Differences in nutrient limitation and grazer suppression of phytoplankton in seepage and drainage lakes of the Adirondack region, NY, U.S.A

PATRICIA A. SAUNDERS*†, WILLIAM H. SHAW‡ AND PAUL A. BUKAVECKAS§
Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A
†*Institute of Ecology, University of Georgia, Athens, GA 30602, U.S.A*
‡*Sullivan County Community College, Loch Sheldrake, NY 12759, U.S.A*
§ *Department of Biology, University of Louisville, Louisville, KY 40292, U.S.A*

SUMMARY

1. For seepage and drainage lakes of the Adirondack mountain region (NY, U.S.A) hydrologic regime is correlated with physical and chemical differences that can affect phytoplankton and planktonic food webs (e.g. presence and influence of wetlands, dissolved organic carbon concentration, anoxia, nutrient cycling). We conducted short-term (48 h), *in situ* enclosure experiments to evaluate the relative importance of macrozooplankton grazing and nutrient limitation of phytoplankton biomass in small Adirondack seepage and drainage lakes ($N = 18$, 1–137 ha). Epilimnetic dissolved organic carbon (DOC) concentrations and pH values represented the diversity of the region. We measured chlorophyll *a* changes in response to grazer removal (> 120 μm) and nutrient addition (~ 10× ambient N, P, or N + P), and evaluated changes with respect to *in situ* light, temperature, NO_3, NH_4, SRP, and crustacean assemblage characters.
2. Nutrient addition stimulated significant increase in chlorophyll *a* concentration at 11 of 18 sites (GLM, Tukey–Kramer). Phytoplankton of clearwater drainage lakes were P-limited, whereas clearwater and brownwater seepage lakes responded to additions of N and/or N + P. Relative light availability explained half the variance in response to nutrient addition in drainage ($r^2 = 0.48$), but not seepage lake experiments ($P > 0.05$).
3. We observed responses to grazer removal at eight of 18 sites, usually clearwater drainage lakes. Crustacean grazing may be as significant as nutrient limitation of [chl *a*] for many drainage lake phytoplankton assemblages. Responses were related to *in situ* density of zooplankton only in drainage lakes. Light explained some variability in response to grazer removal for drainage ($r^2 = 0.35$) and seepage lake experiments ($r^2 = 0.35$).
4. These experiments provide evidence that hydrology may ultimately play an important role in determining nutrient and grazer regulation of phytoplankton. Proximate mechanisms affecting our results may be associated with differences in wetland vegetation, [DOC], and nutrient cycling.

Keywords: Adirondack lakes, dissolved organic carbon (DOC), drainage hydrology, seepage hydrology, nitrogen limitation

Correspondence: Patricia A. Saunders, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF A1C 5S7, Canada. E-mail: patricis@morgan.ucs.mun.ca

Introduction

The dominant flowpath of water through a catchment basin defines its interaction with catchment soils and vegetation, and thus affects lake nutrient concentrations and other chemical characteristics (Newton *et al.*, 1987; Peters & Driscoll, 1987; Newton & Driscoll, 1990). General hydrologic features are used to characterize lakes as seepage ('closed basin') or drainage systems ('open basin'; Driscoll *et al.*, 1991). Seepage lakes rely on groundwater and/or atmospheric exchange, depending on whether the lake is in contact with the local aquifer ('flow-through seepage lakes') or isolated ('mounded seepage lakes'). Drainage lakes are influenced by surface flow through inlet and outlet streams. Within each of these broad categories, lakes vary in the proportion of inputs and outputs accounted for by surface, groundwater or atmospheric exchange. Despite such variation, these designations have been useful to regional assessments of lake water chemistry, and they have provided one useful basis for lake classification in the Adirondack region (Driscoll *et al.*, 1991).

In the Adirondack region, seepage lakes generally show higher dissolved organic carbon concentration (DOC), lower pH, and higher chlorophyll *a* concentration (Driscoll & Newton, 1985; Kretser *et al.*, 1989; Buckaveckas & Robbins, 2000). Elevated DOC concentrations in seepage lakes are attributed to watershed topography and hydrologic flowpaths that create locally stable water surpluses suited to development of shoreline wetlands (Mitsch & Gosselink, 1993). Shoreline wetlands have been shown to play an important role in regulating surface water DOC and pH (Engstrom, 1987; Rasmussen *et al.*, 1989; Hemond, 1990; Cirmo & Driscoll, 1993; Houle *et al.*, 1995). DOC concentration regulates light attenuation and water column temperature, and it is correlated with phosphorus availability (Nurnberg & Shaw, 1998; Williamson *et al.*, 1999; Bukaveckas & Robbins, 2000). Inter-lake variability in light climate due to differences in DOC loading may be a key determinant of phytoplankton production in districts where direct human impacts are minimal (e.g. nutrient loading; Carpenter *et al.*, 1998). DOC and pH have been shown to influence species composition of phytoplankton (e.g. Ilmavirta, 1988; Saunders, 1992; Christensen *et al.*, 1996) and zooplankton (e.g. Pinel-Alloul *et al.*, 1990; Locke, 1992).

Phytoplankton biomass is limited by four major factors that control population growth rates: temperature, irradiance, nutrient limitation, and grazing/predation. Nutrients and grazing have received the most attention, particularly in pelagic food-web studies. Empirical models and experimental manipulations established the importance of phosphorus-limitation of algal biomass (Dillon & Rigler, 1974; Schindler, 1977). Work in temperate and tropical lakes showed that nitrogen also can be a limiting nutrient (e.g. Vincent *et al.*, 1984; Morris & Lewis, 1988; Suttle & Harrison, 1988; Axler *et al.*, 1994; Elser *et al.*, 1995a). However, nutrients do not always limit algal growth. Seasonal and interannual data show that the timing and severity of nutrient limitation is variable within systems (e.g. Elser *et al.*, 1995a).

Differences between observed algal biomass and that predicted by nutrient supply have been attributed to grazing losses (e.g. Carpenter *et al.*, 1991; Cyr & Pace, 1993). Seasonal shifts in the effects of grazers on phytoplankton have been observed and incorporated into qualitative algal succession models. At shorter temporal scales (days to weeks), pelagic consumers are most effective at constraining biomass when growth rates of algal populations are limited (e.g. Sommer *et al.*, 1986; Sager & Richman, 1991). Comparisons of nutrient and grazer-regulation of phytoplankton among pelagic systems emphasize differences in lake nutrient loading or food web structure (e.g. McQueen *et al.*, 1986; Carpenter *et al.*, 1991; Elser & Goldman, 1991), but few studies have simultaneously quantified resource and grazer control of algal biomass in freshwater systems (Vanni & Tempte, 1990; Rosemund *et al.*, 1993; Bukaveckas & Shaw, 1998).

In this study, we evaluated the importance of nutrient and grazing influences on phytoplankton biomass in Adirondack seepage and drainage lakes. We did this using small-scale, *in situ* enclosure experiments conducted in 18 Adirondack lakes during summer (1990, 1991). We quantified the response of epilimnetic phytoplankton assemblages to additions of nutrients (N, P, or N + P) and removal of large herbivores (> 120 μm) during 48-h incubations. We interpreted experimental results with respect to a suite of environmental parameters (lake hydrologic regime, light availability, nutrients, chlorophyll *a*, zooplankton assemblage characteristics).

Table 1 Site characteristics

Site	ALSC numbers	Hydrologic classification†	Bog classification‡	Surface area (ha)	Maximum depth (m)	Shoreline length (km)	DOC (mg L^{-1})§	pH¶	Thermal stratic.	Anoxic hypolim.**
Little Echo	020126	Seepage, mounded	Bog	1	5	0.4	18.8	4.2	Y	Y
Fox	060318	Seepage, mounded	Bog	1	10	0.6	15.1	4.3	Y	Y
Kanacto	040744	Seepage, mounded	Marginal	4	15	1.0	6.9	5.5	Y	Y
Rat	020186	Seepage, flow-through	Nonbog (marsh)	12	9	1.6	6.9	6.1	Y	Y
Wheeler	040731	Seepage, flow-through	Nonbog (marsh)	6	18	0.9	5.7	5.9	Y	Y
Tajeena	040745	Seepage, mounded	Marginal	2	18	0.6	4.4	4.8	Y	Y
Pitchfork	060057 A	Seepage, flow-through	Nonbog (marsh)	16	8	2.8	2.8	6.0	Y	Y
Deer	030372	Seepage, flow-through	Nonbog	10	14	1.6	1.4	6.2	Y	Y
Round	04073 lA	Seepage, mounded	Nonbog	4	7	0.7	0.1	4.7	N	N
Duck	060210	Drainage††	Nonbog††	137	3	7.3	4.2	6.0	N	N
Moss	040746	Drainage, medium till	Nonbog (marsh)	46	15	3.7	3.5	6.5	Y	N
Woods	040576	Drainage, thin till	Nonbog††	25	11	2.4	3.4	6.5	Y	N
McBride	060181	Drainage, medium till	Nonbog (marsh)	2	4	0.7	3.1	6.3	N	N
Cranberry	040575	Drainage, thin fill	Nonbog††	7	9	0.9	2.8	4.8	Y	N
Dart's	040750	Drainage, thin fill	Nonbog (marsh)	52	18	5.0	2.8	5.1	Y	N
Thirsty	040738	Drainage, thin fill	Nonbog (marsh)	11	4	1.8	2.4	5.3	N	N
Twitchell	040584	Drainage, thin fill	Nonbog (marsh)	58	10	6.5	1.9	5.4	Y	Y
Silver	040580	Drainage, thin fill	Nonbog	21	18	2.3	0.01	4.8	Y	N

*Numeric site label assigned by Adirondack Lake Survey Corporation, Ray Brook, NY. Hydrologic class, bog class, surface area, and maximum depth values are from ASLC database (Kretser *et al.*, 1989).

†Seepage and drainage defined as described in Introduction; mounded lake perched above local water table on low periniability sediments, receiving most water direcfly from precipitation; flow-through surrounded by highly permiable stratified drifi, receiving some groundwater input; thin fill and intermediate fill indicate relative volume of glacial till in watershed, determined using [Ca^{2+}] by ALSC (Newton & Driscoll, 1990).

‡Bog class defined by ALSC: bog = *Sphagnum* growing by ≥ 90% of lake perimeter; marginal perimeter 50–90% *Sphagnum*; nonbog = perimeter < 50% *Sphagnum*. The presence of marsh weflands was summarized by the authors from maps provided by ALSC; in all cases marsh weflands grew by 15% or less of the lake's perimeter.

§Dissolved organic carbon concentration, determined mid-summer 1990, for epiliumetic grab samples (at 1 or 3 m depth).

¶pH determined mid-summer 1990, for epilimnetic grab samples (at 1 or 3 m depth).

**For seepage lake sites, we defined anoxia as dissolved oxygen concentrations < 1 mg L^{-1} at some or all depths sampled in hypolimnion at time of experiment(s). For several drainage lakes Duck, Moss, Dart's, Thirsty, Twitchell), we used 1987–88 mid-summer data on oxygen concentration measured 1 m above lake bottom; we used full profiles for Woods (14 Jul 90) and Silver (29 Jul 86), and inferred oxygen condition from lack of stratification at McBride (Bukaveckas unpublished data; Kretser *et al.*, 1989).

†† No further data available.

Methods

Study sites (Table 1)

The Adirondack mountain region is a geologically contiguous dome of granitic gneiss and anorthositic bedrock in upper New York state, USA (~ 43–45°N, 72.5–76°W). Lakes there are typically oligotrophic (Frey, 1963). Our classification of lakes by drainage and seepage hydrology is based on the presence (drainage) or absence (seepage) of a defined surficial outflow and follows that of Newton & Driscoll (1990). We chose nine drainage and nine seepage sites that represented the range of epilimnetic pH and DOC concentrations observed in this region. Each hydro-

logic group included five acidic (pH ≤ 5.5) and four circumneutral (pH ≥ 5.5) sites. In brownwater seepage lakes (DOC ≥ 5 mg L), acidity was due to the combined effects of organic acid inputs and deposition of anthropogenic acids (Gorham *et al.*, 1986; Driscoll *et al.*, 1991). Seepage lakes exhibited a higher range of DOC concentrations than drainage lakes (0.1–19 *vs.* 0.01–4.2 mg L^{-1}). Seepage lakes generally had anoxic hypolimnia, whereas drainage lakes were well oxygenated throughout.

The presence or absence of wetlands was not a criterion for site selection, but there were differences between the two hydrologic groups. Seven of nine seepage sites had areas of either bog or marsh

vegetation, and five drainage sites had marsh vegetation along one or two influent tributaries (Kretser *et al.*, 1989). In all cases, marsh areas included ~ 15% or less of a lake's perimeter. However, seepage lakes generally had larger ratios of shoreline length to epilimnetic volume and were likely more influenced by presence of marsh wetlands. When present, bog vegetation (*Sphagnum*) extended around much larger proportions of a lake's perimeter. This study included two bog lakes (*Sphagnum* $\geqslant$ 90% perimeter) and two marginal bog lakes (*Sphagnum* 50–90%).

The lakes used for our experiments were small to moderate in size (1–134 ha), with drainage lakes generally larger than seepage lakes (X = 40 and 6 ha, respectively). Maximum depths were similar (X = 12 m, drainage; 10 m, seepage). We classified Wheeler Pond as a seepage lake due to its small size, kettle morphometry, and associated wetlands. Kretser *et al.* (1989) had classified Wheeler Pond as a drainage lake, on the basis of a small outlet that appears to be ephemeral (Saunders, personal observation).

In situ limnological data

Temperature was measured with a YSI Model 35 S-C-T meter and dissolved oxygen concentration was determined by Winkler titration (Wetzel & Likens, 1991). Percent light, the proportion of photosynthetically active radiation (PAR) at depth relative to incident light (that measured with upward sensor submerged < 5 cm below lake surface), was quantified with a Protomatic photometer equipped with upward and downward spherical sensors exposed as hemispheres. Measurements of pH were done by glass electrode calibrated with a low ionic strength buffer solution. Epilimnetic nutrient samples were obtained mid-summer 1990 at all 18 sites, and measurements were repeated mid-summer 1991 at six sites. Phosphate (ascorbic acid reduction method), ammonium (phenolhypochlorite method), and nitrate (cadmium reduction method) were determined by autoanalyzer (American Public Health Association (APHA), 1985). DOC was measured by persulfate digestion followed by IR detection of CO_2 (Wetzel & Likens, 1991).

Densities of crustacean zooplankton were enumerated in replicate (N = 2), whole-water column, net-tow samples (120-μm mesh Nitex) collected from 1 m above lake bottom. Biomass estimates were calculated using standard length-dry weight regression equations (Bottrell *et al.*, 1976; Pace & Orcutt, 1981; Downing & Rigler, 1984). In most cases, we collected crustacean zooplankton samples on the day of experimental set-up, but we note exceptions below.

Enclosure experiments

Experiments follow methodology presented in Bukaveckas & Shaw (1998), and drainage lake data are a subset of those data. We used translucent, 10-L, polyethylene, closed containers ('cubitainers') for these small-scale manipulation experiments. Replicated (N = 2 or 3) treatments consisted of zooplankton removal (– Z); zooplankton removal + phosphorus addition (– Z + P); zooplankton removal + nitrogen addition (– Z + N); and zooplankton removal + nitrogen and phosphorus addition (– Z + N + P). We pumped a large (~ 100 L), uniform, pelagic sample from a mid-epilimnetic depth into a mixing container and filled cubitainers with 10-L subsamples. We manipulated nutrient concentrations and/or zooplankton densities, with treatments assigned to containers in a stratified random order. To minimize exposure of phytoplankton to direct sunlight, we kept the mixing container covered and returned each cubitainer to the lake immediately after its treatment. For each set of manipulations performed at a specific site and date (an experiment set), we incubated enclosures at the depth of water collection for 48 h (Table 2). Experiments were performed on 3–5 dates at Darts, Kanacto, and Wood's Lake, and on two dates at Deer, Pitchfork, Rat, Round, and Tajecna Lake (Table 2). Temperature at experimental depth was 20–25 °C.

We assessed grazer suppression of phytoplankton biomass using a zooplankton-removal treatment. For triplicate subsamples, crustacean zooplankton were removed by filtering water through a 120-μm Nitex mesh. This mesh does pass microzooplankton (small nauplii, rotifers, protozoans). To assess phytoplankton response to -Z treatment, we compared mean chlorophyll *a* concentration of -Z enclosures with that of three 'initial samples' obtained from the mixed pelagic sample during the set-up procedure. Due to diel vertical migration, the grazer communities present in the epilimnion at mid-day probably underrepresented total daily grazing pressure. Therefore, it was not possible to establish true control treatments for our manipulations. Instead, we assumed that natural variability in chlorophyll over a 48-h period would be

Table 2 Mean chlorophyll *a* concentration in experimental treatments (± SD). Percent light measured at depth of experiment.

Site	Date of expt	Depth of expt	Percent light	Chlorophyll *a* (μg L^{-1}) initial	MDEZ	– Z	– Z + P	– Z + N	– Z + N + P	Response type
Seepage lakes										
Little Echo	07 Jul 91	1	5	13 ± 0.12	16 ± 0.14	13 ± 2.2	16 ± 0.52	*38 ± 1.1	*#36 ± 2.8	– N
Fox	28 Jul 90	1	4	3.7 ± 0.49	3.2 ± 0.097	4.3 ± 0.66	4.5 ± 0.86	–	5.4 ± 0.36	– –
Kanacto	26 Jul 90	1	19	4.1 ± 0.16	3.6 ± 0.35	4.6 ± 0.22	*5.6 ± 0.39	–	*5.6 ± 0.074	– –
Kanacto	18 Jul 90	1	21	3.4 ± 0.19	*2.4 ± 0.052	3.5 ± 0.091	3.1 ± 0.091	–	3.6 ± 0.14	– –
Kanacto	02 Jul 91	1	23	4.6 ± 0.12	5.4 ± 0.42	5.4 ± 0.24	*7.3 ± 1.2	7.0 ± 0.22	6.5 ± 0.54	– –
Rat	30 Jul 90	1	22	4.1 ± 0.001	3.9 ± 0.30	4.1 ± 0.52	4.2 ± 0.39	–	4.5 (–)	– –
Rat	03 Jul 91	1	24	5.2 ± 0.17	*7.9 ± 0.75	7.5 ± 0.67	7.7 ± 0.21	*9.6 ± 0.59	*#10 ± 0.036	– N
Wheeler	08 Aug 90	1	24	7.2 ± 0.32	8.6 ± 0.97	8.8 ± 0.31	8.5 ± 0.27	–	*10 ± 0.64	– –
Tajecna	09 Aug 90	1	26	4.4 ± 0.81	5.2 ± 0.66	5.6 ± 0.53	5.3 ± 0.084	–	*8.5 ± 1.1	– –
Tajecna	19 Jul 91	1	27	3.8 ± 0.31	3.4 ± 0.18	3.9 ± 0.70	3.7 ± 0.076	4.5 ± 0.18	*#6.5 ± 0.013	– N
Pitchfork	01 Aug 90	1	61	2.9 ± 0.20	3.2 ± 0.088	3.7 ± 0.081	3.5 ± 0.13	–	*#6.4 ± 0.56	– N + P
Pitchfork	03 Jul 91	3	25	2.5 ± 0.017	1.7 ± 0.11	2.4 ± 0.25	2.6 ± 0.18	*#41 ± 0.089	*#5.2 ± 0.071	– N, N + P
Deer	11 Aug 90	1	51	3.6 ± 0.17	4.0 ± 0.18	3.7 ± 0.21	3.8 ± 0.25	–	*#10 ± 0.30	– N + P
Deer	16 Jul 91	3	28	3.6 ± 0.066	4.0 ± 0.43	4.2 ± 0.15	4.2 ± 0.14	*5.6 ± 0.25	*#6.4 ± 0.25	– N
Round	31 Jul 90	1	73	1.3 ± 0.34	1.1 ± 0.17	1.7 ± 0.13	1.7 ± 0.088	–	2.3 ± 0.14	– –
Round	20 Aug 90	1	78	1.4 ± 0.074	2.1 ± 0.004	*2.6 ± 0.096	*#4.7 ± 0.036	–	*#4.4 ± 0.33	Z P
Drainage lakes										
Duck	18 Jul 90	3	23	3.8 ± 0.13	*5.9 ± 0.27	*6.6 ± 0.36	*6.0 ± 0.052	–	*#10 ± 0.0055	Z N + P
Moss	22 Jul 90	3	20	1.4 ± 0.073	1.3 ± 0.073	1.8 ± 0.036	2.4 ± 0.74	–	2.3 ± 0.28	– –
Woods	21 May 90	3	15	3.3 ± 0.070	3.6 ± 0.12	*4.1 ± 0.030	*4.1 ± 0.19	–	*4.0 ± 0.068	Z –
Woods	12 Jun 90	3	15	1.8 ± 0.051	2.1 (–)	*2.5 ± 0.069	*2.8 ± 0.085	–	*2.9 ± 0.14	Z –
Woods	23 Jul 90	3	18	1.3 ± 0.028	1.4 ± 0.10	1.4 ± 0.079	1.7 ± 0.17	–	–	– –
Woods	05 Aug 90	3	18	1.5 ± 0.054	1.3 ± 0.18	1.2 ± 0.056	1.4 ± 0.13	–	*1.3 ± 0.19	– –
McBride	20 Jul 90	3	34	1.9 ± 0.044	–	*3.3 ± 0.047	*#4.4 ± 0.014	–	*#4.1 ± 0.0	Z P
Cranberry	16 Jul 90	3	39	0.50 ± 0.0068	0.38 ± 0.098	0.65 ± 0.023	*#1.1 ± 0.24	–	*0.99 ± 0.032	– P
Darts	19 May 90	3	–	2.1 ± 0.32	2.2 (–)	1.8 ± 0.14	1.9 (–)	–	2.1 ± 0.19	– –
Darts	25 May 90	3	8	2.1 ± 0.10	2.8 ± 0.03	*2.9 ± 0.014	*3.5 ± 0.096	–	*3.3 ± 034	Z –
Darts	13 Jun 90	3	15	1.4 ± 0.067	1.7 ± 0.023	1.7 ± 0.070	*#2.2 ± 0.076	–	*#2.1 ± 0.18	– P
Darts	13 Jul 90	3	22	1.2 ± 0.027	*2.2 ± 0.15	*1.9 ± 0.021	*2.1 ± 0.035	–	1.6 ± 0.29	Z –
Darts	07 Aug 90	3	24	1.6 ± 0.024	*2.6 ± 0.023	*2.8 ± 0.069	*#4.3 ± 0.26	–	*#4.6 ± 0.053	Z P
Thirsty	17 Jul 90	3	32	0.93 ± 0.11	0.48 ± 0.059	*1.6 ± 0.076	*1.7 ± 0.18	–	*1.6 ± 0.12	Z –
Twitchell	19 Jul 90	3	23	1.1 ± 0.015	1.3 ± 0.26	*1.9 ± 0.037	*2.2 ± 0.064	–	*2.0 ± 0.096	Z –
Silver	21 Jul 90	3	64	0.14 ± 0.026	*0.30 ± 0.0060	*0.27 ± 0.012	*#0.66 ± 0.012	–	*#0.68 ± 0.012	Z P

Pair-wise differences were assessed by Tukey–Kramer (α = 0.05): '*' indicates treatment significantly different from initial sample, and '#' indicates that nutrient-addition/zooplankton-removal treatment (– Z + P or –Z + N or – Z + N + P) significantly different form zooplankton-removal treatment (– Z). Response type indicates statistically significant responses to grazer removal (Z) or the quality of significant nutrient response(s) (P, N, P + N) as derived from pairwise statistical comparisons and the rationale described in the text.

low or minimal relative to zooplankton-removal effects. We checked this assumption for each experimental set by incubating two cubitainers in which mid-day, epilimnetic zooplankton (MDEZ) were left intact. As MDEZ incubations represented some variable fraction of total diel grazing pressure, we expected biomass changes in MDEZ incubations to be some fraction of the change in comparable -Z treatments. We also used the MDEZ cubitainers to check for negative enclosure

effects (e.g. sedimentation due to lack of mixing).

Crustacean grazers also were removed from the cubitainers in which we did nutrient addition experiments. By comparing the combined treatment with simple grazer-removal treatments (true controls), we were able to isolate potential nutrient-limitation effects from grazer-regulation effects (Elser & Goldman, 1991). For each experiment set, we augmented inorganic nutrients (P and/or N) in 2 or 3 treatment

Table 3 Nutrient concentrations for midsummer epilimnetic samples

Site	Hydro	Year	Depth (m)	N-NH$_4$ (µmol)	N-NO$_3$ (µmol)	P-PO4 (µmol)	DIN (µmol)	DIN/SRP	Nutrient response
Little Echo	S	91	1	25	0.32	0.75	25	34	N
Fox	S	90	1	23	0.97	0.38	24	64	–
Kanacto	S	90	1	3.9	0.80	0.27	4.7	17	–
Kanacto	S	91	1	7.2	0.48	0.24	7.7	32	–
Rat	S	90	1	5.6	0.32	0.35	5.9	17	–
Rat	S	91	1	14	0.32	0.36	15	41	N
Wheeler	S	90	1	3.9	0.32	0.16	4.2	27	–
Tajecna	S	90	1	5.6	0.32	0.17	5.9	35	–
Tajecna	S	91	1	4.4	0.32	0.25	4.7	19	N
Pitchfork	S	90	1	2.8	0.32	0.16	3.1	20	N + P
Pitchfork	S	91	3	8.3	0.48	0.24	8.8	36	N, N + P
Deer	S	90	1	1.1	0.32	0.14	1.4	10	N + P
Deer	S	91	3	8.3	0.32	0.18	8.6	48	N
Round	S	90	1	3.3	0.80	0.12	4.1	36	–
Duck	D	90	3	2.2	0.32	0.10	2.5	24	N + P
Moss	D	90	3	2.2	28	0.13	30	240	–
Woods	D	90	3	3.3	23	0.095	26	270	–
McBride	D	90	3	0.56	3.7	0.13	4.2	31	P
Cranberry	D	90	3	7.2	16	0.074	24	320	P
Dart's	D	90	3	2.8	32	0.063	35	550	–
Thirsty	D	90	3	2.8	5.5	0.10	8.2	78	–
Twitchell	D	90	3	5.0	14	0.095	20	210	–
Silver	D	90	3	3.9	37	0.032	41	1300	P

Dissolved inorganic nitrogen (DIN) = N-NH4 + N-NO$_3$; soluble reactive phosphorous (SRP) = P-PO$_4$.

combinations, with 2 or 3 replicates per treatment. In 1990, we used P-addition (+ P) and N + P-addition (+ N + P). In 1991, we added an N-addition treatment (+ N). We added 9.8 µmol N-NO$_3$ (as 1000 µg L^{-1} KNO$_3$) and/or 0.58 µmol P-PO$_4$ (as 100 µg L^{-1} K$_2$HPO4) to increase nutrient concentrations to ~ 10X preliminary estimates of ambient concentrations (Bukaveckas & Shaw, 1998; but see Table 3).

Response variables and data analysis

For each initial sample and treatment replicate, we removed a well-mixed, 4-L subsample and stored it at 4 °C in the dark for transport to the lab. Pigment concentrations were determined by filtering 2–3 L of sample through Gelman A/E glass fiber filters (1 µm nominal pore size), grinding filters into a slurry with a Teflon tissue grinder, and extracting overnight in aqueous (90%) acetone. Chlorophyll *a* and pheophytin concentrations were determined from absorbance readings (Varian DMS 70 dual-beam spectrophotometer; Speziale *et al.*, 1984). We compared ratios of pheophytin/chlorophyll *a* (pheo/chl *a*) for initials

and treatments within 16 seepage lake experiment sets. We interpreted pheo/chl *a* as a qualitative indicator of relative algal assemblage condition, as pheophytin is a direct product of chlorophyll decomposition (Parsons *et al.*, 1984).

For comparisons among experiment sets, we calculated the magnitude of response, normalised with respect to chlorophyll concentration in either initial samples (response to grazer removal) or in treatment controls (response to nutrient addition):

$$\text{Response to grazer removal} = [\text{chl } a]_{-Z} / [\text{chl } a]_{\text{initial}} \tag{1}$$

$$\text{Response to nutrient addition} = [\text{chl } a]_{-Z+\text{nutr}} / [\text{chl } a]_{-Z} \tag{2}$$

where [chl *a*]$_{\text{initial}}$ = mean chlorophyll *a* concentration of initial samples, [chl *a*]$_{-Z}$ = mean chlorophyll *a* concentration for zooplankton-removal treatment, and [chl *a*]$_{-Z+\text{nutr}}$ = mean chlorophyll *a* concentration in each of three possible zooplankton-removal/nutri-
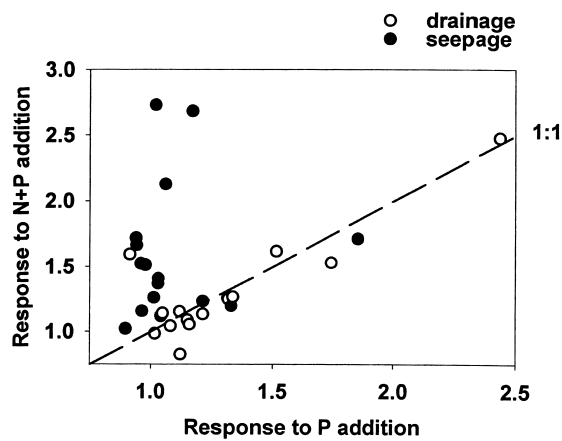
Fig. 1 Normalized responses to nutrient-addition (a,b) and zooplankton-removal treatments (c,d) in drainage (○) and seepage (●) lakes, and their relationship to light availability at experimental depth. Symbols with interior spot in seepage lake figure represent clearwater lakes (epilimnetic DOC < 5 mg L^{-1}). Statistics for drainage lake data based on the model: response = ln (percentage light); those for seepage lake data based on the model: response = percentage light.

ent-addition treatments (− Z + P, -Z + N, -Z + N + P).

For each experiment set, we evaluated P-*vs.* N + P-limitation using pairwise comparisons of mean chlorophyll *a* concentrations, controlled for experimentwise error rate (Tukey–Kramer, α = 0.05, SAS, 1985). If there was a significant response to P or N addition, but there was no additional response to N + P addition, then the assemblage was considered P-limited or N-limited, respectively. If there was a statistically significant difference between N + P addition and the P addition treatment, then the assemblage was considered N + P-or N-limited. We were able to distinguish N + P- from N-limitation in 1991 experiments, when we added the N-addition treatment to our experimental design. To assess the relative short-term importance of grazer regulation and nutrient limitation, we compared the absolute change in [chl *a*] due to grazer removal

$$\left(\Delta Z = \left\{[\text{chl } a]_{-Z} - [\text{chl } a]_{initial}\right\}\right)$$

with that due to nutrient-addition

$$\left(\Delta N = \left\{[\text{chl } a]_{-Z+nutr} - [\text{chl } a]_{-Z}\right\}\right)$$

For each experiment set, we determined significant treatment effects with a one-way ANOVA and Tukey–Kramer pairwise comparisons of treatment

means (α = 0.05). As turnover time of algal and zooplankton assemblages would be short relative to the interval between experiment sets (> month to 1 year at any one site), we assumed independence of data from each set in all statistical analyses. At sites where we did more than one experiment set, chlorophyll responses to treatments varied among dates.

All statistical analyses were done with SAS (1985). We compared hydrologic groups using Student *t*-test and evaluated differences (*P* < 0.05) in light availability, initial chlorophyll, and zooplankton assemblage characteristics. Relationships between responses and environmental variables were evaluated by simple linear regression. Analyses of nutrient responses used the largest mean nutrient response (P, N, or N + P treatment) observed in each experiment set. We checked for outliers and nonlinear patterns using residuals, Student residuals, and Cook's D index. Data were not excluded except where stated explicitly below. *A priori* criteria for exclusion were > ± 2 Student residuals and > 0.5 Cook's D (Table. 2)

Results

Responses to nutrient-addition

Considering all experiments, chlorophyll concentrations increased by as much as 2.73X (treatment means) in response to nutrient addition (*N* = 32; Fig. 1). Chlorophyll responses to P, N, and/or N + P were statistically significant in 14 of 32 experiments, distributed among 11 of 18 sites (Table 2). Pheo/chl *a* ratio was commonly highest in initial samples and lowest in nutrient-addition treatments (9 of 16 seepage site experiments), suggesting a trend of a positive response to nutrient additions. In general, nutrient-limitation of drainage-lake phytoplankton (6 of 16 experiments) was by P alone, and responses to N + P additions were comparable to those for P additions (Fig. 1, Table 2). In contrast, nutrient-limited seepage lake phytoplankton (8 of 16 experiments) typically showed a significantly larger response to the combined nutrient addition, suggesting N or N + P limitation (Fig. 1, Table 2). Seepage lake algal assemblages generally exhibited larger nutrient responses (median = 1.46) than did those of drainage lakes (median = 1.16).

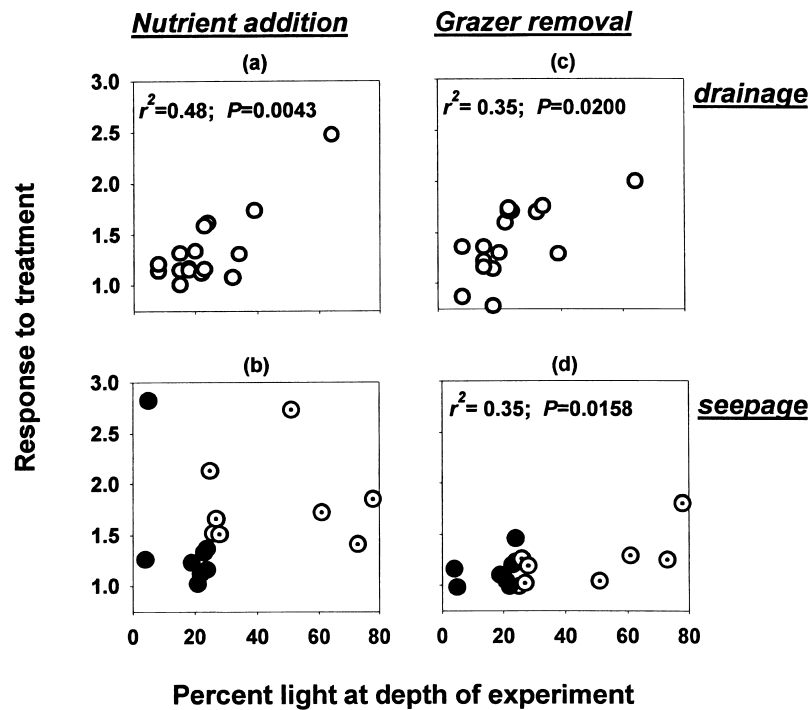**Nutrient addition**   **Grazer removal**

Fig. 2 Normalized response to N + P addition vs. P addition in drainage (○) and seepage (●) Adirondack lakes.

We evaluated treatment responses with respect to relative light availability at experimental depth (% subsurface values, see Method). Experiments were incubated over similar ranges of light availability in
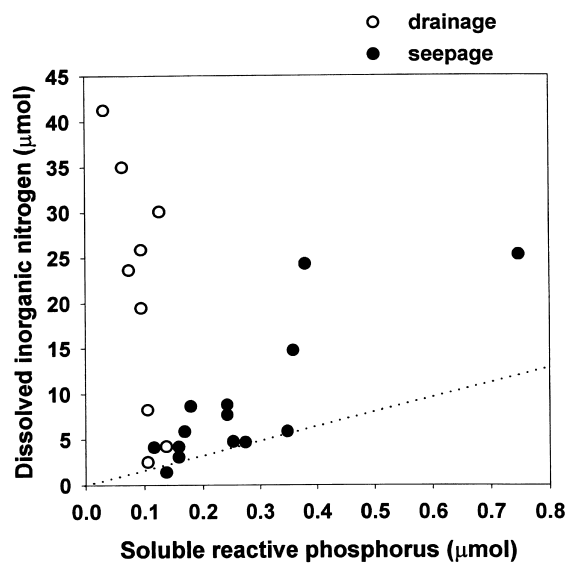

Fig. 3 Concentrations of dissolved inorganic nitrogen (NH$_4$-N + NO$_3$-N + NO$_2$ -N) and soluble reactive phosphorus (P-PO$_4$; mg L^{-1}) from mid-summer, epilimnetic whole water samples from drainage (○) and seepage (●) lakes. Dotted line represents Redfield N/P elemental ratio.

drainage (8–78%) and seepage (4–73%) lakes (Table 2). Over all experiments, percentage light explained 36 percentage of the overall variance in nutrient-response ($P = 0.0005$), after one outlier observation was eliminated from final data analysis following evaluation of model residuals (Little Echo 18 Jul 91 = LE; $r^2 = 0.14$, $P = 0.0387$ if LE included). This relationship (Fig. 2a,b) was strong for drainage lake experiments ($r^2 = 0.48$, $P = 0.0043$) but not for seepage lake experiments (LE omitted: $r^2 = 0.20$, $P = 0.09$; LE included: $r^2 = 0.02$, $P = 0.5934$) (Fig. 2).

Epilimnetic nutrient concentrations were consistent with trends we observed in the type of nutrient-treatment response (Table 3, Fig. 3). Atom ratios for total dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) indicated that DIN/SRP was usually higher in drainage lakes, but DIN/SRP was not a definitive metric of P *vs.* N limitation. In lakes where we observed N or N + P limitation, ambient nitrate + nitrite was < 0.5 μmol. In lakes where we observed P limitation, ambient SRP was < 0.14 μmol (Table 3, Fig. 3).

Responses to grazer removal

Significant responses to grazer removal were more

Table 4 Comparison of mean chlorphyll concentration among samples (intitial), MDEZ (see text) and grazer removeal treatment (– Z).

Site	Date	– Z response	Tukey–Kramer comparison	– Z vs. initial	– Z vs. MDEZ	MDEZ vs. initial
Seepage lakes						
Kanacto	18 Aug 90	1.04	MDEZ initial –Z	–	Z	MDEZ(–)
Rat	03 Jul 91	1.46	Initial –Z MDEZ		–	MDEZ
Round	20 Aug 90	1.80	Initial MDEZ –Z	Z	–	–
Drainage lakes						
Silver	21 Jul 90	2.00	Initial –Z MDEZ	Z	–	MDEZ
Darts	13 Jul 90	1.60	Initial –Z MDEZ	Z	–	MDEZ
Darts	07 Aug 90	1.71	Initial MDEZ –Z	Z	–	MDEZ
Duck	18 Jul 90	1.71	Initial MDEZ –Z	Z	–	MDEZ
Darts	25 May 90	1.36	Initial MDEZ –Z	Z	–	–
Woods	12 Jun 90	1.36	Initial MDEZ –Z	Z	–	–
Thirsty	17 Jul 90	1.70	MDEZ initial –Z	Z	Z	–
Woods	21 May 90	1.22	Initial MDEZ –Z	Z	Z	–
Twitchell	19 Jun 90	1.74	Initial MDEZ –Z	Z	Z	–
McBride	20 Jul 90	1.76	Initial –Z	Z	nd	nd

We show results of Tukey–Kramer comparisons for all experiments with some significant difference among these three means. Response to zooplankton removal was normalized using initial chlorophyll *a* concentration. Underlines show means that were not significantly different. Treatments are listed in order of increasing mean chlorophyll *a* concentration (left to right, see also Table 2). Significant pairwise comparisons are summarized in the last three columns, where treatment acronym indicates a significant difference.

common in drainage lake experiments (10 of 16) than in seepage lake experiments (one of 16; Table 2). We observed statistically significant responses to grazer removal at eight of 18 sites (Table 2). Significant responses to zooplankton removal occurred in clear-water ([DOC] = 0.01–4.2 mg L^{-1}) lakes at experimental light levels between 8 and 78% subsurface irradiance and initial chlorophyll *a* concentrations $\leqslant 3.8$ µg L^{-1}.

With one exception (Thirsty, 17 July 1990), there was a close, linear relationship between the magnitude of chlorophyll response to zooplankton removal and the magnitude of chlorophyll change in MDEZ cubitainers ($N = 31$; $r^2 = 0.64$, $P = 0.0001$). Chlorophyll responses to zooplankton removal were generally about 50% larger than responses to MDEZ conditions ($\beta_1 = 0.65 \pm 0.091$), but the relative increase varied among experiments (Table 4). Data thus supported our assumption that MDEZ treatments would represent some intermediate level of grazing pressure. Chlorophyll concentrations in MDEZ incubations were significantly different from initial samples in 6 of 31 experiment sets, at five of 17 sites (we lack MDEZ data for McBride, which showed a significant -Z response). Statistical difference was due to increased chlorophyll in five experiments (MDEZ > initial) and decreased chlorophyll in one experiment (Kanacto 18 Aug 90) (Table 4).

Light conditions varied among experiments, and we examined the relationship between light availability and chlorophyll response to zooplankton removal. This was significant but highly variable for the combined data set ($r^2 = 0.15$, $P = 0.0336$), because responses to grazer removal were lower in seepage lake *vs.* drainage lake experiments (Fig. 2c,d). Analyzed separately, light availability explained a larger portion of the variance of response to zooplankton-
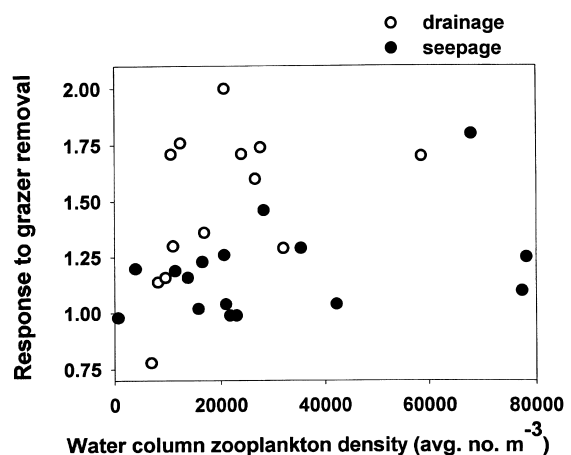

Fig. 4 Normalized response to zooplankton exclosure *vs.* mean water column density of crustacean zooplankton (no. m^{-3}).
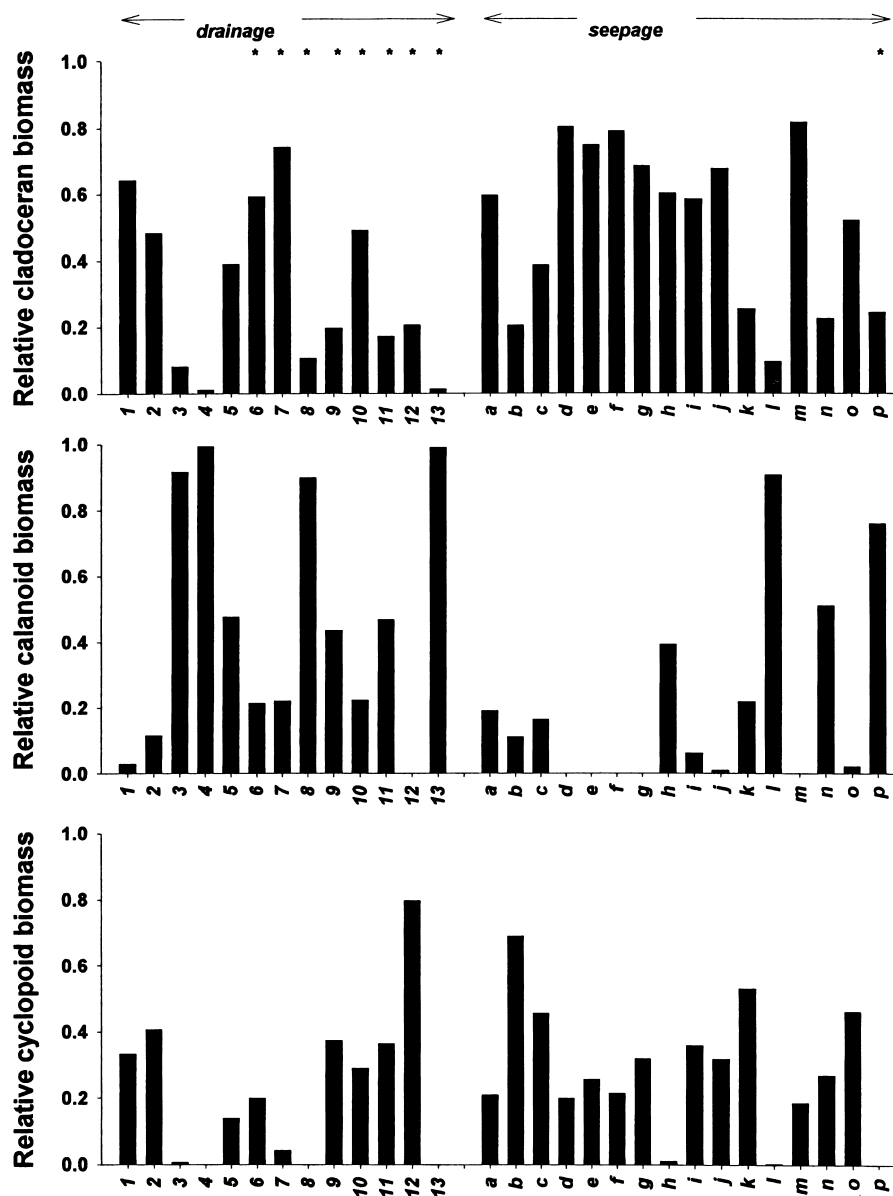
Fig. 5 Relative *in situ* biomass (dry wt.) of cladoceran, calanoid, and cyclopoid species in drainage (left) and seepage lakes (right), arranged in order of normalized response to zooplankton in experimental enclosures. Asterisks (*) indicate lake samples corresponding to experiments with statistically significant grazer-removal responses. Site labels are as follows: (1) Woods, Aug 90; (2) Woods, Jul 90; (3) Dart's, Jun 90; (4) Cranberry; (5) Moss; (6) Woods, Jun 90; (7) Dart's, Jul 90; (8) Thirsty; (9) Dart's, Aug 90; (10) Duck; (11) Twitchell; (12) McBride; (13) Silver; (a) Little Echo; (b) Rat 1990; (c) Pitchfork 1991; (d) Tajecna 1991; (e) Kanacto, Aug 90; (f) Deer 1990; (g) Kanacto, Jul 90; (h) Fox; (i) Deer 1991; (j) Kanacto 1991; (k) Wheeler; (l) Round, Jul 90; (m) Tajecna 1990; (n) Pitchfork 1990; (o)

removal treatments in both drainage lake ($r^2 = 0.35$, $P = 0.0200$; Fig. 2c) and seepage lake experiments ($r^2 = 0.35$, $P = 0.0158$; Fig. 2d).

Responses to zooplankton removal were related to integrated water column density of crustacean grazers for drainage lake experiments (ln-transformed densi-

ties: $N = 13$, $r^2 = 0.33$, $P = 0.0387$; Fig. 4), but not for seepage lake assays ($N = 16$, $r^2 = 0.14$, $P = 0.1$ Fig. 4). Average water column densities of macrozooplankton ($\geqslant 120$ μm) ranged from 612 to 78 186 ind m^{-3}, while biomass estimates ranged from 0.512 to 135 mg m^{-3} (Table 5). We detected no difference in average

Table 5 Average water column density and abundance of crustacean zooplankton. When *in situ* sample was collected on date other than that of experiment set up, the difference (in days) is indicated in parentheses. (*) indicates that associated experiment showed a significant response to grazer removal

Site	Date of in situ sample	Biomass (mg dry wt. m^{-3})	Density (ind m^{-3})
Seepage lakes			
Little Echo	18-Jul-91 (11)	0.53	612
Fox	30-Jul-90 (2)	31.82	13674
Kanacto	26-Jul-90	56.84	77323
Kanacto	18-Aug-90	16.84	20959
Kanacto	30-Jun-91 (2)	4.66	3828
Rat	30-Jul-90	57.70	23014
Rat	03-Jul-91	70.67	28300
Wheeler	08-Aug-90	73.89	16439
Tajecna	09-Aug-90	23.35	20644
Tajecna	19-Jul-91	15.89	15696
Pitchfork	01-Aug-90	66.36	35355
Pitchfork	03-Jul-91	57.11	21738
Deer	11-Aug-90	40.14	42180
Deer	18-Jul-91 (2)	9.44	11259
Round	31-Jul-90	134.57	78186
Round	20-Aug-90	124.64	67868*
Drainage lakes			
Duck	18-Jul-90	65.45	24000 *
Moss	22-Jul-90	20.77	10869
Woods	12-Jun-90	30.46	16789 *
Woods	14-Jul-90 (9)	15.42	8025
Woods	05-Aug-90	21.75	6730
McBride	20-Jul-90	13.63	12326 *
Cranberry	15-Jul-90 (1)	32.58	32002
Darts	15-Jun-90 (2)	20.69	9418
Darts	13-Jul-90	23.86	26653 *
Darts	07-Aug-90	16.08	10524 *
Thirsty	20-Jul-90 (3)	85.75	58553 *
Twitchell	21-Jul-90 (2)	83.93	27674*
Silver	21-Jul-90	35.46	20688 *

Fig. 6 Absolute change in chlorophyll *a* due to zooplankton-exclosure (*y*-axis) *vs.* nutrient-addition, representing drainage (○) and seepage (●) lake experiments.

ments exhibited significant responses to zooplankton removal only. Nine of 32 experiments showed significant chlorophyll increases in response to nutrient addition only. Average initial chlorophyll *a* concentrations were lower in drainage lakes (0.14–3.83 μg L^{-1}) than in seepage lakes (1.33–13.5 μg L^{-1}; $P = 0.0019$). In drainage lake experiments, the magnitudes of responses to zooplankton exclosure were comparable to responses to nutrient addition (Fig. 6). In seepage lake experiments, where we observed only one significant response to grazer removal (Table 2), responses to nutrient addition were larger than responses to grazer removal.

Discussion

Patterns of nutrient-limitation

Phosphorus limitation of phytoplankton biomass was common in clearwater drainage lakes. However, both clearwater and brownwater seepage lakes showed nitrogen limitation (Table 2, Fig. 1). Overall, we found evidence for nutrient limitation of epilimnetic phytoplankton in two-thirds of the 18 Adirondack lakes studied. Our experimental results were consistent with measurements of ambient nutrient concentrations (Fig. 3) and phosphatase activity (Bukaveckas & Shaw, 1998). The magnitude of response was related to light availability in drainage lakes, but not in seepage lakes (Fig. 2), suggesting that another important factor contributes to phytoplankton growth rates in seepage

density or biomass of crustacean assemblages ($P = 0.2$ and 0.3) in drainage *vs.* seepage lakes, but average cladoceran biomass was two-fold higher in seepage lakes ($P = 0.0416$; Fig. 5). Average individual biomass was 0.74–4.49 μg dry wt. ind^{-1} (drainage $\underline{X}$ ± SD = 1.89 ± 0.74 μg ind^{-1}; seepage = 1.71 ± 1.00 μg ind^{-1}) (Table 5, Fig. 5)

Grazer-regulation vs. nutrient-limitation

We observed significant chlorophyll responses to both grazer removal and nutrient addition in five of 32 experiments (Table 2). An additional six of 32 experi-
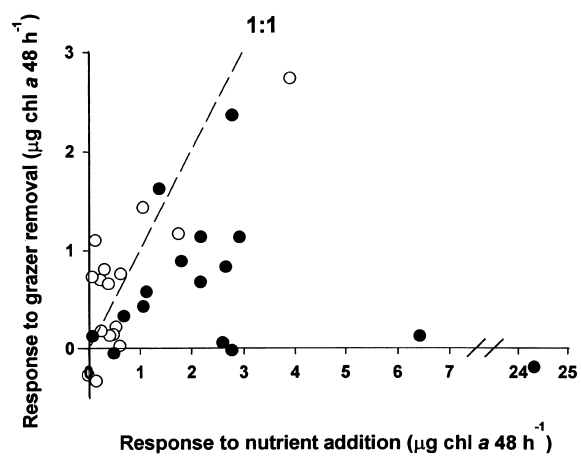
systems. Phytoplankton species typical of brownwater seepage lakes may use osmotrophic or heterotrophic uptake of dissolved or particulate organic carbon for energy and nutrients (Tulonen *et al.*, 1992; Jansson *et al.*, 1996), but mixotrophic species did not dominate the phytoplankton studied at several of these seepage lakes (Arenovski, 1994). Micronutrients may be less available in brownwater lakes (Williamson *et al.*, 1999).

Evidence for N + P-limitation suggests that both elements are available at concentrations close to those needed by phytoplankton. Inorganic nutrients did approach Redfield ratio concentrations in seepage lakes (Fig. 3). Sites with higher DIN/SRP ratios were most likely to exhibit P-limitation, and sites with lower ratios were more likely to be N + P-limited, but ratios were not a clear metric for type of nutrient response (Table 3). Atom ratios are suggestive in this and other studies that detect N or N + P limitation, but because nutrients are often at detection limit concentrations, quantitative use of DIN/SRP ratios can be unreliable (Axler *et al.*, 1994).

Several hypotheses could explain different availabilities of nitrogen and phosphorus in drainage and seepage lakes. Atmospheric inputs can be very important to nutrient budgets of oligotrophic systems, especially in precipitation-dependent seepage lakes and drainage lakes with short hydrologic flow paths (thin and medium till; Rudd *et al.*, 1986; Cole *et al.*, 1990; Axler *et al.*, 1994; Elser *et al.*, 1995b). Precipitation in the Adirondack region has a high N/P ratio because of elevated N in deposition (Driscoll *et al.*, 1991). This would suggest that phytoplankton should be especially prone to P limitation in thin and medium till drainage lakes and lakes dominated by atmospheric inputs (mounded seepage lakes). These experiments found P limitation of phytoplankton at most drainage lakes, and the one P-limited seepage lake (Round) was dilute (< 25 μmhos), acidic (pH = 4.7) and low DOC (Table 1), supporting this generalization. However, our data show that phytoplankton in five other seepage lakes are N or N + P-limited. While this suggests that increased N deposition is likely to have a fertilizing effect on seepage lakes (Axler *et al.*, 1994), it also points to another important influence on nutrient cycling in these lakes.

The presence of adjacent wetlands may influence nutrient availability in these lakes. Most of these seepage lakes have abundant *Sphagnum* vegetation or areas of marsh vegetation that are relatively large *vs.* epilimnetic volume (Table 1). Plants may remove available nitrogen at higher rates than they remove phosphorus (Verhoeven *et al.*, 1990), though true nutrient limitation in wetlands has been difficult to quantify (Urban *et al.*, 1988). Alternatively, carbon inputs from decomposition of wetland plants may enhance the productivity of denitrifying bacteria at the oxic/anoxic interface within wetlands or the open water. Anoxia modifies the chemical environment of a water column, and it can indicate systems where heterotrophic activity is relatively high (Table 1). Most of the seepage lakes in this study had anoxic hypolimnia. The one exception was Round Lake, which was P-limited in August 1990. