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Seasonal changes in the biochemistry of lake seston

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SUMMARY

1. The quantity of seston was measured and the elemental carbon, nitrogen and phosphorus (C, N, P) and biochemical composition (carbohydrate, protein, lipid) of the < 53 μm size fraction in three temperate lakes during one year was analysed. The lakes differed in nutrient concentration and were characterized as oligotrophic, mesotrophic and eutrophic. Linear regression analyses defined associations between seston composition and either lake trophic status, depth or season.
2. The concentration of particulate organic seston was greatest during spring and autumn and lowest during the clear water period in early summer. Seasonal patterns in seston elemental and biochemical percentage composition (quality) were observed to be independent of differences in seston quantity.
3. Concentrations of seston C, N and P were high in most cases in the spring and autumn and low in summer. Concentrations of P were particularly high during late summer and early autumn in the metalimnion, perhaps because of recovery of P from anaerobic sediments and hypolimnetic waters. Because seston C and N did not increase as markedly as P, C : P and N : P ratios both declined in the autumn. Primary production was thought to be co-limited by N and P in all three of these lakes; however, the data suggested that N might be more important as a major limiting nutrient in the eutrophic lake as the metalimnion increased in depth in late summer and autumn.
4. Concentrations of protein, carbohydrate, polar lipid and triglyceride generally increased with lake type as expected (greatest in the eutrophic lake), but showed no relationship with water depth. As the year progressed, no significant changes were measured in protein and carbohydrate concentrations; however, the concentration of polar lipid decreased and triglyceride increased significantly with time of year.
5. The biochemical composition of seston varied during the year and among lakes; for example, in Lake Waynewood the proportion of protein composing the seston (percentage protein by weight) varied from $< 10\%$ to $> 40\%$. No statistically significant patterns in the percentage protein or carbohydrate were found. However, the proportion of seston comprised of triglyceride decreased with lake type and increased during the year; whereas the proportion of seston as polar lipid increased with lake type and decreased during the year. Triglyceride comprised most of the lipid. Both protein : lipid and protein : carbohydrate ratios tended to be greatest in summer and lowest in the spring and autumn.
6. Relationships between samples and biochemical composition analysed by Canonical Correspondence Analysis (Canoco) indicated similar patterns in seasonal changes in seston biochemistry for the three lakes, with samples separated primarily by vectors for

lake type (oligotrophic to eutrophic) and the percentage polar lipid (proportion of total lipid) and secondarily by vectors for date and water depth (epilimnion or metalimnion). 7. These seasonal biochemical changes in the seston food base were compared with biochemical changes known to occur in algae grown under N-or P-limited conditions in the laboratory, and the resultant quality of this algal food for suspension-feeding consumers (zooplankton). It was concluded that zooplankton were likely to be physiologically challenged by these distinct seasonal shifts in the quality of lake seston.

Introduction

Recent efforts by a variety of researchers (Ahlgren *et al*., 1997; DeMott & Müller-Navarra, 1997; Kilham *et al*., 1997b; Lurling *et al*., 1997; Sterner, 1997; Sundbom & Vrede, 1997; Weers & Gulati, 1997) indicate that zooplankton living in northern temperate lakes are sensitive to food quality effects. Most studies that have reported such effects were conducted under defined conditions in the laboratory. In natural systems, however, it is unclear whether generalist feeders like suspension-feeding zooplankton are limited by suboptimal food quality because seston is usually comprised of a heterogeneous mix of particle types quite different from laboratory-reared algae. Compared with marine systems, there have been relatively few reports documenting *in situ* variability in seston composition, and hence, food quality for freshwater zooplankton (Elser & George, 1993; Mazumder, 1994; Sterner & Hessen, 1994; Urabe, 1995).

In marine and estuarine environments, both the availability and biochemical composition of seston are known to be highly variable over tidal, diurnal, lunar and seasonal temporal scales (Mayzaud & Taguchi, 1979; Mayzaud, Taguchi & Laval, 1984; Soniat, Ray & Jeffrey, 1984; Berg & Newell, 1986; Mayzaud, Chanut & Ackman, 1989; Lizotte & Sullivan, 1992; Parrish *et al*., 1995). Variability in seston abundance has obvious implications for secondary production by aquatic suspension-feeders; however, there is mounting evidence that animals in this functional group can be just as limited nutritionally by food quality as food quantity; for example, copepods (Kifrboe, 1989; Roman, 1991; Anderson & Hessen, 1995; Kleppel & Burkart, 1995) and bivalves (Prins & Smaal, 1989; Bayne *et al*., 1993; Kreeger & Langdon, 1993; Cranford, 1995). This is particularly true for zooplankton because of their relatively short life cycle (months) precludes time-averaged optimization of their nutritional balance such as occurs in longer-lived suspension-feeders (Hawkins *et al*., 1985).

The objectives of the present study were threefold: (i) to measure seasonal changes in the elemental and biochemical composition of seston in three lakes differing in trophic status; (ii) to determine whether changes in lake seston biochemistry are consistent with observed changes in laboratory algae grown under nutrient-limited conditions; and (iii) to define seasonal changes in lake seston composition from a view of its food quality for suspension-feeding consumers.

Seston biochemical and elemental composition was studied in three north-temperate lakes: Lakes Giles, Lacawac and Waynewood, situated in the Pocono Mountains, north-east Pennsylvania, U.S.A. Lake Giles is an oligotrophic, low-pH lake with a prevalence of chrysophytes; the dominant zooplankton are *Daphnia catawba* and *Diaptomus minutus*. The hypolimnion has oxygen throughout the year. Lake Lacawac is mesotrophic and has chrysophytes and cryptomonads as dominant phytoplankton, and *Daphnia catawba*, *Holopedium gibberum* and *Diaptomus minutus* as the dominant zooplankton. It is strongly stratified with little oxygen in the hypolimnion during the summer months. Lake Waynewood is eutrophic and has heavy blooms of cyanobacteria, primarily *Aphanizomenon* and *Anabaena*; *Daphnia pulicaria* and *Diaptomus oregonensis* are the dominant zooplankton taxa. Its hypolimnion is anaerobic during summer.

Phytoplankton and zooplankton are most abundant in spring and autumn in all three lakes (Tessier, 1986; Moeller *et al*., 1995). R. Moeller (Lehigh University, personal communication) has found that algal growth in the three lakes generally is co-limited by nitrogen (N) and phosphorus (P). The kinds of biochemical changes found in laboratory monocultures of algae grown under such nutrient limitations are described in Kilham *et al*., 1997a. Are the observed changes in lake seston biochemistry consistent with expectations from these laboratory experiments?

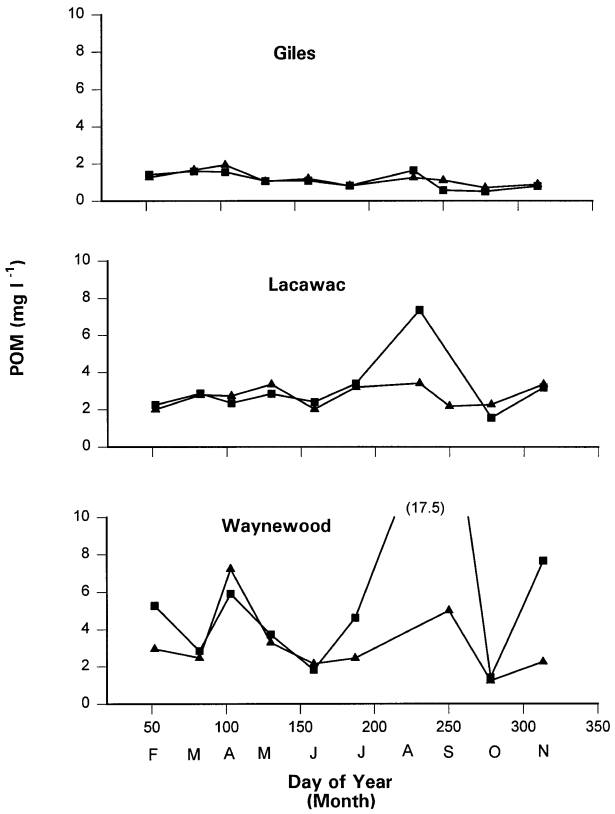

Fig. 1 Mean (± SE) concentration of particulate organic seston (1–53 mm) in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

