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Seasonal changes in particulate and dissolved lipids in a eutrophic prairie lake

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SUMMARY

1. Critical periods of lipid energy transfer from phyto- to zooplankton were inferred by comparing seasonal patterns of particulate and dissolved lipid fractions in lake water with temporal changes in lipid energy reserves of the zooplankton in a hypereutrophic lake.
2. The midsummer phytoplankton community was dominated by the bloom-forming cyanobacterium *Aphanizomenon flos-aquae*. The collapse of the bloom was accompanied by a 2-week period of severe nitrogen deficiency after which there was a marked increase in the concentration of lipid energy reserves in the particulate (algal) fraction.
3. Areal lipid energy reserves of the dominant herbivorous zooplankton responded positively to changes in the tri-and diacylglycerol content of the particulate fraction of lake water in a species-specific manner.
4. Bacterial numbers also peaked in September concomitant with a large increase in free fatty acids in the dissolved lipid fraction probably produced by the decay of the *A. flos-aquae* bloom.
5. The association between periods of nitrogen deficiency and increased energy reserve lipids in the particulate fraction supports observations made with laboratory algal cultures that periods of nutrient deficiency may intensify lipid synthesis in some algal species, thereby enhancing the rate of lipid energy transfer from phytoplankton to zooplankton.

Introduction

Concentrations of specific lipid classes and/or fatty acids in the particulate and dissolved phases of marine waters have, in the past, received considerable attention (see Parrish, 1988 for a review). However, studies which investigate temporal patterns in the concentrations of the various lipid classes (wax esters, triacylglycerols, phospholipids, etc.) in freshwater systems are rare (however, see Kreeger *et al.*, 1997). We were motivated to measure such temporal trajectories in lipid class concentrations because a knowledge of the timing, duration and amplitude of the peaks in the various lipid classes can provide information germane to several fields of investigation.

First, lipids in the dissolved fraction are part of the dissolved organic carbon (DOC), forming an important component of the labile fraction of the DOC available to bacteria. Thus, seasonal periodicity in the synthesis and retention of these biomolecules in the particulate fraction and their subsequent release in the dissolved fraction provides an important measure of the quality and quantity of substrates available for bacteria.

Second, through the process of sedimentation, lipids in the particulate fraction contribute to the accretion of lipids in the sediments of wetlands. Therefore the seasonal periodicity and concentrations of the various lipid classes in the particulate phase will be related to the stratigraphy of lipid biomarkers in the sediments (Meyers & Ishiwatari, 1993). This link between the

production of lipids in the pelagic region and their occurrence and abundance in sediments may provide information crucial to the successful interpretation of palaeolimnological events.

Finally, much of the lipid found in the tissues of freshwater animals is derived from algae which biosynthesize fatty acids. In particular, the synthesis of storage lipids by algae is crucial to the survival of many aquatic invertebrates because these lipids buffer against periods of low food abundance or quality (Goulden, Henry & Tessier, 1982), and furnish an energy reserve for developing neonates (Tessier *et al*., 1983). Since energy-reserve lipids are calorifically dense and often comprise a large fraction of the digestible dry weight of aquatic invertebrates, fundamental life processes such as respiration, reproduction and mobility will usually be linked to the timing and magnitude of lipid transfer and storage between and within trophic levels. The transfer of readily usable energy in pelagic ecosystems is therefore often associated with the transfer of lipid material. Some of these algal lipids (eicosapentaenoic acid 20 : 5ω3 and docosahexaenoic acid 22 : 6ω3) are essential for the growth and development of aquatic invertebrates and vertebrates. We would expect organisms to have evolved strategies to capitalize on periods when lipid production and/or lipid quality is greatest because of their importance in aquatic ecosystems

This paper presents data on phytoplankton numbers and species composition, seasonal patterns in areal lipid reserves of zooplankton, and measurements of lipid class composition in particulate (1.2–153 μm) and dissolved (< 1.2 μm) fractions of lake water from Humboldt Lake, a eutrophic prairie lake. Humboldt Lake was chosen because seasonal data were available on plankton dynamics, including: estimates of primary productivity, standing stocks of algae and zooplankton, and measures of bacterial abundances and productivity, as well as nutrient data. These data were combined with measures of the seasonal trajectories in lipid concentrations in the particulate and dissolved phases in an effort to obtain a better understanding of their relationship to the seasonal dynamics of these key biomolecules.

Materials and methods

Study site

Humboldt Lake (52°09′N, 105°06′W) is situated on a glacio-fluvial outwash plain underlain by Upper Cretaceous shales (Hammer & Haynes, 1978). The lake (locally known as Stoney Lake) has been the focus of numerous and varied limnological and ecological investigations over the past five decades (Rawson & Moore, 1944; Haynes & Hammer, 1978; Hammer, Shamess & Haynes, 1983; Timms, Hammer & Sheard, 1986; Arts, Evans & Robarts, 1992; Robarts, Evans & Arts, 1992; Evans, Robarts & Arts, 1995). The surface area is 17.2 km^2, and the maximum depth is ⊤ 6 m.

The lake is weakly saline (TDS = 2.8 g l^{-1}), hypereutrophic (maximum total phosphorus = 443 μg l^{-1} and maximum chlorophyll = 839 μg l^{-1}) and slightly alkaline (pH = 8.7). The dominant cation and anion (expressed as mEq percentage of the sums of either cations or anions) are magnesium (60.7%) and sulphate (72.3%), respectively, with smaller amounts of sodium (21.9%), calcium (13.4%), and chlorine (9.5%). Ice formation typically begins in early November and the ice attains a thickness of 1.0–1.5 m by January. The ice-free period usually commences in early May.

Bacteria, phytoplankton and nutrient parameters

Water samples (10 ml) were collected at 0.5 m, preserved with Lugol's solution, stained with DAPI (4,6-diamidino-2-phenylindole) and counted with an epifluorescence microscope as in Tumber *et al*. (1993). Aliquots (100 ml) of lake water were collected at 1-m intervals and combined to produce a composite chlorophyll or phytoplankton sample as in Arts *et al*. (1992). Chlorophyll *a* was extracted in ethanol and measured on a spectrophotometer (Pye Unicam, 8–400) with a correction for phaeopigments (Nusch, 1980). Primary productivity was measured at 0, 0.5 and 1 m, and thereafter at 1-m intervals using the ^{14}C light and dark bottle technique as in Robarts *et al*. (1992). These measurements were then integrated for the entire water column to give areal primary production (mgC m^{-2} h^{-1}).

