate T_{10}	0.3	125	0.3

* Each number represents a separate experiment.

mass and LR, there was no significant correlation between Chl concentrations and LR for any of the depths sampled. There was also no significant correlation between LR and lake-water temperature. At present, we have no explanation for the relatively high LR from about September 2002 to February 2003 compared with that of the corresponding period in 2003–2004 (Fig. 5), other than invoking the possibility of different phytoplankton population composition.

The accuracy of the esterase method as a quantitative measure of phytoplankton lysis has been questioned (Riegman et al. 2002). We have attempted to overcome some of the problematic aspects of this assay (e.g., by correcting for nonenzymatic FDA hydrolysis and by direct measurement of ambient PEA), but we note that inaccuracies occur if levels of PEA, DEA, or DEA decay rates are not constant over 24 h. Thus, although the LR, calculated on an hourly basis, may correspond to the actual rate of lysis at the time of sampling, it is uncertain if these rates should be extrapolated to a daily period merely by multiplying by 24. Moreover, incomplete extraction or inadvertent inactivation of PEA from phytoplankton samples collected on GF/C filters could lead to overestimates of LRs. For example, if routine PEA extractions were only ~90% effective, then LRs would be correspondingly overestimated. Further difficulties occur because the esterase method actually follows the flux of esterases from PEA to DEA and does not directly measure phytoplankton cell lysis. Because of these uncertainties, we prefer to regard the LR reported here as apparent LRs. Presumably, more accurate estimates of phytoplankton daily LRs could be obtained if ambient levels of DEA, PEA, and DEA decay rates are measured over short time intervals over a 24-h period, but we realize that this is often logistically infeasible.

The high levels of apparent LRs reported here have profound implications for the patterns of carbon and nutrient fluxes from phytoplankton in Lake Kinneret. If phytoplankton lysis is responsible for considerable losses of particulate organic carbon from phytoplankton, then previous estimates of gross primary production in Lake Kinneret (Berman et al. 2004) may have been underestimated, as has been suggested by Luz et al. (2002). Carbon-based estimates of phytoplankton biomass turnover range from about 1.5 d for most of the year to 7–15 d during *Peridinium* blooms (Stone et al. 1993). An overall average LR of ~0.9 d^{-1} (Table 4) implies that about 60% of PEA is transferred to DEA daily. Only if the ratio between PEA and algal biomass (as Chl, carbon, or other biomass proxy) remains constant does this LR mean that 60% of the actual phytoplankton biomass lyses daily. Such high average daily rates of phytoplankton lysis seem intuitively unrealistic and may be overestimated due to methodological uncertainties detailed above. Nevertheless, irrespective of the precise LR, our observations that perceptible levels of DEA, derived predominantly from algae (Table 5), were always present in lake water (Table 4; Fig. 4), concomitant with rapid rates of DEA decay, imply that phytoplankton lysis was ongoing and responsible for a considerable flux of material from algal cells to dissolved and particulate detrital pools.

In general, rapid phytoplankton LRs might be expected in this lake. Berman et al. (2004) reported that bacterial biomass production in the epilimnion was at ''the high end of rates reported for many aquatic environments,'' with an average of an ~1-d turnover of bacterial biomass. This would be consistent with a high, constant input of readily available DOM such as might be released from lysing algal cells.

In conclusion, we submit that phytoplankton lysis may indeed be responsible for important carbon and nutrient fluxes in Lake Kinneret and other freshwater and marine ecosystems. Moreover, we suggest that at least some part of the organic carbon fluxes from phytoplankton usually attributed to extracellular release from living algae (Berman-Frank and Dubinsky 1999) may, in fact, be due to phytoplankton cell lysis. Increasing evidence of virus-mediated lysis (Brussaard 2004) and functional apoptotic mechanisms in algae and cyanobacteria (Berges and Falkowski 1998; Berman-Frank et al. 2004; Bidle and Falkowski 2004) emphasizes the need for accurate quantitative measures of lytic cell death rates in the natural aquatic environment (Kirchman 1999). The esterase method in its present form has the advantage of being simple and noninvasive but should be modified by more frequent determination of ambient PEA, DEA, and DEA decay rate to improve its reliability as a quantitative measure of daily LRs. Also, more importantly, other means of assessing phytoplankton lysis rates should be developed. Alternatives, such as determining the release of particulate polysaccharides by lipid biomarkers and molecular probes (Baldi et al. 1997) or cell-cycle analysis (Garces and Naso 2001), appear to have limited applicability, but some modification of methods to identify the proportion of moribund or dead cells in a phytoplankton population (e.g., membrane permeability probes, Agusti and Carmen-Sanchez 2002; measurement of caspase activation, Berman-Frank et al. 2004) may offer more feasible approaches. Clearly, the potential importance of phytoplankton cell lysis in cycling organic material in aquatic environments merits further research effort.

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