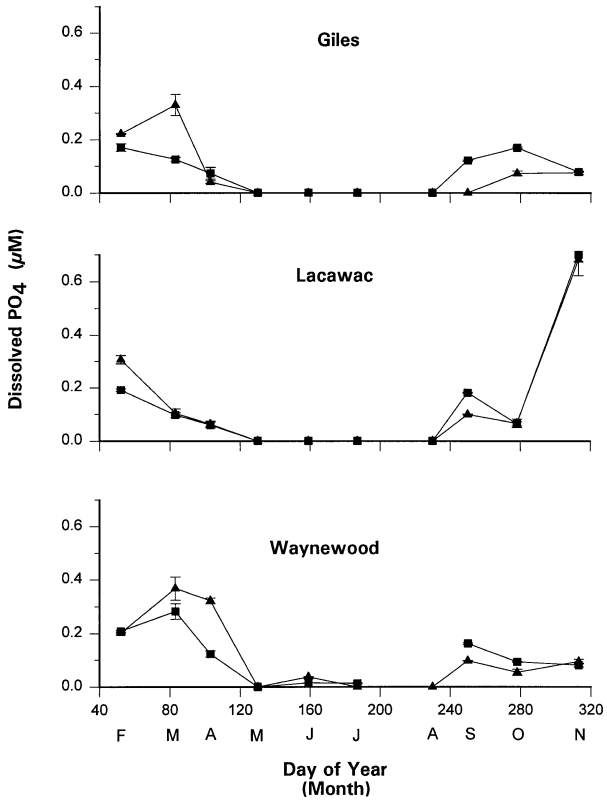

Fig. 2 Mean (± SE) dissolved phosphorus (PO_4^+) concentration in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

Measurements of algal abundance (chlorophyll concentrations) and dissolved nutrient concentrations are essential to understand 'bottom-up' control of primary production in lakes, however, they are of limited value in describing shifts in food quality for suspension-feeders. As with all animals, suspension-feeders require a sufficient amount of dietary energy, protein, lipid and essential amino acids, fatty acids (FA), minerals and vitamins. In natural systems, however, the elemental and biochemical composition of phytoplankton and other seston constituents varies seasonally and inter-and intraspecifically, and so the food value of seston cannot be adequately characterized by measuring concentrations of chlorophyll. The objective of this study was to examine how the food value of seston varies for lake zooplankton during the year, with depth, and among lakes of differing trophic status. To do this, we measured concentrations of various elemental [carbon (C), N, P] and biochemical (protein, carbohydrate, lipid, triglyceride, polar lipid) constituents in seston (< 53 μm fraction) collected from the epilimnion and metalimnion of Lakes Giles, Lacawac and Waynewood during 1995.

Materials and methods

Collection of lake water

Water was collected monthly from each of the three lakes during the period February to November 1995. Water was collected from the deepest station in each lake, from the middle depth of both the epilimnion (2–3 m) and metalimnion (5–9 m). A 2-l Van Dorn bottle was used to collect water, which was added to 20-l polyethylene carboys for transport to the laboratory. Temperature, dissolved oxygen concentration and pH were measured at 1-m intervals at the time of each water collection.

Particulate organic material (POM)

Seston is defined as particulate material that can be efficiently removed from suspension by most lake

Table 1 Elemental composition of particulate seston (1–53 μm) of lake water in 1995. Values in parentheses are SE of means

	Day of year									
	53	83	103	130	159	187	230	250	278	313
C (μmol l^{-1})										
Giles Epi	52.4	69.4	80.5	43.3	49.7	33.5	51.8	45.9	29.7	37.0
Giles Meta	58.8	66.4	64.6	44.4	45.2	33.9	68.1	23.7	21.2	33.0
Lacawac Epi	83.2	115.6	113.5	139.8	84.1	133.2	142.0	90.5	94.1	139.7
Lacawac Meta	93.5	119.3	97.4	118.3	100.1	141.4	306.9	—	65.0	132.4
Waynewood Epi	122.9	102.8	300.9	137.1	89.4	102.2	—	208.4	51.4	93.8
Waynewood Meta	219.5	118.5	246.2	155.2	75.8	192.5	—	729.1	58.9	318.8
N (μmol l^{-1})										
Giles Epi	—	—	—	—	—	—	—	—	—	—
Giles Meta	—	—	—	—	—	—	—	—	—	—
Lacawac Epi	4.27	4.87	6.54	—	3.55	7.02	—	5.67	4.64	4.56
	(0.30)	(0.24)	(0.40)		(0.29)	(1.17)		(1.43)	(0.14)	(0.22)
Lacawac Meta	4.97	3.57	3.07	—	4.64	5.69	—	16.48	3.24	4.94
	(0.87)	(0.49)	(0.86)		(0.91)	(0.16)		(2.22)	(1.45)	(0.82)
Waynewood Epi	3.16	—	1.07	—	—	0.63	—	3.30	—	0.26
	(0.17)		(0.22)			(0.16)		(0.16)		—
Waynewood Meta	1.73	—	0.52	—	—	0.60	—	0.52	—	0.32
	(0.03)		(0.20)			(0.28)		—		(0.02)
P (μmol l^{-1})										
Giles Epi	—	—	—	—	—	—	—	—	—	—
Giles Meta	—	—	—	—	—	—	—	—	—	—
Lacawac Epi	0.109	0.162	0.162	0.110	0.139	0.154	0.201	0.157	0.162	0.218
	(2.4×10^{-3})	(6.2×10^{-3})	(6.4×10^{-3})	(8.8×10^{-4})	(7.4×10^{-3})	(1.0×10^{-2})	(4.4×10^{-3})	(3.7×10^{-3})	(4.7×10^{-3})	(4.5×10^{-3})
Lacawac Meta	0.088	0.161	0.113	0.144	0.156	0.208	0.526	0.635	0.184	0.205
	(2.4×10^{-3})	(8.8×10^{-4})	(7.6×10^{-4})	(4.9×10^{-3})	(4.8×10^{-3})	(9.8×10^{-3})	(5.2×10^{-3})	(9.3×10^{-3})	(4.4×10^{-3})	(1.8×10^{-3})
Waynewood Epi	0.211	0.221	0.219	0.175	0.166	0.150	0.368	0.226	0.204	0.500
	(2.8×10^{-2})	(1.2×10^{-3})	(2.2×10^{-2})	(4.6×10^{-3})	(5.2×10^{-3})	(3.8×10^{-3})	(6.2×10^{-3})	(2.5×10^{-2})	(1.3×10^{-3})	(4.2×10^{-2})
Waynewood Meta	0.694	0.238	0.267	0.253	0.200	0.635	1.862	0.564	0.154	0.564
	(2.0×10^{-1})	(4.0×10^{-4})	(5.4×10^{-3})	(6.3×10^{-3})	(7.2×10^{-3})	(1.1×10^{-2})	(1.5×10^{-2})	(7.7×10^{-2})	(2.2×10^{-3})	(9.7×10^{-3})

zooplankton; that is, particles having diameters between 1 and 53 μm. The concentration of seston POM in each sample of lake water was calculated by summing the respective concentrations of seston protein, carbohydrate and lipid (see methods below). This procedure assumes that biochemical components other than protein, lipid and carbohydrate comprised negligible weight. To check this assumption, POM was periodically measured directly with weight-on-ignition (WOI) analysis of the seston dry weight (DW) and ash-free dry weight (AFDW) concentrations.

For WOI analysis, each water sample was passed through a 53-μm Nitex mesh sieve to remove animals and other large particles, and a known volume of filtrate (50–800 ml, enough to maximize the amount collected without clogging the filter) was then passed through a pre-combusted (500 °C, 4 h) glass fibre filter (25 mm diameter) to concentrate the seston. All filters containing seston were frozen at –80 °C until analysis. Six replicate filters used for WOI analysis were pre-weighed (± 0.01 mg) before use. After seston was filtered, the filters were dried (60 °C, 2 days), reweighed, combusted (500 °C, 4 h), and again reweighed. The difference in weight between the dry, filtered seston and the pre-filtration filter was divided by the filtered volume to calculate the concentration of POM DW in the original water sample. The concentration of POM (mg l^{-1}) was calculated by dividing the volume filtered (l) into the difference in weight between dry and combusted filtered seston (mg AFDW). There were no significant differences (paired student's *t*-test, $\alpha = 0.05$) in POM values obtained directly (WOI analysis) or indirectly (summation of biochemical components), and so only results from the indirect method will be reported.