Total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) were measured following the methods outlined in Environment Canada (1992) from near-surface (0–0.5 m) and near-bottom (5–6 m) water. Stannous chloride was used as the reductant for the phosphorus analyses. Nitrite/nitrate and SRP were also measured at 4 m. Ammonia was determined as in U.S.G.S. (1985), and nitrite (NO_2^-) and nitrate (NO_3^-) as in APHA (1989). Nutrients are reported as the mean for all depths.

Particulate organic carbon (PC) was measured with a CHN analyser (Control Equipment Co., 240XA) (Environment Canada, 1992)

Particulate phosphorus (PP) was estimated as the difference between TP and TDP. The following abbreviations are used: PN, particulate nitrogen; PP, particulate phosphorus; PC, particulate carbon. Empirical ratios of PN : PP ($\mu g\ \mu g^{-1}$), PP : PC ($\mu g\ mg^{-1}$), and PN : PC ($\mu g\ mg^{-1}$) were used to suggest potential periods of phosphorus and nitrogen deficiency, respectively, as in Healey & Hendzel (1980).

Lipid extraction and analyses

The basic methodology follows that of Parrish (1986, 1987), with some slight modifications. All glassware used in the extraction procedures was cleaned with LiQui-Nox detergent (Alconox Inc, New York, USA), rinsed five times with Milli-Q distilled water, acid washed overnight in 1 N HCl, and then subjected to two rinses with acetone followed by two final rinses with DCM (dichloromethane), both BDH 'Omni-Solv' grade. Duplicate water samples for lipid analyses were collected at 0.5 and 5 m depths (four samples) using an 8-l Niskin sampler at ⊤ 2-week intervals in 1989 from mid-May to mid-October. The water was filtered through a 153 μm Nitex mesh to remove net plankton and then placed in 4-l amber-coloured glass bottles in a darkened cooler partially filled with ice.

On each sampling trip duplicate bottles were also filled with 'Milli-Q' water (Millipore Corp., Nepean, Ontario, Canada) and treated identically to the lake water samples. A surrogate spike of ketone (20.0 μg hexadecanone) was added initially to both blanks and samples so that extraction efficiency could be estimated. However, since ketones were also detected in lake samples that were not spiked with hexadecanone, extraction efficiency (84%) could only be estimated from the blanks. Upon returning to the laboratory 1.0 l of the pre-screened lake water was immediately filtered through a pre-combusted (400 °C) Whatman GFC filter. The material retained on the GF/C filter was designated as the particulate fraction and any material contained in the filtered liquid phase was designated the dissolved fraction. Pore size may change slightly on combustion, hence we consider the 1.2 μm pore size of the GF/C to be a nominal pore size.

The GF/C filter containing the particulate fraction was folded using a pair of Teflon-coated tweezers and placed in a 15 ml stoppered centrifuge tube containing 4 ml of chloroform : methanol (2 : 1). The filter was then finely crushed using a Polytron grinder (Model 10–35). The grinding tip was washed with two 1-ml rinses of chloroform : methanol (2 : 1) and these washes added to the centrifuge tube. After centrifuging, the glass fibres were removed by blowing the slurry through a clean pre-combusted GF/C filter using pressurized air. The pellet was washed and resuspended in 3 ml of chloroform : methanol (2 : 1). The wash was then blown through the same GFC filter. The filtrate from the extraction and wash were collected in a 25 ml roto-evaporator flask.

The 1 l of GFC-filtered lake water (dissolved fraction) was transferred to a 1-l separatory flask containing 20 ml of DCM. The flask was shaken vigorously for 1 min and the DCM allowed to settle to the bottom of the flask. The DCM–lipid solution was then released through the stopcock into a 250-ml separatory flask. The water was extracted (1 min) twice more with 10 ml DCM and the contents added to the 250-ml separatory flask. The pH was adjusted to 2.0 by adding ⊤ 0.3 ml of concentrated sulphuric acid (98%). Any remaining water was removed when the DCM–lipid solution had settled and could be released through the stopcock into a 50-ml roto-evaporator flask.

The lipid extracts (both particulate and dissolved) were evaporated to dryness under vacuum at 55 °C using a Büchi (Rotavapor-R) roto-evaporator. The lipid material in the flask was washed out into a 5-ml Vacutainer vial with two 2 ml washes of DCM. The headspace in each Vacutainer vial was purged with nitrogen gas to prevent oxidation of the lipid and the vials were then stored at –75 °C in the dark. A small aliquot of this lipid extract (1–5 μl) was later used for determination of lipid class composition by thin-layer chromatography coupled with flame ionization detection (TLC–FID) after the method of Parrish (1987) on an Iatroscan MK-IV detector (Iatron Labs., Tokyo) equipped with SIII-chromarods (Ancal Inc, Tokyo, Japan). The various lipid classes were quantified by integrating peak areas with a Shimadzu chromatopac (Model C-R6A). Peak areas were converted to lipid concentrations using non-linear regressions derived for each of the lipid classes. Identification of peaks in each sample was based on relative peak retentions obtained from one of the chromarods reserved for a mixed lipid standard. The mixed lipid standard consisted of equal concentrations ($1.0\ \mu g\ \mu l^{-1}$) of the

following lipid classes: hydrocarbon (HC), wax ester (WE), sterol ester (SE), triacylglycerol (TAG), free fatty acid (FFA), fatty alcohol (ALC), sterol (ST), acetone-mobile polar lipid (AMPL) and phospholipid (PL). The trivial names of the standards purchased from Sigma Chemical Co. are: HC, squalane, WE, stearyl palmitate, SE, 3-hexadecanone, TG, tripalmitin, FFA, palmitic acid, ALC, cetyl alcohol, ST, cholesterol, AMPL, α-monopalmitin, PL, lecithin. For each sampling date we report a mean lipid concentration from the two replicates at each depth ($n = 4$) for both particulate and dissolved lipid-class fractions. Standard deviations were calculated for each lipid-class mean from each sampling date. These standard deviations ranged from 1 to 20% of the means, in a non-systematic way, amongst the different lipid classes and sampling dates. The estimate of 20% (SD) provides, therefore, a conservative measure for inter-comparisons amongst the means (see Figs 4 and 5).

Zooplankton

Zooplankton for lipid analyses were collected using a Wisconsin-style zooplankton net (76-μm mesh) towed from near the bottom to the surface at the deepest point in the lake and processed as in Arts *et al*. (1992). The dominant herbivorous zooplankton in Humboldt Lake are: *Daphnia pulex*, *Leptodiaptomus sicilis*, *Diaphanosoma leuchtenbergianum* and *Chydorus sphaericus*. Because most of the seasonal changes in zooplankton lipid are due to changes in energy reserve lipids (triacylglycerols) (Vanderploeg *et al*., 1992; Arts *et al*., 1992, 1993), only the seasonal pattern of energy reserve lipids is presented here. Furthermore, these patterns are expressed in areal energy units (kJ m^{-2}) so that the relative strengths of energy transfers from phytoplankton to each zooplankton species can be assessed separately. We assumed an energy content of 39.3 kJ g^{-1} for triacylglycerol energy reserves (Pond, 1981).

Results

Nutrients

Absolute concentrations of total, dissolved and soluble-reactive phosphorus increased from ice-off until August and then decreased in the autumn (Fig. 1a). Phosphorus peaked during the height of the cyanobacterial bloom in early August. Particulate nitrogen

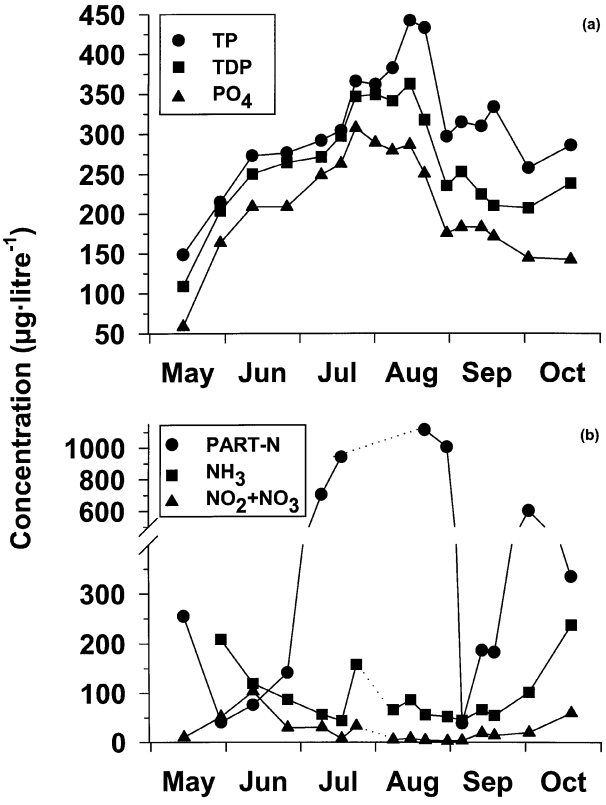

Fig. 1 Absolute concentrations of phosphorus (a) and nitrogen (b) in Humboldt Lake during the ice-free season of 1989.

concentrations exceeded 200 μg l^{-1} throughout the ice-free season, with the exception of late May and early September when concentrations dropped below 50 μg l^{-1} (Fig. 1b). Ammonia and nitrite + nitrate concentrations were greatest in early spring and late autumn and lowest during the late summer cyanobacterial bloom (Fig. 1b).

There were indications of moderate phosphorus deficiency (Fig. 2a) in Humboldt Lake, notably on 18 July when the ratio of PP : PC dropped to 1.5 and the ratio of PN : PP rose to 135. There was also a slight indication of phosphorus deficiency at the end of August (Fig. 2a). Moderate and severe nitrogen deficiency were indicated in late May and from late August to September, respectively (Fig. 2b).

Chlorophyll and primary productivity

Areal chlorophyll concentrations peaked at 1900 mg m^{-2} on 1 August, during the height of the bloom of *Aphanizomenon flos-aquae* (Fig. 3). Rates of primary production were well correlated (91.1%) with chloro-

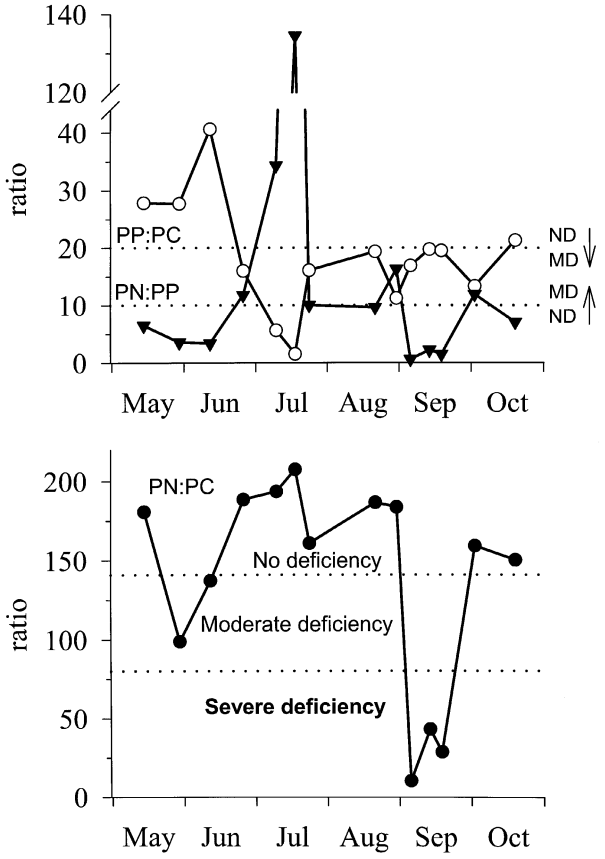

Fig. 2 Seasonal atomic ratios of particulate carbon, phosphorus and nitrogen as PP : PC (○) and PN : PP (▼) for phosphorus (upper panel), and PN : PC (●) nitrogen (lower panel) limitation, respectively. Dotted lines represent divisions between various levels of deficiency and were estimated from Healey & Hendzel (1980, fig. 1). ND, no deficiency and MD, moderate deficiency.

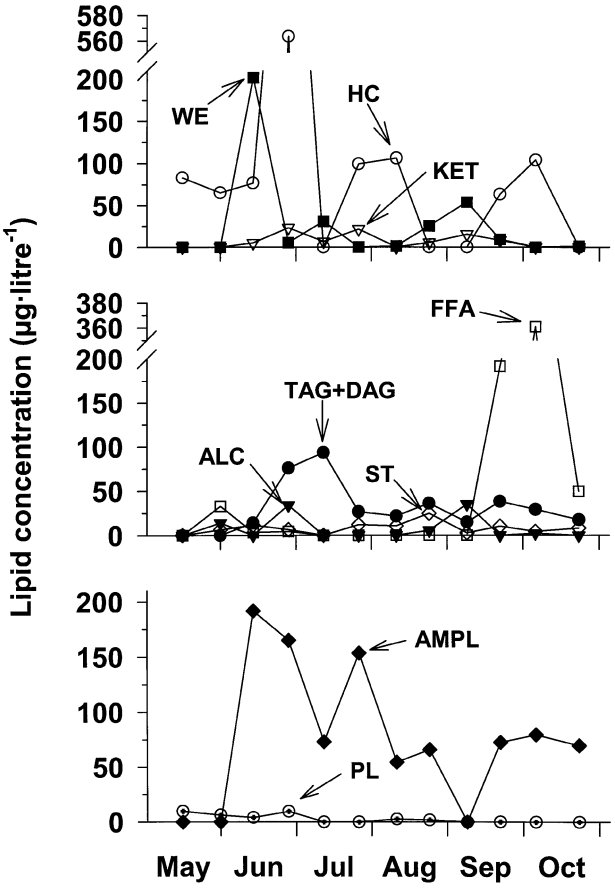

Fig. 4 Concentrations of dissolved lipids (< 1.2 µm) in water from Humboldt Lake during the ice-free season of 1989. Polarity increases from top to bottom. HC = hydrocarbons (○), WE = wax esters (■), KET = ketones (▽), TAG = triacylglycerols (●), DAG = diacylglycerols (●), FFA = free fatty acids (□), ALC = free fatty alcohols (▼), ST = sterols (◇), AMPL = acetone mobile polar lipids (◆), PL = polar lipids (⊙).

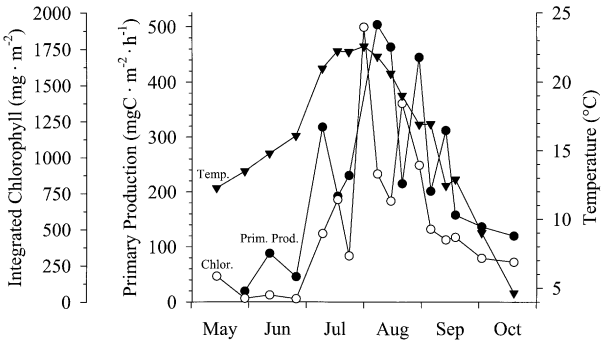

Fig. 3 Chlorophyll *a* (○), primary productivity (●) and temperature (▼) during the ice-free season in Humboldt Lake in 1989. Note the different scales.

phyll concentrations in this lake (Robarts *et al.*, 1992) and therefore also peaked in early August (Fig. 3) when Humboldt Lake was at ⊤ 22 °C.

Particulate and dissolved lipids

The development procedure of Parrish (1987) results in three separate chromatograms representing lipid classes of increasing polarity (Figs 4 and 5). In the dissolved fraction hydrocarbons peaked on 26 June (563 µg l^{-1}), 24 July–8 August (106 µg l^{-1}) and 2 October (104 µg l^{-1}) (Fig. 4). Wax esters reached maximum concentrations on 12 June (202 µg l^{-1}) and 5 September (54 µg l^{-1}); tri-and diacylglycerols peaked on 10 July

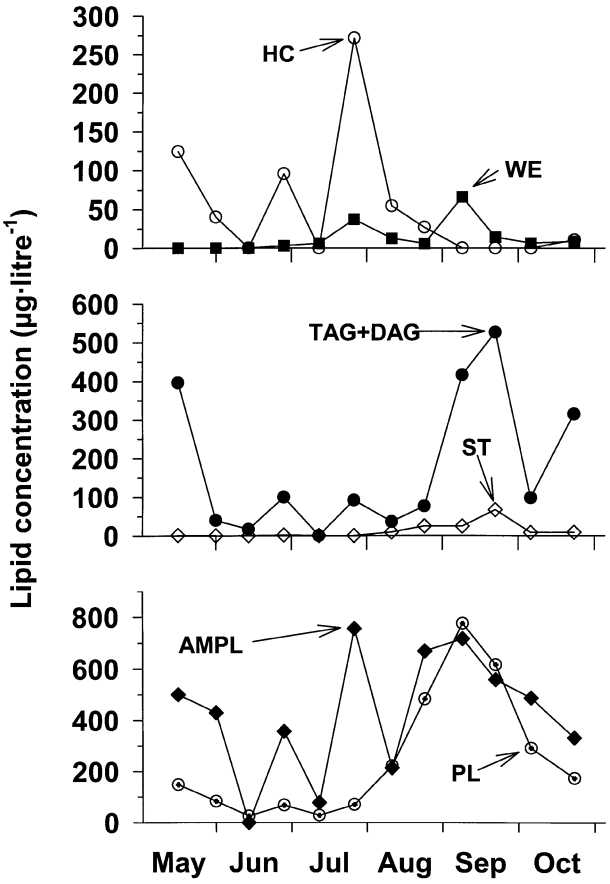

Fig. 5 Concentrations of lipids from the particulate (> 1.2 µm) fraction of Humboldt Lake water during the ice-free season of 1989. HC = hydrocarbons (○), WE = wax esters (■), KET = ketones (▽), TAG = triacylglycerols (●), DAG = diacylglycerols (●), FFA = free fatty acids (□), ALC = free fatty alcohols (▼), ST = sterols (◇), AMPL = acetone mobile polar lipids (◆), PL = polar lipids (⊙). Note: ketones, free fatty alcohols and free fatty acids are not shown because they were minor constituents of the particulate fraction.

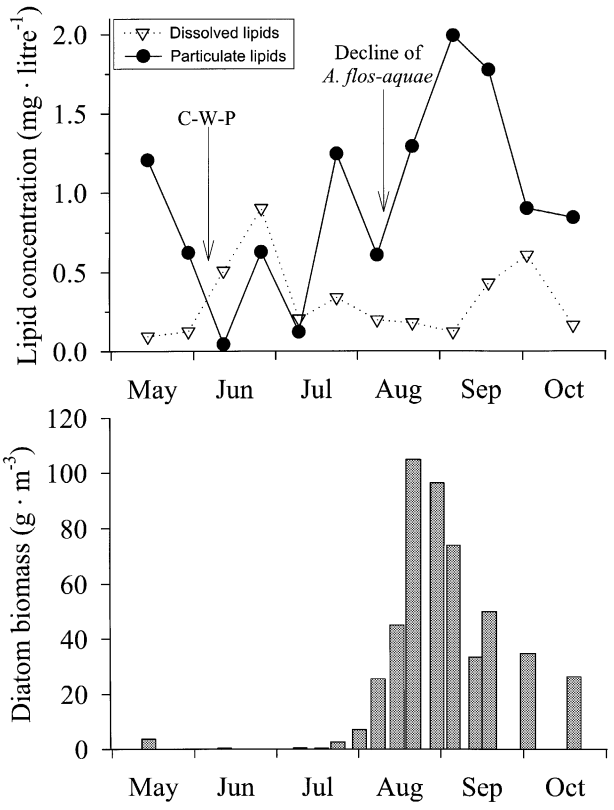

Fig. 6 (a) Concentration of total dissolved and particulate lipids in Humboldt Lake during the ice-free season. CWP = clear-water-phase. (b) Diatom biomass (wet weight).

(93 µg l^{-1}); free fatty acids peaked in late September to early October (361 µg l^{-1}). The acetone mobile polar lipids were most abundant on 12 June (192 µg l^{-1}), 24 July (153 µg l^{-1}), and from mid-September through October (79 µg l^{-1}).

In the particulate fraction hydrocarbons peaked on 15 May (124 µg l^{-1}), 26 June (96 µg l^{-1}) and 24 July (272 µg l^{-1}) (Fig. 5). The tri-and diacylglycerols reached their highest concentration in spring [15 May (396 µg l^{-1})] and again in autumn [18 September (526 µg l^{-1}) and 19 October (315 µg l^{-1})]. Acetone mobile polar lipids fluctuated greatly over the ice-free season, whereas polar lipids were low early in spring and early summer and then increased to a maximum concentration of 775 µg l^{-1} on 5 September.

Some lipid classes were relatively minor constituents of the total lipid pool (< 50 µg l^{-1}) during the ice-free season. These included the ketones, free fatty alcohols, sterols, and polar lipids in the dissolved fraction (Fig. 4). In the particulate fraction, ketones, free fatty acids and free fatty alcohols ranged from the detection limit to 30 µg l^{-1}, whereas wax esters and sterols peaked only in September (Fig. 5).

Overall, total dissolved and particulate lipids were lowest during the clear-water-phase (CWP). Both fractions increased immediately following the CWP (Fig. 6a) under conditions of moderate nitrogen deficiency (Fig. 2). Total particulate lipids peaked in July (Fig. 6a) due primarily to large amounts of acetone-mobile polar lipids (chlorophyll *a*) present during the height of the bloom of *A. flos-aquae* (Fig. 5). Total particulate lipid concentrations were highest in early September under conditions of severe nitrogen deficiency (Fig. 2). The diatom bloom during August and

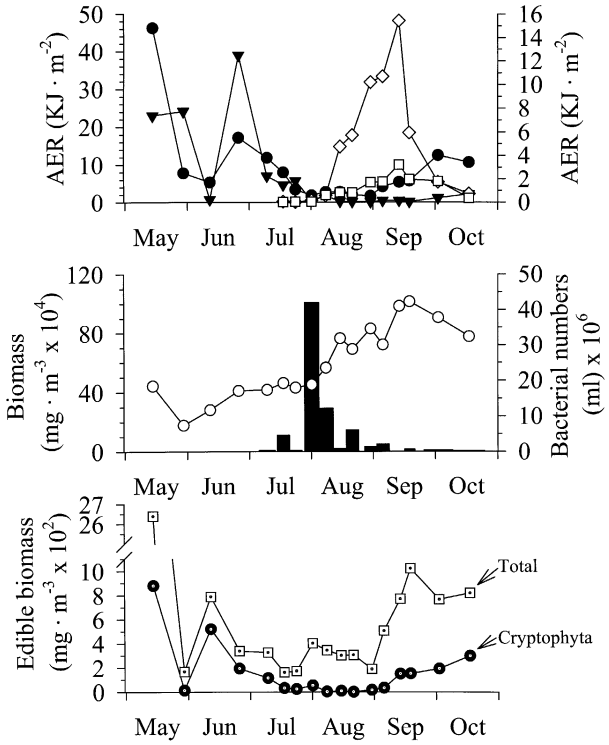

Fig. 7 Seasonal patterns in (a) areal energy reserves (AER) for *Leptodiaptomus sicilis* (●) and *Daphnia pulex* (▼) (upper panel, left scale) and *Diaphanosoma leuchtenbergianum* (◇), and *Chydorus sphaericus* (□) (upper panel, right scale), (b) biomass of *A. flos-aquae* (middle panel, solid histogram) and bacterial numbers (○) (middle panel), and, (c) total edible (⊡) and cryptophyte (ʘ) biomass (lower panel).

September (Fig. 6b) was probably largely responsible for most of this observed peak in total particulate lipid.

Zooplankton areal energy reserves

Areal energy reserves (AER) of *L. sicilis* and *D. pulex* populations were very high in spring (Fig. 7a). The sharp drop in population AER levels in these two species in early June was preceded by a sharp drop in edible algae (CWP) (Fig. 7c). AER of *L. sicilis* and *D. pulex* recovered briefly following CWP and then declined markedly (Fig. 7a) during the bloom of *A. flos-aquae* (Fig. 7b). Cryptophytes accounted for a significant fraction of edible algal biomass in May and June (Fig. 7c). The drop in AER during July and August was due to a combination of per animal decline in lipid content (Arts *et al.*, 1992) and a decrease in the density of adults of these species (M.S. Evans, unpublished data). Although per animal lipid concentrations recovered in the autumn (Arts *et al.*, 1992), AER showed only a modest increase compared to the spring, primarily because population densities of *L. sicilis* and *D. pulex* were lower in the autumn.

Maximum seasonal AER was greater for *L. sicilis* or *D. pulex* than for either *D. leuchtenbergianum* or *C. sphaericus*. However, AER in the latter two species increased markedly (Fig. 7a) soon after they appeared in significant numbers in Humboldt Lake in mid-July immediately following the decline of the cyanobacterial bloom (Fig. 7b). These increases also coincided with an increase (Fig. 7c) of edible algae dominated by small, single-celled algae (< 40 μm) of the Chlorophyta, Chrysophyta, and Cryptophyta (see Arts *et al.*, 1992, table 2). The period of maximum bacterial abundance (Fig. 7b) also coincided with the peak AER for both *D. leuchtenbergianum* and *C. sphaericus* (Fig. 7a). AER of both species declined significantly as the density of their populations decreased. Neither *D. leuchtenbergianum* nor *C. sphaericus* persist over the winter in Humboldt Lake.

Discussion

Dissolved lipid classes

Although all lipids are hydrophobic they are also, to some degree, soluble in water. This water-soluble component, together with the lipids adsorbed onto particles smaller than 1.0 μm and capable of passing through a glass-fibre filter, constitute what has been operationally defined as the dissolved lipid fraction (Parrish, 1986). Changes in the concentrations and class of dissolved lipid will be influenced by: (i) anthropogenic inputs, as, for example, is often the case for hydrocarbons (Parrish, 1986); (ii) biogenic inputs in the form of algal cell exudates (Baines & Pace, 1991) or, by direct release through 'sloppy feeding' of zooplankton (Jürgens, 1994); (iii) other autochthonous (DOC released from macrophytes) sources, and; (iv) allochthonous sources, for example, decomposition of wood and leaves (Robarts, 1986) or resuspended benthic organic matter (e.g. Findlay *et al.*, 1991). Together these dissolved lipids contribute to the labile component of the DOC utilized as substrate by bacteria and picoplankton.

The greatest overall concentrations of dissolved lipids occurred in June and again in early October (Fig. 6a). These periods correspond roughly to the

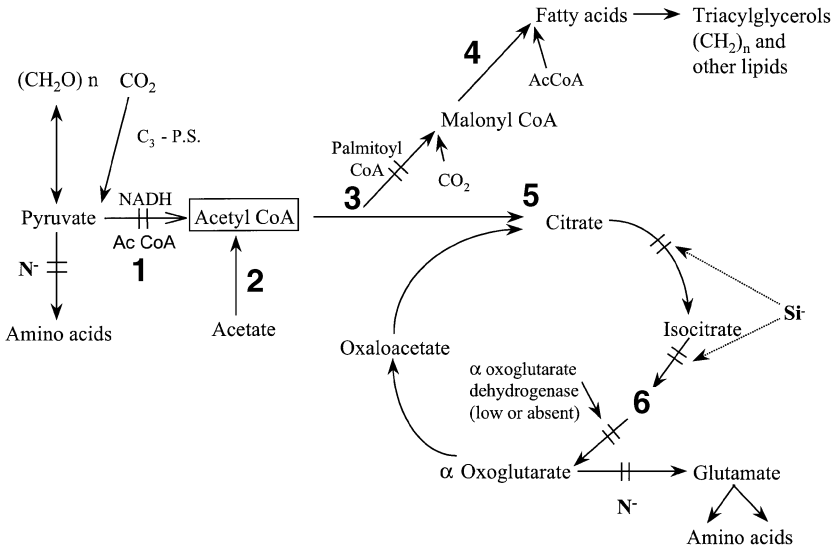

Fig. 8 Schematic showing the major biosynthetic pathway for lipids in some microalgae. Symbols are as follows: 1, pyruvate dehydrogenase; 2, acetyl-CoA carboxylase; 3, fatty acid synthase; 4, citrate synthase; 5, α-oxoglutarate dehydrogenase; 6, acetyl CoA synthase; (CH2O)2 = carbohydrate; N^- = nitrogen limitation; Si^- = silica limitation; % = inhibition; C3-P.S. = photosynthesis.

spring and autumn phytoplankton blooms. The dominant lipid classes in terms of concentration were hydrocarbons, tri-and diacylglycerols, free fatty acids, and pigments (e.g. chlorophyll) represented by the acetone-mobile-polar-lipid (AMPL) class (Fig. 4). We suspect that the hydrocarbon peaks in June, July August and October represent the release of material from decomposition of the spring, midsummer and autumn algal blooms, respectively. Consistent with this, the AMPL class that includes plant pigments such as chlorophyll also peaked at these times. Bacterial numbers in Humboldt Lake increased steadily throughout summer, cresting in the autumn of 1989 (Fig. 7b) when the concentration of dissolved free fatty acids reached ⊤ 360 μg l^{-1} (Fig. 4). The steady increase in bacterial numbers throughout the ice-free season suggests that the dissolved hydrocarbons and free fatty acids liberated from the decay of the spring, midsummer and autumn algal blooms (Figs 3 and 7b) were probably utilized as substrate by the bacterial population in Humboldt Lake. Robarts *et al*. (1994) reported that the rates of bacterial productivity were correlated with rates of primary productivity which peaked during the height of the bloom of *A. flos-aquae* (Fig. 3).

Particulate lipid classes

Lipids comprise a substantial portion of the chemical energy entrained in pelagic particles. Their high energy density makes them an ideal energy storage substance for small-bodied aquatic organisms. Energy reserve lipids (tri-and diacylglycerols and wax esters) represent the most variable component of a given species' lipid, exhibiting strong seasonality in many invertebrate populations (Gardner *et al*., 1985; Cavaletto, Vanderploeg & Gardner, 1989; Arts *et al*., 1992, 1993). The underlying causes of these seasonal patterns depend, to varying degrees, on temperature and its effect on metabolic rate, food quality and quantity, and reproductive state (Vanderploeg *et al*., 1992). Food quantity and quality is probably the major proximate factor regulating population lipid energy reserve content of zooplankton in lakes of similar latitudes.

It is important to consider the potential triggers that might stimulate the synthesis of lipid energy reserves in the particulate fraction, as well as the consequences of algal species succession and collapse of algal blooms on the type and quantity in the dissolved fraction, because, together, these phenomenon contribute to the production and release of energy-rich lipid materials in lentic systems. Algae synthesize long-chain free fatty acids and other lipids from simple carbon substrates following carbon fixation and possibly, in some cases, from the labile fraction of dissolved organic matter. Several factors have been shown to affect lipid synthesis or fatty acid composition, including: temperature (Thompson, Guo & Harrison, 1992); light level (Thompson, Harrison & Whyte, 1990); and nutrient (nitrogen, phosphorus and silica) concentration (Shifrin & Chisholm, 1981; Groeger & Kimmel, 1988; Kilham *et al*., 1997). However, the relative importance

of each of these factors as regulators of lipid biosynthesis in relation to photosynthesis remains the subject of vigorous research.

Nitrogen limitation may increase the relative rate of lipid biosynthesis by reducing the flow of carbon to amino acids. In particular, nitrogen deficiency may act to inhibit the formation of glutamate from α-oxoglutarate, as well as the synthesis of other amino acids from pyruvate (Fig. 8).

The total amount of lipid and its fatty acid composition, formed as a result of synthesis under different environmental conditions, is quite variable and often species-specific; for example, many freshwater and marine algae can be induced, in the laboratory, to increase their rate of lipid synthesis under conditions of nutrient limitation. This lipid induction effect can, under certain conditions, be quite dramatic; for example, Shifrin & Chisholm (1981) demonstrated two-to threefold increases in the lipid content of green algae grown in batch cultures under nitrogen-limited conditions. Diatoms displayed both increased and decreased lipid contents with nitrogen limitation, depending on the species (Shifrin & Chisholm, 1981). Groeger & Kimmel (1988) provided field data which showed that phytoplankton in a Tennessee reservoir responded to summer N-deficiency by increasing rates of lipid synthesis from 10–15% up to 20–35% of the total C fixation.

Due to runoff from surrounding agricultural lands and the historical inflow of treated sewage effluent, nutrient concentrations in Humboldt Lake are high (Fig. 1). Healey & Hendzel's (1980) indices suggest that there was a moderate phosphorus deficiency in Humboldt Lake in July, 1989 (Fig. 2a). This probably occurred as a result of the rapid growth rate of *A. flos-aquae* and concurrent incorporation of phosphorus into cyanobacterial tissue. Nitrogen appears to have been moderately deficient at the end of May just before CWP in early June (Fig. 2b). However, severe nitrogen deficiency was indicated following the collapse of the *A. flos-aquae* bloom (Fig. 2b), probably as a result of the removal of particulate nitrogen (Fig. 1b) when the nitrogen-fixing cyanobacterial cells settled to the bottom and were washed ashore.

Energy reserve lipid concentrations in the particulate fraction of Humboldt Lake water increased dramatically (Fig. 5b) in response to the sustained period of nitrogen deficiency in September (Fig. 2b). This was accompanied by a sharp increase in diatom (Fig. 6b.) and edible algal biomass (Fig. 7c) and a rapid increase in the densities of *D. leuchtenbergianum* and *C. sphaericus*. Small-bodied cladocerans such as *D. leuchtenbergianum* and *C. sphaericus* are especially adept (Geller & Müller, 1981) at filtering out small food particles, such as bacteria (Fig. 7b) and small algae ($\leqslant 25\ \mu m$), which proliferated following the crash of the *A. flos-aquae* bloom (see Arts *et al*., 1992, table 2). Small chrysophytes, such as *Ochromonas* sp., *Heterochromonas polystricta* and *Mallamonas coronata*, and cryptophytes, such as *Rhodomonas minuta* and *Cryptomonas* sp., dominated the edible algae in September and October 1989. Stewart & Wetzel (1986) speculated that cryptophytes may be crucial in the stabilization of planktonic communities. This is because of their ability to supplement photosynthesis by 'scavenging' fine particulate-organic matter (Smith, 1950) during the intervening periods between blooms of more numerically dominant species of algae and by their ability to grow at low light levels. Cryptophytes such as *R. minuta* and *Cryptomonas* sp. were always a significant portion of the edible biomass (Fig. 7c) during periods when AER of the dominant zooplankters *D. pulex* and *L. sicilis* were greatest (Fig. 7a). Certainly the high nutritional quality of flagellated cryptophytes such as *R. minuta* and *C. erosa* is well documented (Chen & Folt, 1993; Hart & Santer, 1994; Santer & Van den Bosch, 1994).

Although moderate nitrogen deficiency was briefly observed at the end of May, no large increase in the concentration in energy-reserve lipids in the particulate fraction was observed (Fig. 5b). We suspect that intense grazing pressure resulting from the high densities of *D. pulex* and *L. sicilis* (Evans *et al*., 1995) reduced edible algal cell density (Fig. 7c) during CWP, to the extent that potential increases in per-cell lipid biosynthesis rates were masked by the sharp declines in cell density (Fig. 7c).

Very little field data exist on nutrient-induced triggers of lipid synthesis in freshwater systems. Results of the present study are similar to Groeger & Kimmel's (1988) findings of a lipid induction effect in algae following a period of nitrogen deficiency in a mesotrophic reservoir, and contrast with the findings of Wainman *et al*. (1993) in which no lipid induction effect was observed following P and N limitation in three small oligotrophic to mesotrophic lakes.

A generalized schematic provides a conceptual

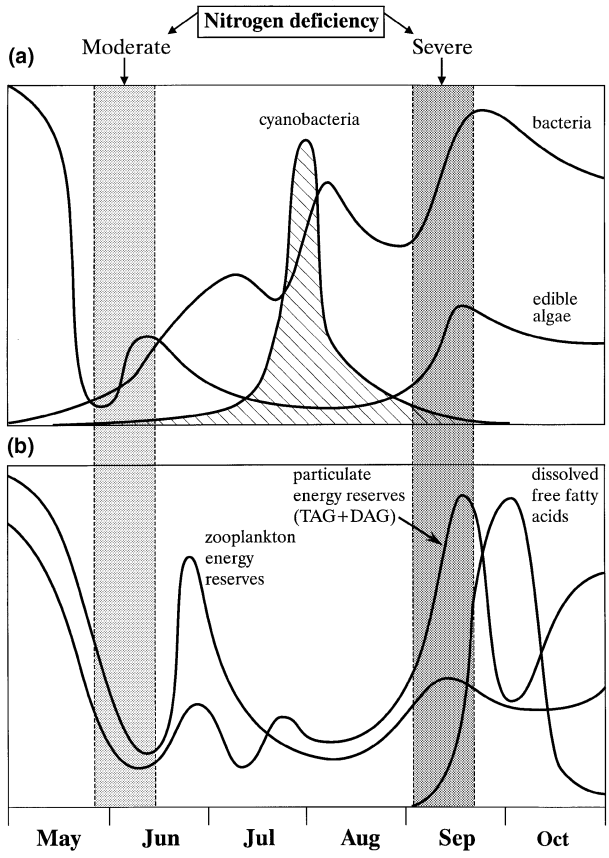

Fig. 9 Model of key parameters implicated in lipid energy transfers from primary producers to secondary consumers in a hypereutrophic lake. Note: (a) parameters in top panel represent biomass units; zooplankton energy reserves are represented as areal units; particulate and dissolved lipid fractions are expressed as concentrations, and, (b) the diagram is not drawn to scale; e.g. cyanobacterial biomass is two orders of magnitude greater than edible algal biomass (see preceding figures for absolute values).

summary of the key parameters implicated in promoting lipid energy transfers from primary producers to secondary consumers in a hypereutrophic lake (Fig. 9). Energy reserve lipids of the particulate fraction increase slightly following CWP under conditions of moderate nitrogen limitation (Fig. 9). Zooplankton biomass is still high at this time and therefore zooplankton rapidly incorporate algal lipid for a brief period prior to the onset of the cyanobacterial blooms of midsummer. Following the demise of the bloom-forming cyanobacteria there is a period of severe nitrogen deficiency accompanied by a fivefold increase in the concentration of lipid energy reserves in the phytoplankton and a latent release of free fatty acids from the decomposition of *A. flos-aquae*. These phenomenon are tightly linked to the appearance of lipid-rich taxa such as diatoms and several small-bodied species of cryptophytes, chlorophytes and chrysophytes which dominate the edible biomass following the decline of *A. flos-aquae*. Lipid energy transfers from algae to zooplankton occur at this time, however, the responses are modulated by the demographics of each individual species; for example, areal energy reserves of *D. leuchtenbergianum* increase rapidly because the population density of this species is high at this time. Although there is a strong increase in per animal lipid concentration of *D. pulex* and *L. sicilis* (Arts *et al.*, 1992) the population responses (AER) are more muted because the density of large-bodied adults is lower than in the spring.

It is important to recognize that, from these data alone, we cannot state equivocally that the elevated levels of energy reserve lipids that we observed arise as a direct consequence of nutrient limitation, although this remains a plausible explanation. The algal species composition changes noted above undoubtedly played an important role in determining lipid levels in this lake. Also, there can be no doubt that light levels play a crucial role in determining the relative allocation of algal carbon to lipid, protein and polysaccharide (Cuhel, Ortner & Lean, 1984; Thompson *et al.*, 1990). Underwater light levels were undoubtedly higher during CWP and also following the demise of *A. flos-aquae* in late summer (Robarts *et al.*, 1992). Finally, it would have been desirable to have information on the percentage of the primary productivity incorporated into protein and polysaccharide to compare with carbon fixed as lipid.

Data of this sort are usually obtained during short-term incubations with ^{14}C as a tracer. Short-term tracer studies provide information on what the algae were doing metabolically around the time they were collected but they are not necessarily good indicators or integrators of metabolic activity beyond more than a few hours. In addition, if incubation experiments are conducted late in the day or very early in the morning the results could differ because algae are known to alter their relative allocation of carbon in response to changing light levels (Cuhel *et al.*, 1984; Thompson *et al.*, 1990). In some tracer studies algae are first concentrated by tangential flow filtration and then incubated in the laboratory, which introduces further uncertainty. The approach used here, which seeks to

measure standing stocks of lipid in the algal communities based on field collection, may be a better integrator of past condition and may therefore provide a better composite of what the algae had experienced over the last day or two prior to collection rather than the last few hours. This is because this approach measures stored lipid reserves rather than the alga's instantaneous ability to synthesize new lipid. The drawback of this approach is that it does not provide information on carbon partitioning or on short-term production rates and that it does not discriminate between living and dead particles. Both approaches have their merit, depending on the nature of the question being asked.

In conclusion, the data provide some empirical support for laboratory data derived from algal cultures in which nutrient deficiency resulted in increased lipid production. In eutrophic systems, characterized by bloom-forming, nitrogen-fixing cyanobacteria, strong seasonal variations in nutrient concentrations, in particular nitrogen, will be common. These seasonally predictable events have the potential to trigger lipid synthesis in algae over the short-term, resulting in transient increases in the availability of essential, energy-rich lipids to higher trophic levels. Although more detailed studies are required to determine the generality validity of these observations the seasonal recurrence of these phenomenon could provide the evolutionary basis for an adaptive response on the part of the zooplankton which must maximize their lipid energy consumption in order to overwinter, either in the active stage or as resting eggs.

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