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Seston composition and the potential for *Daphnia* growth

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Abstract

A field survey was conducted to study the relationships amongst the composition of the seston, the nutritional value of the seston for herbivorous zooplankton (*Daphnia*), and selected water clarity parameters. Sixteen ponds in a wetland area and seven larger lakes, all located in south central Saskatchewan, Canada, were sampled for seston. The phytoplankton species were identified, and various biochemical seston variables were measured. A biotest using the zooplankter *Daphnia magna*, was employed to assess the nutritional value of the seston. The best seston variable to explain *Daphnia* growth was the phospholipid content (simple linear regression analysis: $R^2_{\text{adj}} = 0.50$). The water absorbance ratio A250/A365 was a good predictor of lipid content of the seston. Both the absorbance ratio A250/A365 and the dissolved organic carbon (DOC) concentration were negatively correlated with *Daphnia* growth. We hypothesize that the penetration of visible and ultraviolet radiation is an important determinant of seston quality, especially the phospholipid content, and that this has important implications for determining ultimate growth rates of herbivorous zooplankton.

Introduction

Zooplankton nutrition and, in particular, deciphering what controls food limitation for zooplankton is a topic with a rich history in aquatic research. However, despite an abundance of studies (especially laboratory studies), our understanding of what controls food quality limitation in freshwater and marine systems remains incomplete. Most studies on zooplankton have focused on mineral limitation, especially phosphorus (P) limitation (e.g. Hessen, 1992; Sterner, 1993; Sterner & Schulz, 1998), or on biochemical limitation, especially the content of polyunsaturated fatty acids (PUFA) (e.g. Ahlgren et al., 1990; Brett & Müller-Navarra, 1997; Müller-Navarra, 1995; Weers & Gulati, 1997). The importance of phosphorus content for *Daphnia* can be explained by the higher P-demand of *Daphnia* (Andersen & Hessen, 1991). The importance of fatty acids in the food of *Daphnia* can be explained by the fact that fatty acid synthesis is generally low in daphnids. Goulden and Place (1990), for example, demonstrated that most of the accumulated lipid in the diet of *Daphnia* was derived from the diet with only a small amount synthesized *de novo*.

Ahlgren and co-workers (1997) found that the polyunsaturated fatty acid (PUFA) content of lake seston was strongly correlated to the seston's P-content. They concluded that the controversy concerning biochemical versus mineral limitation of zooplankton may not be relevant, because high-quality phytoplankton apparently contain large amounts of phospholipids which, it is important to realize, contain the highest concentrations of ω3-PUFA (Ahlgren et al., 1997; Sargent et al., 1987). We agree with Gulati & DeMott (1997) who point out that much work needs to be done in order to delineate the specific conditions under which phosphorus limitation and/or fatty acid limitation will control growth and reproduction

in zooplankton (and not just in Daphnids). Conceptual models do exist; for example, the 'rule of thumb' proposed recently that zooplankton, feeding on sestonic with atomic C:P ratios >300, will be deficient in P (Sterner & Schulz, 1998). Under those conditions, elemental nutrient limitation and stoichiometry relationships will likely be important determinants of zooplankton growth rates.

Unlike the systems studied by Sterner and coworkers, the lakes and wetland ponds (sloughs) on the Canadian Prairies are typified by high elemental N and P concentrations (Bierhuizen & Prepas, 1985; Campbell & Prepas, 1986; Barica, 1987; Robarts et al., 1995). The lakes and ponds in our study are located in fertile soils developed on glacial deposits. High nutrient concentrations in prairie wetlands can further be explained by a negative water balance with respect to the atmosphere, decomposition of macrophyte vegetation, and occasional resuspension of sediments (LaBaugh, 1989; Winter, 1989). In our study ponds, for example, atomic seston C:P ratios were <100, C:N ratios were <10, total dissolved phosphorus concentrations were well above 0.100 mg l^{-1}, and total dissolved nitrogen concentrations were >1.2 mg l^{-1}. An extensive temporal and spatial study in this area showed that nutrients were sufficient in all ponds for the majority of the ice-free season (Waiser, NWRI, Saskatoon, pers. comm.). In these systems, therefore, seston quality is not likely to be solely determined by phosphorus or nitrogen limitation. Instead, other factors, such as the concentration of essential fatty acids, are likely to be the most important determinants of zooplankton growth potential. It has further been suggested that the influence of light on algal lipid synthesis, for example, is likely to be far larger than variations in nutrient supply (Wainman et al., 1999). Therefore, since phytoplankton in our survey ponds and lakes were nutrient-sufficient, light climate was likely playing an important role in determining seston quality; and in particular lipid production and fatty acid composition. Thus, these systems provide a good field test of the hypothesis that food quality, especially as determined by the fatty acid composition, can under certain conditions (i.e. nutrient replete conditions), play a major role in determining zooplankton growth rates.

The light climate in aquatic systems is affected by physical and chemical factors, such as the concentration of dissolved organic carbon (DOC), chlorophyll, particles, and humic substances (Kirk, 1994a). The DOC concentration, in particular, is important in the attenuation of UV-B radiation (280–320 nm) (Scully & Lean, 1994). However, solar UV radiation can also cause a decrease in DOC, and shift molecular size downwards indicating photodegradation of humic substances (e.g. Allard et al., 1994; De Haan, 1993; Morris & Hargreaves, 1997). These two phenomena, coupled with recent declines in stratospheric ozone concentration and the concomitant threat of increased UV-B radiation (280–320 nm), have stimulated renewed research into the effects of UV radiation on aquatic organisms and ecosystems (Williamson & Zagarese, 1994).

UV radiation can have negative effects on phytoplankton, typically resulting in a decrease of photosynthetic rate (e.g. Smith et al., 1992). Both laboratory and field studies have demonstrated that the allocation of photosynthates into lipid, protein and carbohydrates is affected by UV radiation. The results are typically species-specific, therefore it is not possible to identify one aspect of photosynthate allocation as consistently diagnostic of UV stress (Arts & Rai, 1997; Smith et al., 1998). Other effects of UV-B on phytoplankton include changes in the morphology and biochemical composition (e.g. Hessen et al., 1997). These types of morphological and biochemical changes might also affect herbivorous zooplankton, as shown in laboratory studies with *Daphnia* (De Lange & Van Donk, 1997).

In their recent paper Sterner and Schulz (1998) call for 'more correlative studies between measures of food quality and the abundance, growth or community structure of zooplankton in the field'. Hence, our procedure was to use a common laboratory strain of the herbivorous zooplankter *Daphnia magna*, as a biotest organism to assess the nutritional quality of natural seston obtained from sixteen ponds in a wetland area and seven larger lakes in south central Saskatchewan, Canada. *D. magna* is considered to be a non-selective herbivorous filter-feeder, feeding mainly on particles smaller than 40 μm (Lampert, 1987).Therefore, this biotest is a useful tool to compare differences in lake seston quality/quantity relationships for herbivorous zooplankton (cf. Müller-Navarra, 1995; Vijverberg, 1976).

Our objective was to correlate biochemical measures of the seston with *Daphnia* growth rate to test the hypothesis that parameters related to lipids would best explain growth rate in nutrient replete systems such as prairie lakes and ponds. Further, in a preliminary way, we attempt to assess the effect of light climate on seston biochemistry by examining correlations amongst seston biochemical parameters, absorbance and dif-

fuse attenuation coefficients for UV-B (280–320 nm), UV-A (320–400 nm) and photosynthetically active radiation (PAR = 400–700 nm), and growth rates of *Daphnia magna*.

Materials and methods

Sample procedure

In a period of 8 weeks from May to July 1997, 16 ponds and 7 lakes were sampled in south central Saskatchewan, Canada. The ponds were all located in St. Denis National Wildlife Area, a wetland area of 361 ha, located ca. 40 km east of Saskatoon, Saskatchewan, Canada. There are numerous ponds in this area, ranging in size, water chemistry and UV penetration (Arts et al., 2000). Two ponds (pond 15 and pond 4857) were sampled 3 times during the 8 week period, the other ponds and lakes were sampled once. In general, the ponds were small, shallow, and without fish. The lakes were large, deep, and with fish.

Water samples (10 l) were collected from the surface, and taken back to the laboratory. The samples were filtered through a 40-μm mesh to remove zooplankton and to retain the edible fraction for *Daphnia*. Various biochemical seston variables were measured (see below), and *Daphnia* biotests were performed with the samples.

Daphnia magna *biotest*

Daphnia magna was obtained from Aquatic Research Organisms, New Hampshire (USA). This culture was originally from the Environmental Protection Agency, Duluth, Minnesota (USA). *Daphnia magna* was cultured in artificial ADAM medium (Klüttgen et al., 1994), in 1-litre jars at a density of 15 to 20 individuals l^{-1}. Feeding was daily with *Scenedesmus acutus* f. *alterans* (Chlorophyceae, strain T10 from University of Texas Culture Collection) at a concentration of 1 to 2 mg C l^{-1}. The daphnids were cultured at 20 °C, at a light-dark cycle of 12:12 h, and a PAR intensity of 25 μmol m^{-2} s^{-1}. Juveniles were collected every day, and cultured in cohorts under the same conditions. Once a week, all animals were transferred into clean jars and fresh medium.

Scenedesmus was cultured in WC medium (Guillard & Lorenzen, 1972), in 2 l chemostats, with a dilution rate of 0.4 d^{-1}. *Scenedesmus* was present mostly as single cells. PAR intensity was 120 μmol m^{-2} s^{-1} at the vessel surface, with a light:dark cycle of 16:8 h.

For each sample site a laboratory biotest was performed with *Daphnia magna* feeding on pond water (containing natural seston at ambient concentrations). The biotest started with 3-d old juveniles from one cohort. Immediately prior to each experiment, 15 juveniles from the cohort were dried at 60 °C for at least 4 h, and weighed using a Cahn C-30 microbalance (accuracy 1 μg), to obtain a reliable starting weight. Twenty juveniles were placed in 1 litre of pond water. Pond water was refreshed daily and, simultaneously, 3 individuals were removed, dried at 60 °C, and weighed. Each biotest lasted 5 d. Pond water was stored cool and dark. As a control with each biotest, 20 juveniles were placed in ADAM medium with *S. acutus* at a concentration of 1 mg C l^{-1} and processed in the same way as with the natural pond water biotests.

Growth rate per day as dry weight increase was calculated by plotting the individual weights on natural log scale versus day and fitting a linear line. The slope of that line was used as a measure of daily dry weight increase. This growth rate for each pond was also compared with the growth rate in the *Scenedesmus* control, resulting in a relative growth rate. This was done to correct for possible differences between the biotest experiments, because the ponds and lakes were sampled over an 8 w period, and the total of 27 sites were tested in 12 biotest experiments.

Water analyses

Water samples for DOC analysis were filtered through a 0.2 μm cellulose acetate filter (Sartorius). Samples were acidified with 0.4 ml concentrated phosphoric acid (85–87% W/W) and stored until analysis. DOC concentration was measured using a Shimadzu (Model TOC-5050AXX) carbon analyser connected to a Shimadzu autosampler (Model ASI-5000A) following combustion of the aqueous sample and detection of the CO_2 gas in a non-dispersive infrared gas analyser.

Absorbance was measured on filtered water samples (Whatman GF/C) using a Sargent-Welch Pye Unicam 8-400 UV/VIS spectrophotometer at 250 nm, 365 nm and 390 nm. The absorbance at 250 nm (A250) is usually strongly correlated with the DOC concentration (De Haan & De Boer, 1987). The ratio of absorbance at 250 nm and absorbance at 365 nm (A250/A365) gives an indication of the size of the humic acid molecules. A high ratio (around 9) indicates the presence of small molecules, a low ratio (around 5) indicates the presence of large molecules (De Haan & De Boer, 1987). The ratio can also be regarded as

a proxy for UV attenuation, a higher ratio means that shorter wavelengths are attenuated more rapidly than longer wavelengths. pH was measured with a Fisher Scientific pH meter (model: Accumet 915).

Seston analyses

Duplicate samples for protein, carbohydrate, lipid, and chlorophyll-*a* analyses, and single samples for fatty acid and pigment analyses, were filtered on precombusted GF/C filters (Whatman). The pigment and chlorophyll samples were encased in aluminium foil to protect them from the light. All filters were stored at $-70\,^{\circ}$C until analysis. Samples (100 ml dark bottles) for phytoplankton species analysis were preserved with 1 ml Lugol's solution.

The size spectra (biovolume) of the seston was determined with an electronic particle counter (Coulter Multisizer II, aperture 100 μm) in the following equivalent spherical diameter (ESD) size fractions: 2–3 μm, 3–10 μm, 10–20 μm, 20–30 μm, and 30–40 μm. Phytoplankton species composition and abundance was determined using an inverted microscope (Reichert, Germany), with 500$\times$ magnification. Chlorophyll-*a* was determined by boiling the filter in ethanol. Chlorophyll-*a* fluorescence was then measured on a Turner fluorometer (Model 10-005R) without correction for phaeopigments.

Particulate carbohydrate was measured by a phenol-sulphuric acid method (Dubois et al., 1956) as described in Pick (1987). The procedure was standardized with reagent grade glucose (Sigma G5767). Total proteins were analysed using a protein assay kit (Sigma, P5656), with an extra extraction with 1 ml 0.5 N NaOH. Absorbance was measured at 750 nm. The procedure was standardized with bovine serum albumin.

Lipids were extracted overnight with 3 ml chloroform:methanol (2:1 v/v). An aliquot of this lipid extract was used for determination of lipid class composition by thin-layer chromatography coupled with flame ionization detection (TLC-FID) using an Iatroscan MK-IV detector (Iatron Labs., Tokyo), as described in Arts et al. (1992). Fatty acids were extracted with 4 ml chloroform:methanol (2:1 v/v). Heneicosaenoic acid (C21:0) was added as internal standard. The chloroform contained 0.003% butylated hydroxytoluene as an antioxidant. The extract was vortexed and centrifuged (10 min, 2500 rpm). This procedure was repeated twice, and the supernatants were combined and washed with demineralized water with 0.88% NaCl (Folch et al., 1957). Lipid esters were transmethylated at 80 °C for 4 h with 1 ml of 3% H_2SO_4 in dry methanol, and extracted with hexane. The fatty acids were analysed on a Hewlett Packard 5890 gas chromatograph, with a very polar 50 m silica column (OSGE BPX-70). Peaks were identified using retention times of known standards, and calculating equivalent chain length (ECL) values. The concentration of each fatty acid was calculated relative to the internal standard, and expressed as μg l^{-1}.

Pigments were extracted with acetone:methanol:water (80:15:5 v/v), and the pigment composition was analysed with reversed-phase HPLC techniques, as described in Leavitt et al. (1989). Particulate organic matter (POM) was calculated as the sum of lipids, carbohydrates, and proteins. Kreeger et al. (1997) found a good relation between the actual measurement of particulate matter, and the sum of lipids, carbohydrates and proteins. An estimate of energy value of the seston was made with the following equation (Lemcke & Lampert, 1975):

$$\text{energy content (J l}^{-1}) = 17.50 * \text{carbohydrates (mg l}^{-1}) + 25 * \text{proteins (mg l}^{-1}) + 39 * \text{lipids (mg l}^{-1}).$$

Statistical analyses

Multivariate analysis of the phytoplankton species data was performed with CANOCO (Ter Braak, 1988, 1990), using direct ordination (CCA = canonical correspondence analysis). The data were ln transformed, and rare species were downweighted. Growth rates from the *Daphnia* biotest were compared with the physical and (bio)chemical variables of the pond water, using correlation and (multiple) regression (Genstat, 1993). Correlations were calculated with the total seston variables (sum of lipid classes, sum of pigment classes, total number of phytoplankton, total biovolume, protein, carbohydrate, chlorophyll-*a*). Significant product-moment correlation coefficients were determined using sequential Bonferroni techniques, to reduce the type I error (Rice, 1989).

Linear regressions were calculated with the individual classes of each seston variable (lipid classes, phytoplankton taxa, biovolume classes, FA classes, pigment classes, chlorophyll *a*, total carbohydrate, total protein, energy content, POM). Ln-transformed seston variables were the explaining variable and *Daphnia* growth rate the response variable (equation: $y = a + b * \ln(x)$). This proved to be the best relation

to describe the data, and it implies that at increasing food concentration, the growth rate levels to a plateau, which is likely to be the physiological maximum growth rate. Multiple linear regression models were selected with an iterative procedure in Genstat, selecting the best ln-transformed seston variables to explain *Daphnia* growth (Genstat, 1993). In this procedure all possible models are calculated. The best models are selected on criteria of minimal number of predictor variables, and minimal residual mean sum of squares ($MS_{residual}$) (Montgomery & Peck, 1982).

Results

Daphnia *biotest results*

Table 1 gives an overview of the sample sites and the *Daphnia* growth rates obtained from the laboratory biotests. The average growth rate in the *Scenedesmus* control was 0.44 d^{-1} ($n = 12$, SE=0.01). Further analysis was done with the actual growth rate for each sample. The chlorophyll-*a* concentration and dominating phytoplankton taxon for each sample site illustrate the diversity in the sampled systems (Table 1).

Multivariate analysis

Direct ordination (CCA) with CANOCO showed that absorbance at 250 nm and the ratio A250/A365 were significant variables in the ordination of the different sampling points based on their phytoplankton species composition (Figure 1). The variance explained by the CANOCO analysis was 27.8%. The lakes were grouped together in the upper right corner of the graph, with the ratio A250/A365 as ordinating variable. In the upper left corner the three samples of pond 15 were grouped separate from the other ponds, with the absorbance at 250 nm as ordinating variable.

Correlation

Product-moment correlations were calculated for the physicochemical variables, total seston variables and *Daphnia* growth (Table 2). DOC concentration and absorbance at 250 nm were correlated ($r = 0.84$). The ratio A250/A365 was negatively correlated with total lipid concentration ($r = -0.73$), and with *Daphnia* growth ($r = -0.64$). Of the seston variables, total lipids ($r = 0.64$), protein concentration ($r = 0.62$) and chlorophyll-*a* concentration ($r = 0.62$) produced a good correlation with *Daphnia* growth.

Simple regression

The regression between absorbance at 250 nm and DOC gave the following relation: DOC (mg l^{-1}) $= 12.88 + 22.21 * A250$ (cm^{-1}). This relation explained 61% of the variation in DOC concentration. Table 3 gives the significant regressions of (ln-transformed) biochemical seston variables versus *Daphnia* growth. Of the total seston variables, total biovolume ($R^2_{adj} = 0.43$), particulate organic matter ($R^2_{adj} = 0.40$), total lipids ($R^2_{adj} = 0.39$) and energy content ($R^2_{adj} = 0.39$) gave good explanations for *Daphnia* growth.

Analysing the detailed individual classes (e.g. biovolume size fractions, phytoplankton taxa, lipid classes, etc.), the concentration of phospholipids emerged as the best predictor for *Daphnia* growth ($R^2_{adj} = 0.50$) (Figure 2). Other variables that gave a reasonable prediction of *Daphnia* growth were Bacillariophyceae, sterol content, and sum of polyunsaturated fatty acids. The relative abundance of Cyanobacteria had a negative relation with *Daphnia* growth, the relative abundance of Bacillariophyceae had a positive relation with *Daphnia* growth (Table 3).

Regressions with the physicochemical variables versus *Daphnia* growth were calculated. There was a significant negative relation between the ratio A250/A365 and *Daphnia* growth (growth = $0.47 - 0.03 * \text{ratio}$; $R^2_{adj} = 0.38$). Also between DOC concentration and *Daphnia* growth a significant negative relation was observed (growth $= 0.42 - 0.005 * \text{DOC}$; $R^2_{adj} = 0.11$).