Table 2 Dissolved elemental concentrations (≤0.4 μM) of lake water in 1995. Values in parentheses are SE of means

	Day of year (month)									
	53 (Feb.)	83 (Mar.)	103 (Apr.)	130 (May)	159 (Jun.)	187 (Jul.)	230 (Aug.)	250 (Sep.)	278 (Oct.)	313 (Nov.)
P (μmol l^{-1})										
Giles Epi	0.222 (2.0×10^{-3})	0.330 (4.0×10^{-2})	0.041 (5.0×10^{-3})	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)	0.074 (9.0×10^{-3})	0.076 (6.0×10^{-3})
Giles Meta	0.171 (1.4×10^{-2})	0.126 (8.0×10^{-3})	0.074 (2.2×10^{-2})	0.000 (0)	0.001 (1.0×10^{-3})	0.000 (0)	0.000 (0)	0.122 (2.0×10^{-3})	0.170 (3.0×10^{-3})	0.079 (2.0×10^{-3})
Lacawac Epi	0.307 (1.6×10^{-2})	0.104 (1.6×10^{-2})	0.063 (1.1×10^{-2})	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)	0.099 (2.0×10^{-3})	0.066 (1.6×10^{-2})	0.618 (5.9×10^{-2})
Lacawac Meta	0.191 (5.0×10^{-3})	0.097 (9.0×10^{-3})	0.060 (1.1×10^{-2})	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)	0.181 (2.0×10^{-3})	0.064 (8.0×10^{-3})	0.699 (2.7×10^{-2})
Waynewood Epi	0.202 (3.0×10^{-3})	0.367 (4.4×10^{-2})	0.321 (1.0×10^{-2})	0.000 (0)	0.037 (2.0×10^{-3})	0.000 (0)	0.000 (0)	0.097 (1.0×10^{-3})	0.054 (1.1×10^{-2})	0.094 (9.0×10^{-3})
Waynewood Meta	0.209 (3.0×10^{-3})	0.282 (2.9×10^{-2})	0.123 (1.2×10^{-2})	0.000 (0)	0.015 (1.5×10^{-2})	0.013 (2.0×10^{-3})	—	0.162 (3.0×10^{-3})	0.093 (2.0×10^{-3})	0.081 (7.0×10^{-3})
Si (μmol l^{-1})										
Giles Epi	4.00 (1.5×10^{-2})	0.61 (7.4×10^{-2})	5.20 (2.1×10^{-2})	—	5.16 (5.4×10^{-2})	1.92 (2.4×10^{-1})	1.52 (1.1×10^{-2})	2.13 (1.5×10^{-2})	2.18 (4.6×10^{-2})	3.10 (2.7×10^{-2})
Giles Meta	4.00 (2.0×10^{-3})	0.53 (9.0×10^{-3})	5.10 (2.8×10^{-2})	—	4.27 (4.9×10^{-2})	2.32 (3.8×10^{-2})	2.02 (3.0×10^{-3})	1.12 (4.9×10^{-2})	7.52 (7.1×10^{-2})	2.99 (8.7×10^{-2})
Lacawac Epi	12.76 ($2.8\ 10^{-2}$)	3.19 (2.3×10^{-2})	9.32 (9.8×10^{-2})	—	2.29 (5.6×10^{-2})	3.79 (4.4×10^{-2})	6.30 (5.1×10^{-1})	6.14 (1.5×10^{-1})	6.14 (5.0×10^{-3})	12.76 (3.3×10^{-2})
Lacawac Meta	12.68 (9.1×10^{-2})	2.63 (9.1×10^{-2})	9.62 (2.3×10^{-1})	—	4.24 (3.4×10^{-2})	8.70 (1.5×10^{-1})	20.36 (7.0×10^{-2})	19.83 (3.4×10^{-1})	7.39 (4.8×10^{-2})	12.62 (8.2×10^{-2})
Waynewood Epi	25.72 (4.9×10^{-2})	21.95 (8.5×10^{-1})	24.28 (1.9×10^{-1})	—	10.70 (1.2×10^{-1})	7.65 (6.0×10^{-2})	14.48 (4.5×10^{-1})	11.64 (2.6×10^{-1})	3.40 (3.3×10^{-2})	24.99 (9.1×10^{-2})
Waynewood Meta	26.23 (1.5×10^{-1})	19.92 (8.2×10^{-1})	24.34 (5.3×10^{-1})	—	19.72 (2.9×10^{-1})	37.93 (3.1×10^{-1})	44.23 (6.0×10^{-1})	33.46 (3.6×10^{-1})	6.60 (4.9×10^{-2})	24.48 (8.4×10^{-2})

Dissolved elements in lake water

Dissolved phosphate concentrations in lake water samples were determined in triplicate by the ascorbic acid method (APHA, 1985). Concentrations of dissolved silicate in lake water were determined in triplicate by the molybdosilicate method (Strickland & Parsons, 1972). Dissolved nitrogen was not measured.

Particulate elements in lake seston

Concentrations of C, N and P in particulate seston were analysed for Lakes Lacawac and Waynewood, but not for Lake Giles.

Nitrogen and carbon. Three seston samples for N and C determination were collected from each water sample on glass fibre filters as stated above for POM analysis. Filters were dried at 60 °C for 24 h and then held in a desiccator until elemental analysis. Subsamples were punched from the filters with a bore tube, packed into pre-cleaned (acetone-washed) tin capsules, and then analysed on a Carlo Erba 1106 elemental analyser fitted with an autosampler by D. Morris at Lehigh University.

Phosphorus. For determination of seston P concentration, known volumes of each lake water sample were passed through triplicate polycarbonate filters (Costar, 0.4 μm, 25 mm diameter) instead of glass fibre filters (as for all other analyses). These filters were digested with 5% w/v persulphate at 121 °C for 30 min, and then P was determined by the ascorbic acid method (APHA, 1985).

Biochemical analysis of lake seston

For each water sample, nine seston samples were collected on pre-combusted glass fibre filters (as described above for POM analysis) for the following biochemical analyses (replication per sample in paren-

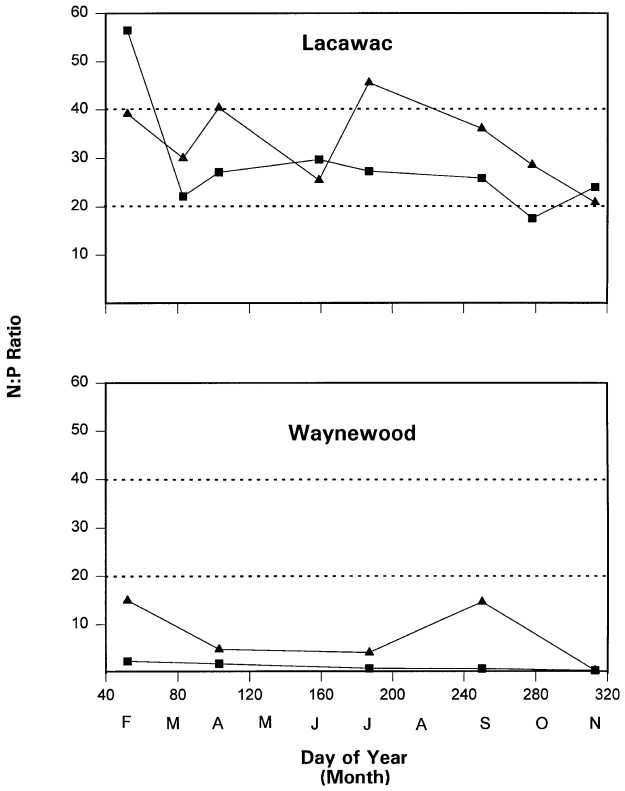

Fig. 3 Elemental N : P ratio (μM : μM) of seston (1–53 μm) collected from the epilimnion (▲) and metalimnion (■) of Lakes Lacawac and Waynewood during 1995.

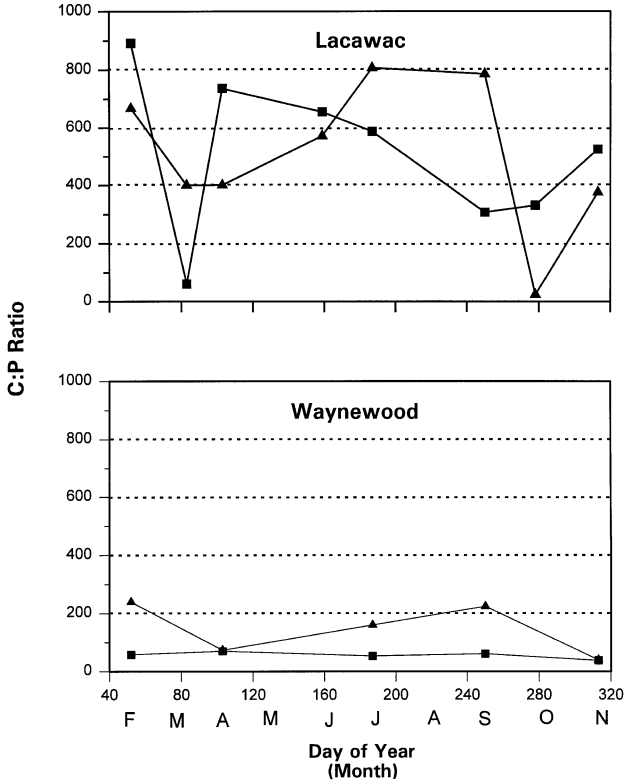

Fig. 4 Elemental C : P ratio (μM :μM) of seston (1–53 mm) collected from the epilimnion (▲) and metalimnion (■) of Lakes Lacawac and Waynewood during 1995

theses): carbohydrate (n = 3), protein (n = 3) and lipid (n = 3). All filters containing seston were frozen at –80 °C until biochemical analysis, except those for lipid analysis, which were added to 2 : 1 chloroform : methanol in 1-ml vials before freezing at –80 °C.

Carbohydrate. Seston carbohydrate (CHO) was determined spectrophotometrically using the Dubois *et al*. (1956) procedure, which was standardized with potato starch (soluble grade, Baker, 4006–4). Each seston filter was combined with 2 ml laboratory-pure water in a 15-ml borosilicate glass test tube, and the Dubois *et al*. (1956) reagents were added accordingly. Tubes were mixed 5 s with a Vortex Genie, and a 200 μl subsample was transferred to three replicate wells on a 96-well microplate for spectrophotometric analysis at 490 nm with a microplate reader.

Protein. Seston protein was determined spectrophotometrically using a Pierce test kit (BCA 23225) based on the procedure of Lowry *et al*. (1951), and standardized with bovine serum albumin. Filtered seston was prepared for analysis by first suspending the filter in 4 ml 0.1 M NaOH in a 15-ml polypropylene test tube. The filter was ground in a tissue homogenizer (Polytron, maximum power) for 60 s and sonicated for 10 s to transform the filter into a slurry. This procedure helped to degrade walls of algal cells. Samples were diluted to 8 ml with 0.1 M NaOH, heated to 60 °C for 45 min, mixed for 10 s with a Vortex Genie, and then centrifuged (800 ***g***, 5 min). Samples were held on ice whenever possible throughout the procedure. Samples (10 μl) of each supernatant were transferred to each of triplicate wells in a 96-well microplate. BCA reagent (200 μl) was added to each well, and after a 30 min incubation period at 37 °C, the microplate was analysed at 562 nm with a microplate reader (Molecular Devices, Thermomax).

Lipid class composition. Concentrations of six major classes of lipids were measured in each sample of lake seston, and total concentration of seston lipids was then calculated for each sample by summation. Prior to class analysis, lipids were extracted and purified

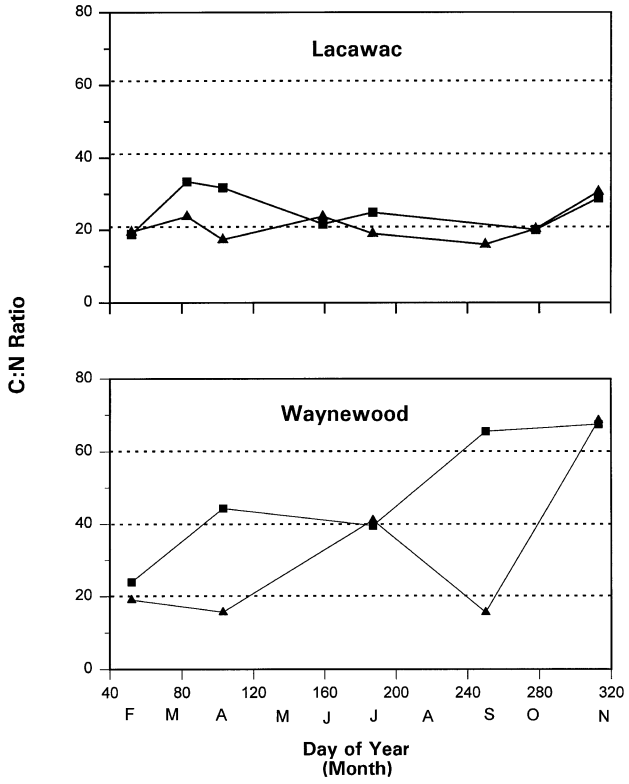

Fig. 5 Elemental C : N ratio (μM : μM) of seston (1–53 μm) collected from the epilimnion (▲) and metalimnion (■) of Lakes Lacawac and Waynewood during 1995.

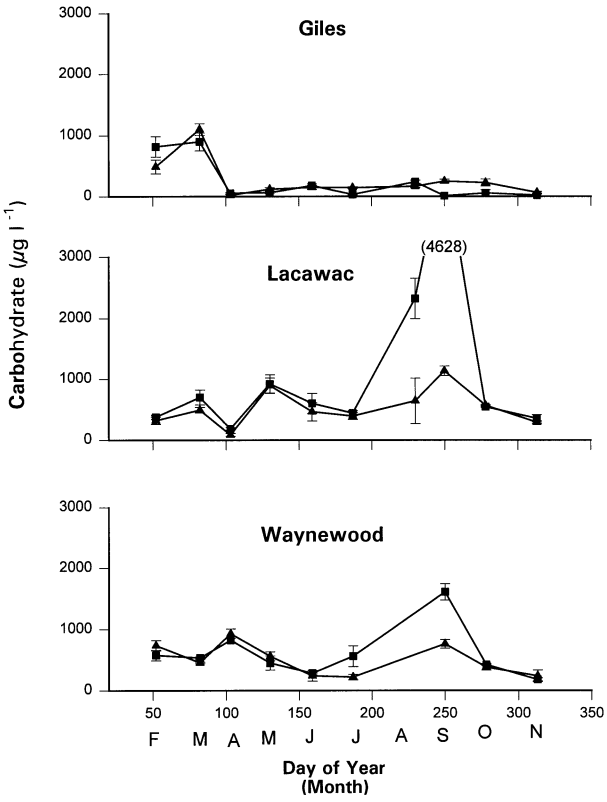

Fig. 6 Mean (± SE) concentration of particulate (1–53 μm) carbohydrate in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

from seston filters which had been stored in 2 : 1 v/v chloroform : methanol under a N_2 atmosphere (to prevent oxidation of FA) at –80 °C. Each filter was ground for 5 min in approximately 4 ml of 2 : 1 v/v chloroform : methanol with a tissue grinder (10-ml Potter-Elvehjem with Teflon pestle, Wheaton No.358039). During grinding, an internal standard of 2% w/v 3-hexadecanol (not produced by plants or animals) in 2 : 1 chloroform : methanol was added. Each ground suspension was then centrifuged (1000 *g*, 5 min), and the supernatant containing extracted lipid was transferred to a second test tube. A 20% v/v aliquot of 0.88% KCl was then added to each sample to effect an aqueous/organic phase separation. Each tube was then centrifuged for 2 min at 1000 *g*, and the lipid-containing chloroform layer was then transferred with a 22-cm (9 inch) Pasteur pipet to a 5 ml conical centrifuge tube for rapid drying in a vacuum evaporator (Savant Speed-vac). After drying, the lipid was resuspended in 100 μl 2 : 1 v/v chloroform : methanol, sealed in an amber 1-ml vial flushed with N_2, and stored at –80 °C until lipid class analysis.

Lipid classes were quantified using an Iatroscan Analyser (Mark III or IV) with methods described by Parrish (1987) and modified by Goulden & Place (1990). Wax esters, triglycerides, diglycerides, FA, sterols and polar lipids (phospholipids, galactolipids) were quantified in each lipid sample using standards of cetyl oleate, triolein, dipalmitin, oleic acid, cholesterol, digalactocyl diglyceride, and phosphatidyl choline, respectively.

Statistical analyses

Absolute concentrations of each elemental and biochemical fraction of particulate seston were divided by the total concentration of POM and multiplied by 100% to calculate that fraction's percentage composition. Percentage data were arcsine square root transformed prior to all statistical analyses (Sokal & Rohlf, 1981).

Concentrations and percentage composition values were both analysed by linear regression (SYSTAT for Windows, v. 2.0) and also by canonical correspondence

Table 3 Results of linear regression analyses of seston biochemistry as a function of either lake type (ordered as oligo-, meso- and eutrophic), water depth (ordered as epi- as metalimnion), collection day during 1995, or polar lipid concentration (see text for explanation). b = slope of the regression, P = significance level

Biochemical component	Lake type	Water depth	Day of year	Polar lipid concentration
Carbohydrate concentration	$b = 152$	$b = 79$	$b = -0.619$	$b = -0.189$
	$P = 0.024$	$P = 0.487$	$P = 0.356$	$P = 0.025$
Protein concentration	$b = 162$	$b = 39$	$b = 0.173$	$b = 0.179$
	$P = < 0.001$	$P = 0.641$	$P = 0.294$	$P = 0.004$
Protein (% of POM)	$b = -2.24$	$b = -1.345$	$b = -0.012$	$b = -0.003$
	$P = 0.066$	$P = 0.508$	$P = 0.310$	$P = 0.039$
Polar lipid concentration	$b = 436$	$b = 190$	$b = -6.31$	–
	$P < 0.001$	$P = 0.287$	$P < 0.001$	–
Polar lipid (% of total lipid)	$b = 2.898$	$b = 1.73$	$b = -3.54$	–
	$P = 0.060$	$P = 0.502$	$P < 0.001$	–
Triglyceride concentration	$b = 663$	$b = 376$	$b = 3.58$	$b = 1.070$
	$P = 0.002$	$P = 0.312$	$P = 0.101$	$P = < 0.001$
Triglyceride (% of lipid)	$b = -2.791$	$b = -1.965$	$b = 0.051$	–
	$P = 0.049$	$P = 0.407$	$P = < 0.001$	–

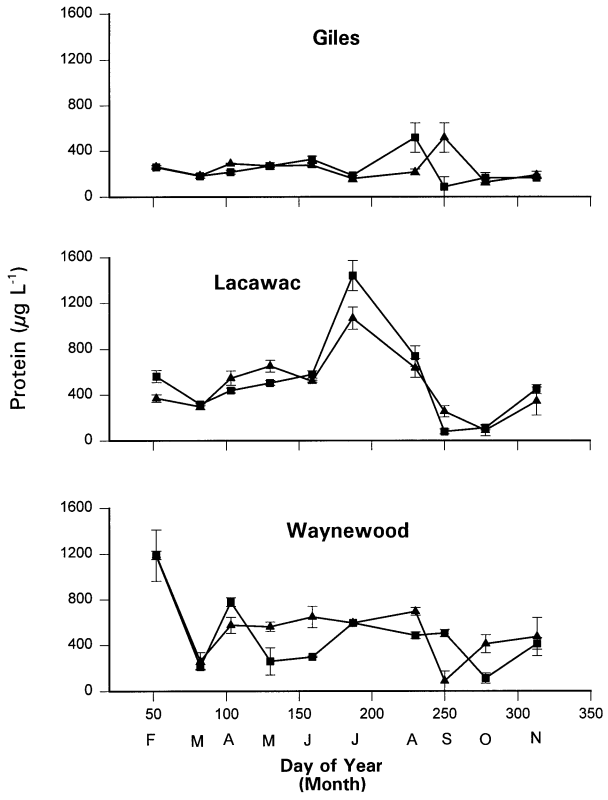

Fig. 7 Mean (± SE) concentration of particulate (1–53 μm) protein in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

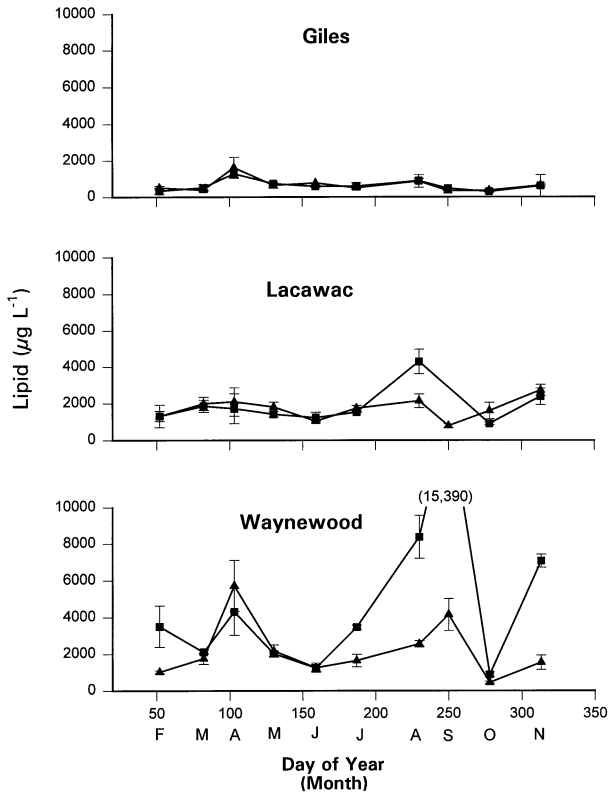

Fig. 8 Mean (± SE) concentration of particulate (1–53 μm) lipid in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

analysis (Canoco; ter Braak & Verdonschot, 1995; P characteristic with four independent variables. Lake type, depth and time of year comprised three of these predictors. For statistical analysis, lake type was ordered as Giles = 1, Lacawac = 2, Waynewood = 3). Because polar lipids compose biological membranes, this group was assumed to be the most conservative biochemical indicator of algal biomass in the seston;

Table 4 Polar lipid and triglyceride as a percentage of seston lipid of lake water in 1995. Values in parentheses are SE of means

	Day of year (month)									
	53 (Feb.)	83 (Mar.)	103 (Apr.)	130 (May)	159 (Jun.)	187 (Jul.)	230 (Aug.)	250 (Sep.)	278 (Oct.)	313 (Nov.)
Polar lipid (%)										
Giles Epi	30.13	47.73	29.95	23.55	9.77	29.66	11.36	26.85	26.63	13.59
	(4.09)	(2.74)	(1.69)	(2.30)	—	(0.81)	(1.03)	(2.96)	(1.59)	(0.65)
Giles Meta	51.85	32.29	30.22	26.16	46.70	33.26	10.54	36.77	24.52	14.57
	(11.85)	(1.45)	(1.81)	(3.32)	(2.23)	(6.85)	(0.91)	(2.43)	(2.30)	(0.32)
Lacawac Epi	41.33	24.79	51.26	33.54	29.11	14.06	29.00	19.69	25.36	9.56
	(4.19)	(2.07)	(7.54)	(2.15)	(0.62)	(0.37)	(1.16)	(2.56)	(3.77)	(0.34)
Lacawac Meta	37.99	30.48	30.88	38.24	30.74	14.34	23.93	—	30.70	12.51
	(5.55)	(1.06)	(6.49)	(3.83)	(3.78)	(1.30)	(0.16)	—	(3.06)	(2.20)
Waynewood Epi	65.60	27.99	32.24	34.50	42.61	32.29	32.07	19.11	3.56	34.28
	(3.93)	(2.51)	(5.27)	(1.35)	(2.09)	(2.69)	(1.35)	(0.48)	(5.05)	(3.88)
Waynewood Meta	85.94	17.87	64.47	24.86	57.89	32.28	29.03	17.45	27.49	15.29
	(4.80)	(0.49)	(2.21)	(1.21)	(1.57)	(0.53)	(1.10)	(0.38)	(1.90)	(3.10)
Triglyceride (%)										
Giles Epi	58.77	30.27	56.42	68.80	87.31	50.38	73.04	58.97	52.81	72.62
	(4.29)	(2.97)	(2.71)	(3.90)	—	(0.85)	(2.45)	(1.77)	(2.62)	(1.99)
Giles Meta	41.52	46.30	48.97	62.74	39.84	50.75	71.78	49.81	57.01	71.89
	(10.94)	(2.06)	(1.46)	(5.40)	(1.15)	(7.82)	(1.56)	(1.26)	(2.22)	(0.38)
Lacawac Epi	50.28	53.21	35.87	54.34	52.29	70.53	45.56	63.23	59.42	77.42
	(6.28)	(3.03)	(7.78)	(3.11)	(5.40)	(0.88)	(2.45)	(5.09)	(5.67)	(1.25)
Lacawac Meta	47.91	60.52	56.09	54.93	51.71	69.63	60.45	—	50.80	74.15
	(4.44)	(1.21)	(8.21)	(3.77)	(4.37)	(1.80)	(2.49)	—	(6.00)	(4.74)
Waynewood Epi	21.42	49.88	52.46	53.99	45.94	57.96	52.29	53.64	46.22	71.16
	(1.03)	(3.34)	(0.93)	(1.87)	(4.73)	(4.17)	(3.91)	(0.45)	(4.20)	(4.82)
Waynewood Meta	10.96	64.82	27.96	59.69	26.56	48.85	45.87	56.80	48.12	73.06
	(2.20)	(3.82)	(1.87)	(2.58)	(1.67)	(1.67)	(0.15)	(1.19)	(3.90)	(3.99)

therefore, its concentration was used as the fourth independent variable for comparison.

Results

Seasonal changes in the concentration of POM in the seston (< 53 μm diameter) of Lakes Giles, Lacawac and Waynewood are shown in Fig. 1. As expected, POM was less abundant in oligotrophic Lake Giles (< 2 mg l^{-1}) than in mesotrophic Lake Lacawac (1.6–7.4 mg l^{-1}) or eutrophic Lake Waynewood (1.2–17.5 mg l^{-1}). POM varied considerably in Lacawac and Waynewood with peaks occurring in spring (Waynewood) and late summer (Lacawac and Waynewood). During the late summer peak in POM concentration in Lacawac and Waynewood, POM in the metalimnion greatly exceeded that in the epilimnion, but at all other times, POM did not vary appreciably with depth.

Elemental concentrations of C, N and P in seston from Lakes Lacawac and Waynewood varied seasonally in the same manner as POM concentration, being greatest in early spring and autumn and lowest in summer (Table 1). The concentration of seston C ranged from 61 to 195 μM in Lake Lacawac and from 16 to 51 μM in Lake Waynewood. With the exception of a late summer peak in the metalimnion (16.5 μM), seston N concentration varied between 3 and 7 μM in Lacawac (Table 1). In Waynewood, N concentration ranged from 0.5 to 3.3 μM. Seston P concentration was generally greater in the metalimnion than epilimnion and was markedly higher during the late summer peak in POM; however, during most of the year P concentrations were < 0.2 μM (Table 1). Although Lake Waynewood is more eutrophic than Lake Lacawac, C and N concentrations were higher in Lacawac than Waynewood; whereas, P concentrations were greater in Waynewood.

The concentration of dissolved inorganic P in lake water from Lacawac and Waynewood was greatest at the start and end of the year (Fig. 2, Table 2); hence,

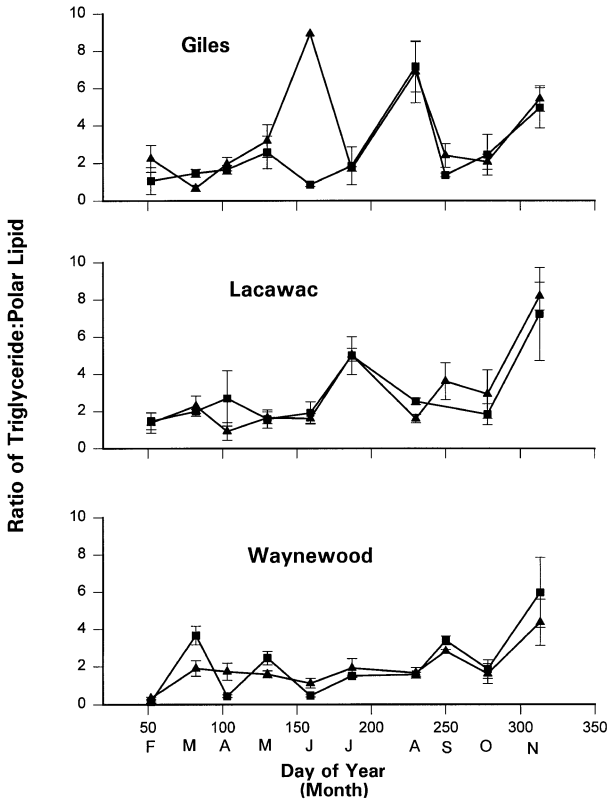

Fig. 9 Mean (± SE) ratio of triglyceride : polar lipid comprising seston (1–53 μm) lipids in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

dissolved P appeared inversely related to the concentration of seston P. Dissolved silica concentration was low (< 10 μM) at all times in Giles, during most of the spring and summer in Lacawac, and only briefly in the fall in Waynewood (see Table 4).

The stoichiometric ratios of C : N, N : P and C : P in the seston were calculated. Coincident with the observed increases in seston P in late summer and autumn (Table 1), corresponding decreases in the N : P and C : P ratios were found (Figs 3 and 4, respectively). In contrast, C : N ratios increased during the year, especially in Waynewood (Fig. 5). Ratios of C:P were high (> 300) throughout most of the year in Lake Lacawac, and low (< 300) during the entire year in Lake Waynewood. In contrast, C : N ratios were higher in Waynewood (mostly > 30) than Lacawac (mostly < 30).

Seston biochemistry was more variable during the year than the elemental composition of seston. Carbohydrate concentrations varied more than tenfold, but followed the same seasonal pattern as for POM concentrations (Fig. 6). Seston in Giles had an early peak (February–March) in carbohydrate (1.1 mg l^{-1}), but thereafter remained very low (< 0.3 mg l^{-1}). Lakes Lacawac and Giles had much higher carbohydrate concentrations (up to 4.6 mg l^{-1}) during late summer when POM concentrations were greatest. Because of this variability among lakes in seasonal trends, regression analysis did not indicate that there was a significant linear trend in carbohydrate concentration with time of year (Table 3).

In contrast to carbohydrate, seston protein concentrations (Fig. 7) exhibited a different seasonal pattern. In Giles, protein concentration showed a clear peak in late summer (0.5 mg l^{-1}); whereas, POM and carbohydrate (and lipid, see below) concentrations in Giles were invariable after early summer. Like POM and CHO (and lipid, see below) concentrations, protein concentrations in Lacawac seston peaked during the summer (1.4 mg l^{-1}); however, the peak in protein preceded (July) that for the other biochemical parameters (August). Protein concentrations in both the epilimnion and metalimnion of Lacawac were much more variable (twelve-and eighteenfold, respectively) during the year than either lipid or carbohydrate concentrations (which varied by four-to fivefold). Unlike Lacawac, in Waynewood there was no summer maximum for protein concentration; rather, protein was in greatest abundance in February (1.2 mg l^{-1}). As with carbohydrate, this variability among lakes in the seasonality of seston protein concentration precluded significant linear relationships ($P > 0.05$) over time (Table 3).

The seasonal pattern for seston lipid concentration (Fig. 8) was similar to that for POM. Lipids appeared to directly parallel POM concentrations with low concentrations (0.3–1.6 mg l^{-1}) in Giles, generally low concentrations (< 2.7 mg l^{-1}) with a late summer metalimnion peak (4.3 mg l^{-1}) in Lacawac, and highly variable (especially in the metalimnion) concentrations in Waynewood (1.2–15.4 mg l^{-1}). Analysis of the lipid class composition indicated that most of the lipid was either triglyceride or polar lipid (phospholipid and galactolipid) (Table 4). Although variable during the year, triglyceride comprised an increasing share of lipid, and polar lipid a decreasing share as the year progressed; that is, in all lakes, the proportion of lipid as triglyceride was lowest between February and April and highest in November (Table 4). The proportion of lipid as polar lipid peaked between February and April and was lowest in November. Both of these

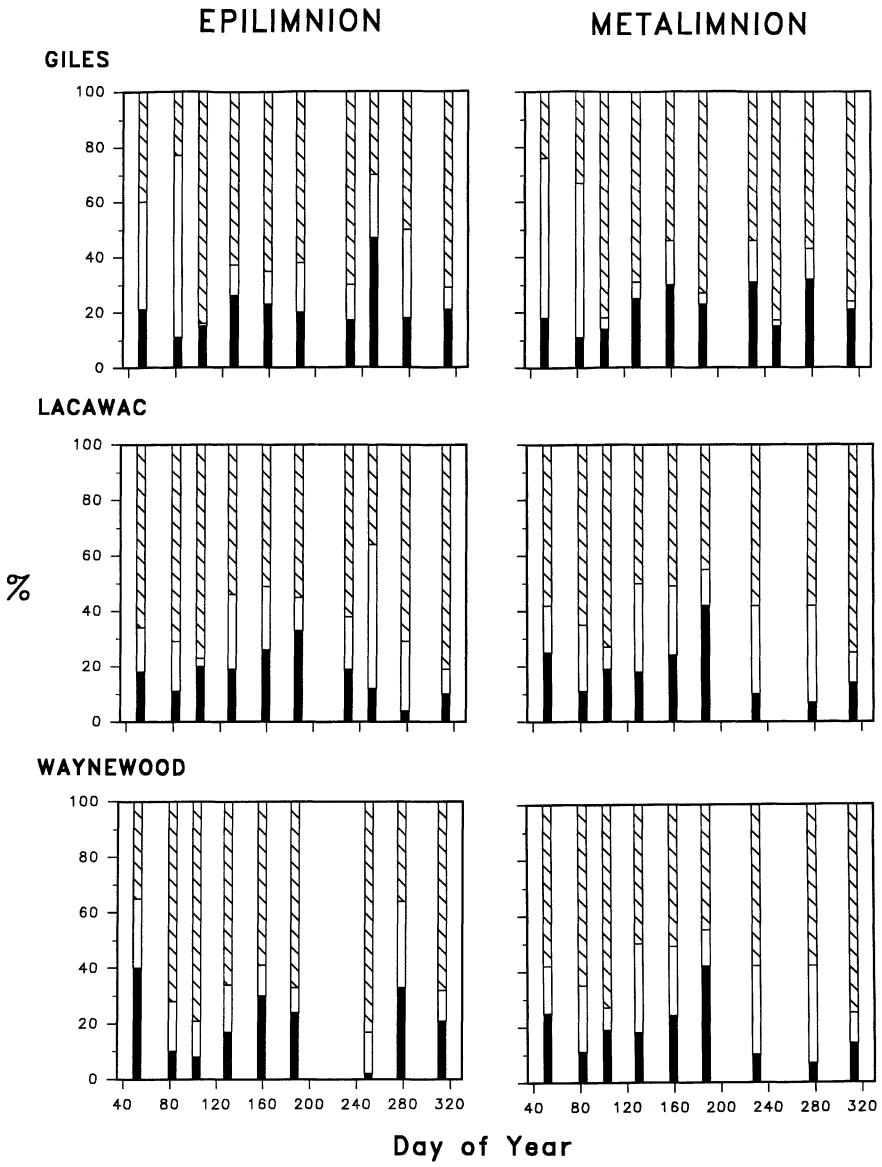

Fig. 10 Relative percentage composition of carbohydrate (□), protein (■) and lipid (▨) in seston (1–53 μm) collected from the epilimnion and metalimnion of Lakes Giles, Lacawac and Waynewood during 1995.

seasonal trends were highly significant ($P < 0.001$) as determined by linear regression analyses (Table 3). The absolute concentration of polar lipids also decreased significantly ($P < 0.001$). Triglyceride concentrations trended upward but were not significantly ($P > 0.05$) associated with increasing time of year, probably because they comprised the bulk of the lipid fraction, and overall lipid concentrations peaked in late summer (when POM concentration peaked) and then declined. The shift in lipid class composition is depicted by the increasing ratios of triglyceride : polar lipid (Fig. 9).

Nearly every parameter of seston biochemistry exhibited a significant ($P < 0.05$) relationship with lake type (the slopes of the regression equations for the concentrations of carbohydrate, protein, triglyceride and polar lipid were positive; Table 3). Hence, the absolute amount of each biochemical increased from oligotrophic to mesotrophic to eutrophic types, as expected. Interestingly, the relative balance of carbohydrate, protein and lipid in the seston (Fig. 10) differed among lakes as well. Statistical evidence for these differences (Table 3) was clouded by high seasonal variability (Fig. 10); however, some trends with lake type were noted, for example, although not statistically significant ($P = 0.066$), the proportion of POM as protein (hereafter 'percentage protein') decreased with lake type (Table 3). Lipid composition also differed in that the proportion of lipid comprised

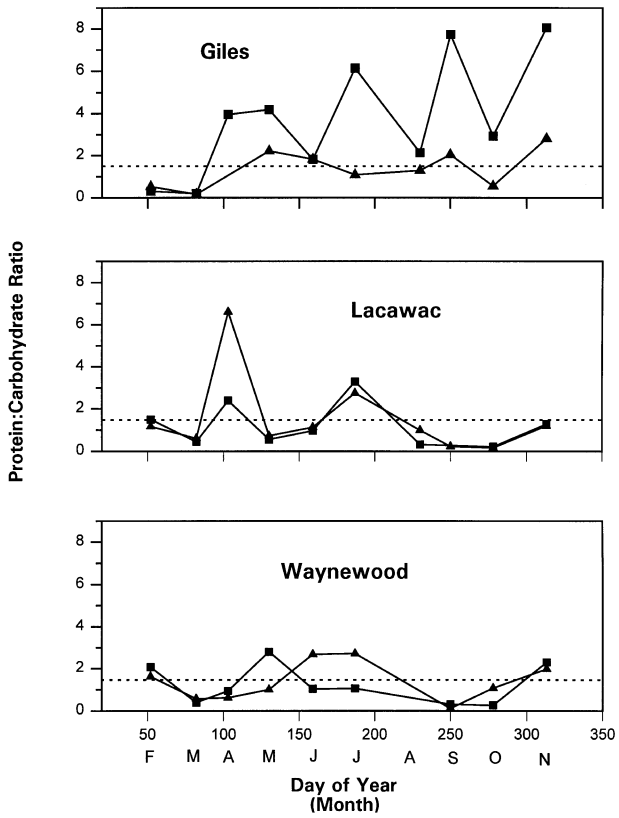

Fig. 11 Ratio of protein : carbohydrate comprising particulate (1–53 μm) seston in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

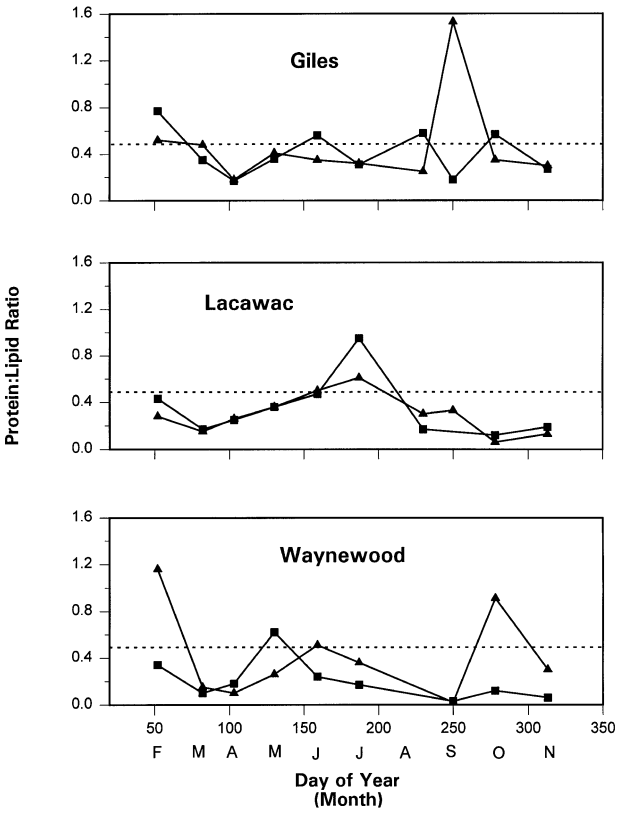

Fig. 12 Ratio of protein : lipid comprising particulate (1–53 μm) protein in the epilimnion (▲) and metalimnion (■) of Lakes Giles, Lacawac and Waynewood during 1995.

by triglyceride decreased and the percentage polar lipid increased with increasing lake trophic status (Table 3).

In general, lipid comprised a greater fraction of the seston (usually $> 50\%$) than protein or carbohydrate (Fig. 10). Percent protein values rarely exceeded 40% and were typically 15–25%. Percent carbohydrate values were usually less than 20%. The relative balance of the biochemical constituents varied considerably, however, during the year. In Lake Giles, for example, the lipid fraction of the seston was small and the carbohydrate fraction was large at the start of the year. Also, in Lakes Lacawac and Waynewood the percentage protein in the seston was exceptionally low ($< 10\%$) in September and October when the ratios of protein : carbohydrate and protein : lipid dropped to less than 0.3 and 0.1, respectively (Figs 11 and 12).

Cannonical correspondence analysis (CCA)

The four independent variables (lake, date, depth and polar lipid concentration) associated strongly into two main vector directions: date and depth, and lake type and polar lipids (Fig. 13). Polar lipids and lake type were the longest vectors and appeared to separate the sample data best. Date also strongly separated the data, but depth had a weak effect. Samples were clustered in an array within the relationships of these four independent variables. Samples were aligned with date (vertically), and with lake type (horizontally) across the axis, suggesting the discreetness of seston biomass in the three lakes and the seasonal changes in its composition. Epilimnion samples for all three lakes formed the left side of each envelope, indicating that the epilimnion seston had higher polar lipid values than the metalimnion. However, it is clear that the seston in all three lakes underwent similar kinds of transformations during the year. This change shows a strong positive relationship with triglyceride composition (LTG) in a vertical direction with the vector for date. At the same time, there was a negative shift of concentration and percent composition for most of the components with either set of vectors. The percentage polar lipid (i.e.% of total lipid)

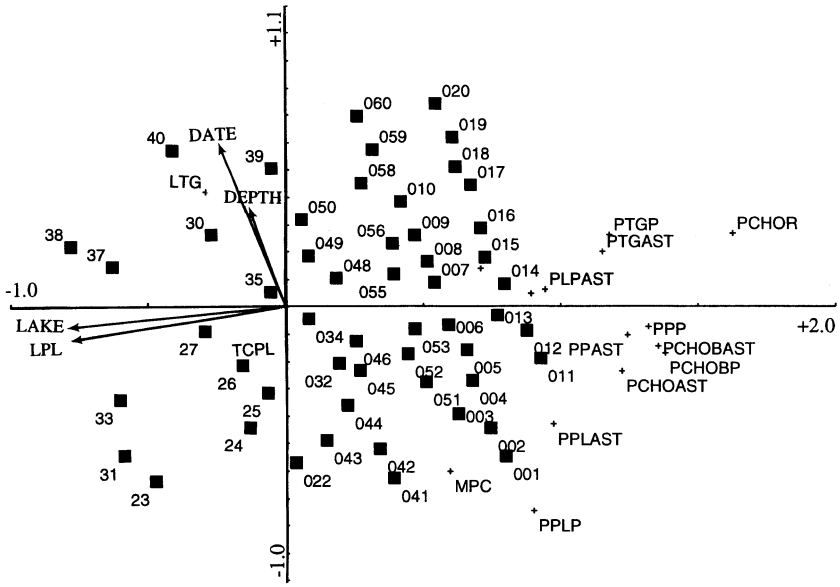

Fig. 13 Triplot of results of canonical correspondence analysis for 1995 lake seston data. Some data are not shown because of overlap in position. The following labels represent vectors: Lake, Polar Lipids (LPL), Date, Depth. Environmental variables are triglyceride concentration (LTG),% carbohydrate in seston (PCHOBP), arcsine square-root transformed percentage carbohydrate (PCHOBAST), ratio of triglyceride to polar lipid (TGPL), percentage polar lipids (PPLP), arcsine square-root transformed percentage polar lipids (PPLAST), percentage tryglycerides (PTGP), arcsine square-root transformed percentage triglycerides (PTGAST), carbohydrate concentration (MCHO), protein concentration (MPC), lipid concentration (MLC), total particulate organic matter concentration (TCPL),% carbohydrate (PCHOP), arcsine square-root transformed percentage carbohydrate (PCHOAST),% protein (PPP),% lipid (PLP), arcsine square-root transformed percentage lipid (PLPAST), ratio of protein : lipid (PLR), ratio of protein : carbohydrate (PCHOR). Sample numbers 001–020 were from Lake Giles, 021–039 from Lake Waynewood, and 040–059 from Lake Lacawac.

was more closely aligned with date and depth; whereas, the percentage protein and carbohydrate (i.e.% of total POM) lined up with lake and percentage polar lipid (% of total lipid) in a negative association. These results are supported by the finding from linear regression analysis (Table 3) that polar lipid concentration was negatively associated with percentage protein (Fig. 13).

Discussion

The data in the present study confirmed previous reports that Lakes Giles, Lacawac and Waynewood differ in trophic status (Moeller *et al.*, 1995). Concentrations of particulate organic material (POM; Fig. 1) were much greater (up to 17.5 mg l^{-1}) and more variable during the year (six- and twelvefold in epilimnion and metalimnion, respectively) in eutrophic Waynewood than in mesotrophic Lacawac (< 7.4 mg l^{-1}; two- to fivefold variability) or oligotrophic Giles (< 2 mg l^{-1}; two- to threefold variability). Peak POM concentrations occurred in the epilimnia in spring (April) and in the metalimnia in late summer (August–September).

Concentrations of the various biochemical constituents in seston (carbohydrate, protein, lipid) co-varied with overall POM concentration, as expected. The relative proportions of the different constituents also varied widely during the year, and not necessarily in relation to seston abundance. In particular, lipid class composition exhibited a marked shift as the year progressed: triglycerides increased and polar lipids (phospholipid, galactolipid) decreased (Fig. 9). Interestingly, as polar lipids decreased in late summer in both Lacawac and Waynewood, there was an apparent increase in P concentration, and this increase was greater than the coincident increase in POM. Therefore, C : P and N : P ratios of the seston appeared to decrease during the year (Figs 3 and 4). Both absolute concentrations and relative proportions of all elemental and biochemical constituents of the seston varied substantially, particularly between the spring and late-summer peaks in POM, suggesting that the algal species composition also differed markedly between these pulses of seston.

Productivity of algal populations in these lakes is

thought to be limited by both N and P, despite their different trophic status (Moeller *et al*., 1995). However, the increase in P in the seston of Lacawac and Waynewood in late summer and autumn (Fig. 6) with little corresponding increase in N, clearly indicates that N and P cannot be equally limiting throughout the year. Elemental ratios of N : P in Waynewood declined notably to levels indicative of strong N limitation. The source of this P increase remains unclear. Dissolved phosphate concentrations also increased in the autumn (Fig. 2), but only after particulate P increased. The P increase was greater in the metalimnion than in the epilimnion, and might have resulted from entrainment of P from the anaerobic hypolimnion. In contrast to P, N concentrations were relatively constant in the seston throughout the year (Table 1), except in the metalimnion of Lacawac, where an increase in seston N concentration occurred in September. As a result, algae might have been more N limited than co-limited by N and P, particularly in the metalimnion of Waynewood, where C : P ratios were low (< 300) and C : N ratios were high (20–60). This suggestion is buoyed by the finding that the percentage protein in the seston tended to decrease (linear regression, $P = 0.066$, Table 2) with increasing lake trophic status and was less than 10% in the metalimnion of Waynewood from September to November.

The low percentage protein values seen after August might have resulted from nutrient limitation of algae in the seston; for example, cells of the green alga, *Ankistrodesmus falcatus*, cultured under N-limited conditions (15% of maximum growth rate) in the laboratory have been shown to contain substantially lower protein ($< 20\%$ w/w POM) than cells cultured under nutrient-replete conditions (35% w/w POM) (Kilham *et al*., 1997a). In contrast, *A. falcatus* cells cultured under P-limited conditions had percentage protein values similar to cells reared on non-limiting media. Hence, if the biochemical responses to nutrient limitation in such laboratory-reared algae are assumed indicative of nutrient limitation of primary producers *in situ*, then we infer that lake seston could have been N-limited later in the year, particularly in Lakes Lacawac and Waynewood, where percentage protein values remained below 20% after August.

Results from this study's laboratory cultures of nutrient-limited green algae (Kilham *et al*., 1997a) are not useful in explaining the seasonal shifts in lipid class composition seen in lake seston. *A. falcatus* had a similar lipid composition when cultured under both nutrient-limited and nutrient-replete conditions; yet, in lake seston it was observed that significant seasonal shifts occurred in the relative abundance of the dominant classes (Table 1). Triglyceride increased and polar lipid decreased during the year. It remains unclear whether these seasonal shifts in lipid class composition result from intraspecific changes in cellular biochemistry resulting from nutrient limitation (as blooms senesce) or from shifts in the species composition of dominant primary producers (and perhaps non-producers).

Suspension-feeding consumers such as lake zooplankton that are reared in the laboratory are known to be negatively affected by suboptimal diet quality, such as when their algal diet is limited by N or P (Kilham *et al*., 1997b; and other contributions this special issue). It remains equivocal whether zooplankton can be limited qualitatively *in situ* by the elemental or biochemical composition of seston. Based on results from various laboratory studies, there are several qualitative factors of natural seston that could affect secondary production by zooplankton; for example, Sterner & Hessen (1994) suggested that seston elemental ratios were useful indicators of seston quality based on the finding that C : P ratios exceeding 300 negatively impact production of zooplankton such as *Daphnia*. Kilham *et al*. (1997b) found, however, that *D. pulicaria* fecundity and population growth rate were better correlated with the protein : lipid and protein : carbohydrate ratios of the diet rather than elemental ratios, and the effect of diet biochemistry on daphniid nutrition was found to be independent of the quantitative effect of ration size. Hence, they suggested that a knowledge of the balance of biochemical constituents can be more powerful in predicting zooplankton production than either the absolute concentration of a specific biochemical constituent or the overall elemental ratio of the diet.

Regardless of which element of food quality is used, results of this study clearly suggest that zooplankton populations in Lakes Giles, Lacawac and Waynewood are likely to be just as limited qualitatively as quantitatively. Both the elemental and biochemical composition of the seston in all three lakes appeared suboptimal for zooplankton during most of the year; for example, in Lake Lacawac elemental ratios of both C : P and C : N were high (usually greater than 300 and 20, respectively). Elemental ratios of C : N were particu-

larly high in Lake Waynewood (20–60). Hence, according to Sterner & Hessen (1994), zooplankton should have been significantly impacted by low P availability in Lacawac, but in Waynewood, N was more likely to have been the primary limiting nutrient, as the N : P ratio dropped below 1 late in the year.

With these elemental ratios, seston collected from Waynewood late in the season understandably contained virtually no protein. Indeed, protein : lipid and protein : carbohydrate ratios of this seston were < 0.15 and < 0.5, respectively, in September and October. Kilham *et al*. (1997b) reported reduced fecundity and population growth rate by *D. pulicaria* when laboratory-reared microalgae had protein : lipid ratios < 0.5 and protein : carbohydrate ratios < 1.7. With brief exceptions, both protein : carbohydrate and protein : lipid ratios of lake seston were well below these thresholds during most of the year in both the epilimnion and metalimnion of all three lakes (Figs 11 and 12). This study concludes that the population dynamics of suspension-feeding consumers with short life cycles, such as the freshwater cladocera, are likely to be directly impacted by these fundamental seasonal shifts in the elemental and biochemical composition of lake seston.

Acknowledgments

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