Multiple regression

Table 4 gives the results of the multiple regression with the total dataset with detailed individual classes of biochemical variables versus *Daphnia* growth. Three predictors are sufficient to obtain a significant regression. The concentration of acetone mobile polar lipids and phospholipids (both polar lipids) and number of Bacillariophyceae result in a R^2_{adj} of 0.63. Phospholipids and proteins were interchangeable in this 3-predictor model.

Discussion

From the simple regressions predicting *Daphnia* growth, the concentration of phospholipids emerged

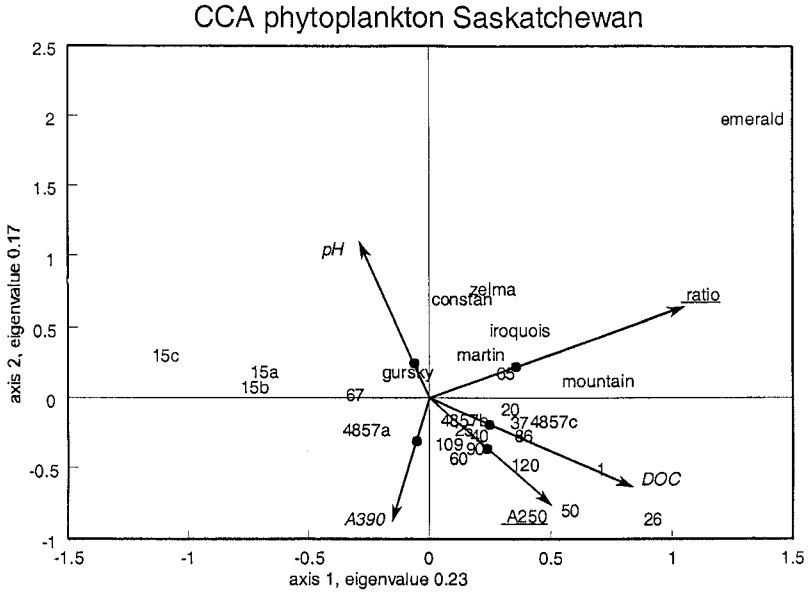

Figure 1. CCA plot of the sample points. Arrows in the plot refer to the direction of ordination by the physical variable. The distance of the large dot from the origin indicates the strength of the physical variable. Underlined variables are significant, variables in italics are not significant. Variance explained = 27.8%.

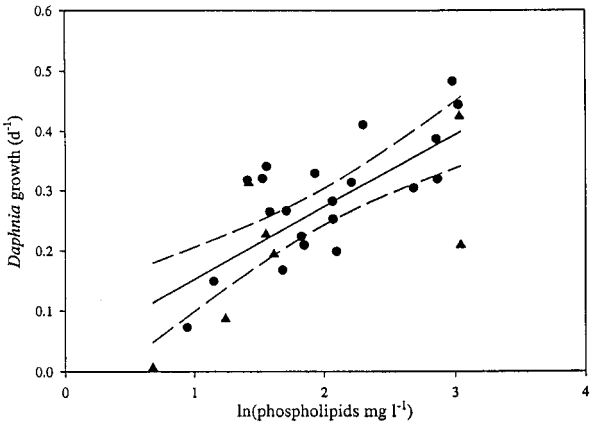

Figure 2. Linear regression of ln phospholipid concentration versus *Daphnia* growth. Dashed lines indicate 95% C.I. Symbols used are circles for ponds and triangles for lakes.

as the best predictor, explaining 50% of the variation in *Daphnia* growth. Phospholipids are primarily structural lipids within cell membranes, and, of all the lipid classes, are the best source of eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) (Ahlgren et al., 1998; Sargent et al., 1987). *Daphnia* growth was further explained both by variables more related to food quality (diatom abundance, energy content, total lipids, sterol esters, PUFA), and variables that are primarily a measure of food quantity (total biovolume, particulate organic matter). The phytoplankton composition plays a role as well, the relative abundance of Cyanobacteria had a negative, and the relative abundance of Bacillariophyceae had a positive relation with *Daphnia* growth. We tested other relative measures as potential food quality index (like protein:carbohydrates, lipids:carbohydrates, phospholipids:total lipids), but none of these had a significant relation with *Daphnia* growth.

The significant negative relation of the ratio A250/A365 versus *Daphnia* growth is most likely due to the negative relationship between this ratio and seston lipid content, and the positive relationship between seston lipid content and *Daphnia* growth. The DOC concentration was negatively related to sestonic lipid content and *Daphnia* growth, but with larger variation.

In the multiple regression results, protein and acetone-mobile polar lipids also emerge as important predicting variables. Food protein content is a better measure for food quality than N-content, because N cannot be used by daphnids in elemental or inorganic form, and must be metabolized as proteins and amino acids (Brett, 1993).

Unfortunately, we don't have stoichiometric analysis for all study sites. For the 7 pond samples where we measured elemental nutrient concentrations, neither the C:N or the C:P ratio showed a relation with the *Daphnia* growth, suggesting that there was no P

Table 1. Sample sites, *Daphnia* growth rate, chlorophyll-*a* concentration (μg l^{-1}), and dominant phytoplankton taxon (based on cell counts); mv indicates missing value

Site		*Daphnia* growth rate		Chlorophyll-*a*	Dominant phyto-plankton taxa
		d^{-1}	% of control	μg l^{-1}	
St. Denis	Pond 1	0.07	17	1.1	Cyanobacteria
	Pond 15	0.32	65	16.0	Chrysophyceae
	Pond 15	0.39	86	58.8	Bacillariophyceae
	Pond 15	0.48	113	26.3	Bacillariophyceae
	Pond 20	0.25	59	2.2	Bacillariophyceae
	Pond 25	0.27	49	1.5	Cyanobacteria
	Pond 26	0.15	33	1.7	Cyanobacteria
	Pond 37	0.31	72	3.5	Cyanobacteria
	Pond 40	0.21	48	1.0	Cyanobacteria
	Pond 50	0.20	50	2.3	Cyanobacteria
	Pond 60	0.32	69	1.3	Chlorophyceae
	Pond 65	0.33	60	3.0	Cyanobacteria
	Pond 67	0.41	75	5.8	Cryptophyceae
	Pond 86	0.30	76	0.8	Chlorophyceae
	Pond 90	0.34	85	3.3	Cryptophyceae
	Pond 109	0.28	61	2.2	Cyanobacteria
	Pond 120	0.26	57	1.0	Cyanobacteria
	Pond 4857	0.32	75	7.0	Chlorophyceae
	Pond 4857	0.22	56	2.7	Cyanobacteria
	Pond 4857	0.17	39	1.4	Cryptophyceae
Lakes	Constance	0.01	1	3.3	Chrysophyceae
	Emerald	0.23	57	2.6	Chrysophyceae
	Gursky	0.21	54	32.3	Chlorophyceae
	Iroquois	0.09	18	4.0	Cyanobacteria
	Last Mountain	0.19	48	0.6	Cryptophyceae
	Martin	0.31	78	4.4	Chrysophyceae
	Zelma	0.43	88	mv	Chrysophyceae

or N limitation (Figure 3). Further, as mentioned previously, it is well established that prairie lakes and ponds are very nutrient rich (Bierhuizen & Prepas, 1985; Campbell & Prepas, 1986; Barica, 1987; Robarts et al., 1995; Waiser, NWRI, Saskatoon, pers. comm.), so there is good reason to believe that our study systems were nutrient replete.

The scatter in the regressions can be explained by the fact that these are field data. Outliers (see Figure 2) can generally be explained by the phytoplankton species. For example, Lake Constance seston supported only a very low *Daphnia* growth rate (0.01 d^{-1}). The phytoplankton density was low (1500 cells ml^{-1}), but was comparable to the other lakes. However, species such as *Aphanotece* sp. and *Anabaena* sp. (cyanobacteria) and *Kephyrion* sp. and *Syncrypta* sp. (chrysophytes) that are hard to ingest or digest predominated. This may explain the much lower growth rate than would be expected on the basis of biochemical composition alone.

The evidence that EPA (C20:5ω3) limits *Daphnia* growth (Müller-Navarra, 1995) was not directly confirmed by this study. However, the strong link between *Daphnia* growth and the PUFA-rich phospholipids is supportive. In another study by Rothhaupt et al. (1998), the fatty acid linolenic acid (C18:3ω3) was found to be the best predictor for *Daphnia* growth in Lake Constance (Germany) over one season. Those two studies followed one system during one season, and each identified a different fatty acid as limiting. This indicates that it is difficult to extrapolate from one system to another. Our study described 23 different systems, and we found phospholipids as the best predictor for *Daphnia* growth.

Table 2. Correlation matrix of physical variables, seston variables (In transformed) and *Daphnia* growth, bold figures indicate significant correlation coefficient at $\alpha = 0.05$ after sequential Bonferroni technique ($n = 27$, threshold $r = 0.626$); ratio is A250/A365, Chl-a is chlorophyll *a*, CHO is carbohydrates, Prot is protein, Lip is lipids, Biov is seston biovolume, FA is fatty acids, Phyto is total phytoplankton abundance, Pigm is pigments

pH	−0.15														
A250	−0.22	**−0.72**	–												
A365	0.05	**−0.83**	**0.90**	–											
A390	0.11	**−0.81**	**0.84**	**0.95**	–										
ratio	**−0.64**	0.49	−0.13	−0.25	−0.25	–									
DOC	−0.36	−0.32	**0.84**	0.62	0.58	0.39	–								
Chl-a	0.62	0.26	−0.48	−0.30	−0.28	−0.52	−0.42	–							
CHO	0.47	0.11	−0.43	−0.20	−0.12	−0.46	−0.41	**0.82**	–						
Prot	0.62	−0.22	−0.12	0.12	0.26	−0.53	−0.26	**0.65**	**0.66**	–					
Lip	**0.64**	−0.28	−0.15	0.16	0.15	**−0.73**	−0.34	0.47	0.50	0.47	–				
Biov	0.49	−0.13	0.04	0.25	0.30	−0.57	−0.01	**0.78**	**0.67**	0.47	0.60	–			
FA	0.40	−0.09	−0.33	−0.24	−0.19	−0.17	−0.39	**0.72**	0.52	0.38	0.27	0.25	–		
Phyto	0.45	−0.12	0.07	0.28	0.25	−0.52	−0.06	0.62	0.44	0.21	**0.64**	**0.80**	0.11	–	
Pigm	0.31	0.23	−0.26	−0.20	−0.03	−0.15	−0.00	**0.85**	**0.79**	0.59	0.19	0.58	0.55	0.20	
	Growth	pH	A250	A365	A390	ratio	DOC	Chl-a	CHO	Prot	Lip	Biov	FA	Phyto	

Table 3. Significant ($p<0.05$) simple regressions of biochemical variables versus *Daphnia* growth (g, d^{-1}), as $g = a + b\ln(x)$; equation for the phytoplankton ratios was $g = a + b(x)$. PUFA is polyunsaturated fatty acids, POM is particulate organic matter. Units are mg l^{-1} for all variables, except phytoplankton (cells l^{-1}), seston biovolume ($\mu m^3\ l^{-1}$), energy content (J l^{-1}), chlorophyll *a* ($\mu g\ l^{-1}$)

Class	*x*	*a*	*b*	R^2_{adj}
Lipid classes	phospholipid	0.03	0.12	0.50
	sterol	0.47	0.13	0.39
	acetone mobile polar lipid	0.20	0.04	0.24
	hydrocarbon	0.23	0.09	0.11
	total lipids	0.01	0.09	0.39
Phytoplankton taxa	Bacillariophyceae	−0.02	0.05	0.40
	total phytoplankton	−0.29	0.06	0.17
	Cyanophyceae:total	0.34	−0.18	0.14
	Bacillariophyceae:total	0.25	0.33	0.10
Fatty acids	PUFA	0.39	0.04	0.30
	EPA (20:5ω3)	0.46	0.04	0.22
	total ω3-FA	0.39	0.03	0.20
Pigment classes	fucoxanthin	0.34	0.09	0.27
biovolume	3–10 μm ESD	−1.56	0.09	0.21
	10–20 μm ESD	−1.09	0.07	0.19
	20–30 μm ESD	−0.80	0.06	0.14
	30–40 μm ESD	−0.84	0.06	0.19
	total biovolume	−0.24	0.13	0.43
Totals	POM	−0.03	0.10	0.40
	energy content	−0.37	0.09	0.39
	chlorophyll *a*	0.22	0.05	0.36
	total proteins	0.38	0.07	0.36
	total carbohydrates	0.22	0.06	0.19

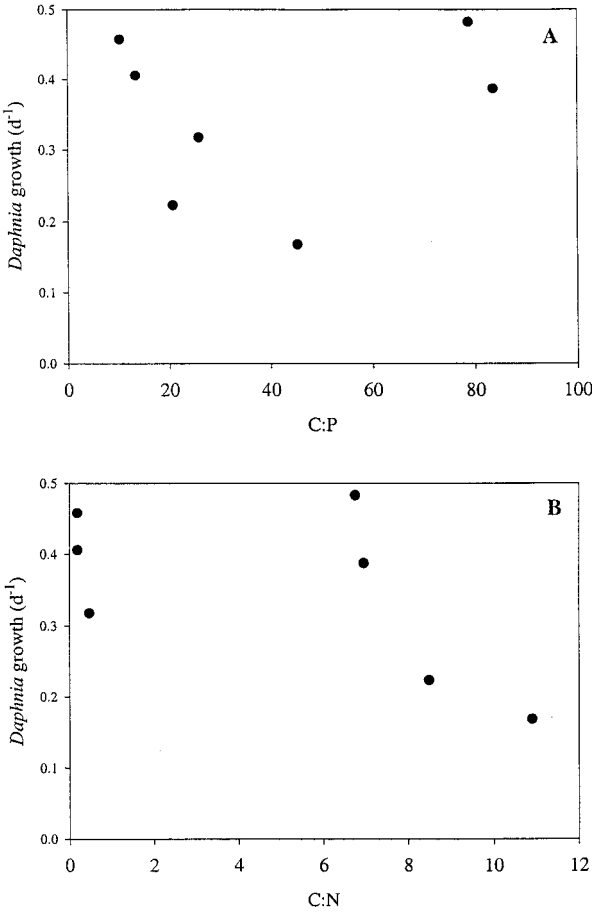

Figure 3. Atomic ratio versus *Daphnia* growth; panel A = C:P, panel B = C:N.

Table 4. Significant ($p<0.05$) multiple regressions of biochemical variables versus *Daphnia* growth (g, d^{-1}), with $g = a + b_i * \ln(x_i)$; AMPL is acetone-mobile polar lipids, PL is phospholipids

a	b_I	x_i	R^2_{adj}
−0.08	0.02	AMPL	0.63
	0.08	PL	
	0.03	Bacillariophyceae	
0.15	0.05	protein	0.63
	0.03	AMPL	
	0.03	Bacillariophyceae	

The multivariate analysis with CANOCO suggested that the phytoplankton species composition was influenced by the light climate of the water (Figure 1). Both A250 and the ratio A250/A365 were significant variables in the ordination of the sample points. The regression analysis confirmed this, the ratio A250/A365 was negatively correlated with biochemical seston variables and with *Daphnia* growth. The positive relation between A250 and DOC found here is comparable with literature values (De Haan & De Boer, 1987; Rostan & Cellot, 1995).

The DOC concentration is important in defining the underwater light climate. It is a quantitative measure of a highly heterogeneous group of compounds. The absorption spectrum of DOC is characterized by an exponentially rising absorption towards the blue and ultraviolet part of the solar spectrum (Kirk, 1994b). The ratio A250/A365 of the lakes was generally higher than that of the ponds. This indicates that the composition of the organic carbon molecules is different in the lakes, and generally consists of smaller molecules. Two explanations can be given for this difference. First; the residence time is longer in the lakes, giving more time for photodegradation processes. Second; ponds most likely will have more highly coloured allochthonous DOC, and the lakes more low-coloured autochthonous DOC. Studies by Morris & Hargreaves (1997) in a variety of Pennsylvania lakes (USA), for example, showed that, following exposure to solar radiation, the A250/A365 ratio increased as result of photochemical degradation processes. The magnitude of the photochemical degradation depended on the source and composition of organic carbon, allochthonous DOC was more affected by photochemical degradation than autochthonous DOC.

The ponds in the St. Denis National Wildlife Refuge occur on a small parcel of land (< 400 ha) and are quite similar in size, hydrology, nutrient loading, and seston stoichiometry. Despite this, the DOC concentration varied amongst the ponds, the biochemical composition of the seston from these similar ponds was quite different, as was the resulting *Daphnia* growth in the biotest. Differences in light climate as a result of the differences in DOC might thus be responsible for the differences in the nutritional quality of the seston (see Sterner et al., 1997).

The *Daphnia* growth results can be related to PAR and UV attenuation measurements that were made in 19 of the sites we sampled (Arts et al., 2000; Figure 4). *In situ* irradiance measurements in that study were made using a scanning spectroradiometer (Optronics OL-754) with a submersible sphere assembly (OL IS-470-WP). Calculations for diffuse attenuation coefficients (K_d) were made according to Kirk (1994a).

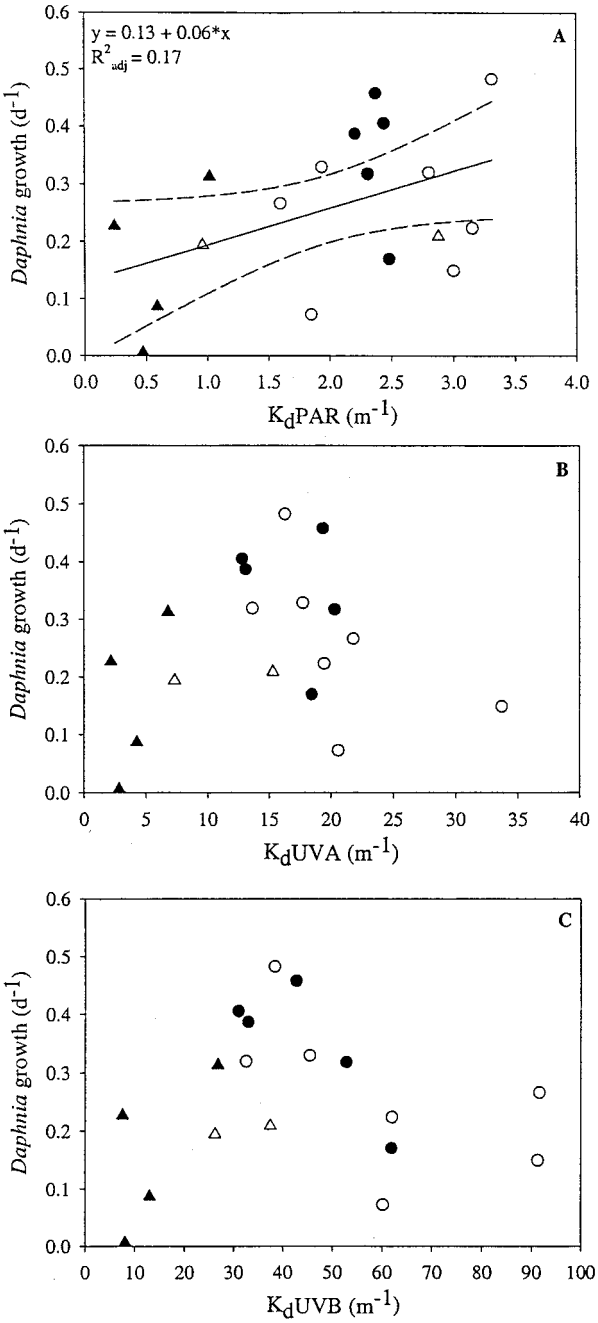

Figure 4. K_d values (Arts et al., 1999) versus *Daphnia* growth (g, d^{-1}); (A) K_dPAR, dashed line indicates the 95% confidence interval of the linear regression; panel (B) K_d UVA; panel (C) K_d UVB. Symbols as in Figure 2. Open symbols indicate that the K_d measurement was not taken at the same day as the seston was sampled.

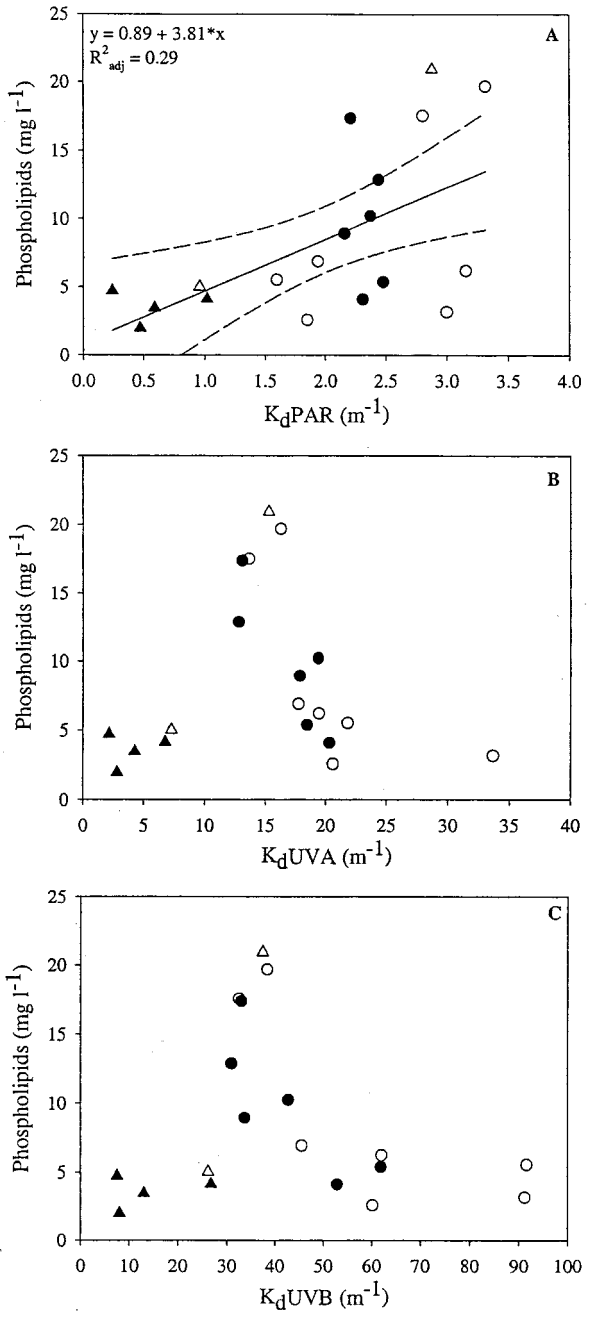

Figure 5. K_d values (Arts et al., 1999) versus phospholipid concentration; (A) K_d PAR, dashed line indicates the 95% confidence interval of the linear regression; (B) K_d UVA; (C) K_d UVB. Symbols as in Figure 4.

It is important to note that the K_d measurements were not always made at the same time as the seston was sampled (indicated with open symbols). Bearing in mind this caveat, the following observations can be made. The relation between K_d AR and *Daphnia* growth is positive and almost significant (linear regression: $p = 0.052$). Sample points with higher K_dPAR values have seston that better support *Daphnia* growth. It is also clear that there is a distinction between the lakes and ponds, with the lakes having lower K_dPAR values. The relation between K_dUVA and K_dUVB versus *Daphnia* growth is more bell-shaped, with an increase in *Daphnia* growth at lower K_d values (which are the lake samples), and a decrease at higher K_d values (pond samples). There appears to be an optimum range of 25 to 55 (m^{-1}) for K_dUVB, and 12 to 22 (m^{-1}) for K_dUVA where *Daphnia* growth is supported best. This apparent optimum range may be explained by the difference in ponds and lakes samples only. It might also be explained by increasing photoprotection against UV-A and UV-B at the low K_d range. Beyond the optimum range higher K_d values and decreased light penetration may result in a decrease in phytoplankton with lower nutritional value, resulting in a lower *Daphnia* growth. The same pattern can be observed in similar relations between K_d and the phospholipid concentration (Figure 5). The highest phospholipid concentrations occur in the range of 30 to 45 (m^{-1}) for K_dUVB, and 12 to 17 (m^{-1}) for K_dUVA.

Conclusions

Our aim with this field study was to assess the strength of the relation between seston composition and potential *Daphnia* growth. We showed that from the measured seston variables, the phospholipid concentration was the best prediction of *Daphnia* growth. Both total lipid and phospholipid concentration showed relations with the underwater light climate.

We hypothesize that in these systems, light climate and especially the penetration of ultraviolet radiation is important in determining the phospholipid content of the seston. Because of the high nutritional value of phospholipids, it is a good predictor for *Daphnia* growth. These proposed relationships between light climate, phospholipid content of the seston, and *Daphnia* growth need experimental confirmation.

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References

Ahlgren G, Lundstedt L, Brett M and Forsberg C (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. J Plankton Res 12: 809–818

Ahlgren G, Goedkoop W, Markensten H, Sonesten L and Boberg M (1997) Seasonal variations in food quality for pelagic and benthic invertebrates in Lake Erken – the role of fatty acids. Freshw Biol 38: 555–570

Ahlgren G, Zeipel K and Gustafsson I-B (1998) Phosphorus limitation effects on the fatty acid content and nutritional quality of a green alga and a diatom. Verh Internat Verein Limnol 26: 1659–1664

Allard B, Boren H, Petterson C and Zhang G (1994) Degradation of humic substances by UV radiation. Env Int 20: 97–101

Andersen T and Hessen DO (1991) Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol Oceanogr 36: 807–814

Arts MT, Evans MS and Robarts RD (1992) Seasonal patterns of total and energy reserve lipids of dominant zooplanktonic crustaceans from a hyper-eutrophic lake. Oecologia 90: 560–571

Arts MT and Rai H (1997) Effects of enhanced ultraviolet-B radiation on the production of lipid, polysaccharide, and protein in three freshwater algal species. Freshw Biol 38: 597–610

Arts MT, Robarts RD, Kasai F, Waiser MJ, Tumber VP, Plante AJ, Rai H and De Lange HJ (2000) The attenuation of ultraviolet radiation in high DOC waters of wetlands and lakes on the northern Great Plains. Limnol. Oceanogr. 45: 292–299

Barica J (1987) Water quality problems associated with high productivity of prairie lakes in Canada: a review. Wat Qual Bull 12: 107–129

Bierhuizen JFH and Prepas EE (1985) Relationship between nutrients, dominant ions, and phytoplankton standing crop in prairie saline lakes. Can J Fish Aquat Sci 42: 1588–1594

Brett MT (1993) Comment on 'Possibility of N or P limitation for planktonic cladocerans: An experimental test' (Urabe and Watanabe) and 'Nutrient element limitation of zooplankton production' (Hessen). Limnol Oceanogr 38: 1333–1337

Brett MT and Müller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic food web processes. Freshw Biol 38: 483–499

Campbell CE and Prepas EE (1986) Evaluation of factors related to the unusually low chlorophyll levels in prairie saline lakes. Can J Fish Aquat Sci 43: 846–854

De Haan H (1993) Solar UV-light penetration and photodegradation of humic substances in peaty lake water. Limnol Oceanogr 38: 1072–1076

De Haan H and De Boer T (1987) Applicability of light absorbance and fluorescence as measures of concentration and molecular size of dissolved organic carbon in humic lake Tjeukemeer. Wat Res 21: 731–734

De Lange HJ and Van Donk E (1997) Effects of UVB irradiated algae on life history traits of *Daphnia pulex*. Freshw Biol 38: 711–720

Folch J, Lees M, and Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509

Genstat 5 Committee (1993) Genstat 5 Release 3 Reference Manual. Oxford University Press

Goulden CE and Place AR (1990) Fatty acid synthesis and accumulation rates in Daphniids. J Exp Zool 256: 168–178

Guillard RRL and Lorenzen CJ (1972) Yellow-green algae with chlorophyllide C. J Phycol 8: 10–14

Gulati RD and DeMott WR (1997) The role of food quality for zooplankton: remarks on the-state-of-the-art, perspectives and priorities. Freshw Biol 38: 445–768

Hessen DO (1992) Nutrient element limitation of zooplankton production. Am Nat 140: 799–814

Hessen DO, De Lange HJ and Van Donk E (1997) UV-induced changes in phytoplankton cells and its effects on grazers. Freshw Biol 38: 513–524

Kirk JTO (1994a) Light & Photosynthesis in Aquatic Ecosystems, Second Edition. Cambridge University Press, Cambridge

Kirk JTO (1994b) Optics of UV-B radiation in natural waters. Ergeb Limnol 43: 1–16

Klüttgen B, Dülmer U, Engels M and Ratte HT (1994) Adam, an artificial freshwater for the culture of zooplankton. Wat Res 28: 743–746

Kreeger DA, Goulden CE, Kilham SS, Lynn SG, Datta S and Interlandi SJ (1997) Seasonal changes in the biochemistry of lake seston. Freshw Biol 38: 539–554

LaBaugh JW (1989) Chemical characteristics of water in Northern prairie wetlands. In: Van der Valk A. (ed.), Northern Prairie Wetlands (pp. 56–90) Iowa State University Press/AMES

Lampert W (1987) Feeding and nutrition in *Daphnia*. In: Peters RH and De Bernardi R (eds.) *Daphnia*. Memorie dell'Istituto Italiano di Idrobiologia 45: 143–192

Leavitt PR, Carpenter SR and Kitchell JF (1989) Whole-lake experiments: The annual record of fossil pigments and zooplankton. Limnol Oceanogr 34: 700–717

Lemcke HW and Lampert W (1975) Veränderungen im Gewicht und der Chemischen Zusammensetzung von *Daphnia pulex* im Hunger. Arch Hydrobiol Suppl 48: 108–137

Montgomery DC and Peck EA (1982) Introduction to Linear Regression Analysis. John Wiley & Sons, New York

Morris DP and Hargreaves BR (1997) The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau. Limnol Oceanogr 42: 239–249

Müller-Navarra D (1995) Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. Arch Hydrobiol 132: 297–307

Pick FR (1987) Carbohydrate and protein content of lake seston in relation to plankton nutrient deficiency. Can J Fish Aquat Sci 44: 2095–2101

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223–225

Robarts RD, Donald D B and Arts MT (1995) Phytoplankton primary production of three temporary northern prairie wetlands. Can J Fish Aquat Sci 52: 897–902

Rostan JC and Cellot B (1995) On the use of UV spectrophotometry to assess dissolved organic carbon origin variations in the Upper Rhone River. Aquat Sci 57: 70–80

Rothhaupt KO, Wacker A and von Elert E (1998) What limits *Daphnia* growth in Lake Constance: mineral limits or fatty acids? Abstract book ASLO/ESA meeting

Sargent JR, Parkes RJ, Mueller-Harvey I and Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleigh MA (ed.) Microbes in the Sea, (pp. 119–138). Ellis Horwood Ltd., Chichester

Scully NM and Lean DRS (1994) The attenuation of ultraviolet radiation in temperate lakes. Ergeb Limnol 43: 135–144

Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z and Waters KJ (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255: 952–959

Smith REH, Furgal JA and Lean DRS (1998) The short-term effects of solar ultraviolet radiation on phytoplankton photosynthesis and photosynthate allocation under contrasting mixing regimes in Lake Ontario. J Great Lakes Res 24: 427–441

Sterner RW (1993) *Daphnia* growth on varying quality of *Scenedesmus*: Mineral limitation of zooplankton. Ecology 74: 2351–2360

Sterner RW, Elser JJ, Fee EJ, Guildford SJ and Chrzanowski TH (1997) The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. Am Nat 150: 663–684

Sterner RW and Schulz KL (1998) Zooplankton nutrition: recent progress and a reality check. Aquat Ecol 32: 261–279

Ter Braak CJF (1988) CANOCO – A FORTRAN program for Canonical Community Ordination by [Partial] [Detrended] [Canonical] Correspondence Analysis, Principal Component Analysis and Redundancy Analysis (Version 2.1). Technical Report, LWA-88-02, GLW-Agricultural Mathematics Group, Wageningen

Ter Braak CJF (1990) Update Notes: CANOCO Version 3.10. GLW-Agricultural Mathematics Group, Wageningen

Vijverberg J (1976) The effect of food quantity and quality on growth, birth-rate and longevity of *Daphnia hyalina* Leydig. Hydrobiol 51: 99–108

Wainman BC, Smith REH, Rai H and Furgal JA (1999) Irradiance and lipid production in natural algal populations. In: Arts MT and Wainman BC (eds), Lipids in Freshwater Ecosystems (pp. 45–70) Springer-Verlag, New York

Weers PMM and Gulati RD (1997) Effect of the addition of polyunsaturated fatty acids to the diet on the growth and fecundity of *Daphnia galeata*. Freshw Biol 38: 721–730

Williamson CE and Zagarese HE (eds.) (1994) Impact of UV-B radiation on pelagic freshwater ecosystems. Ergeb Limnol 43: 1–226

Winter TC (1989) Hydrological studies of wetlands in the Northern prairie. In: Van der Valk A. (ed.), Northern Prairie Wetlands (pp. 16–54). Iowa State University Press/AMES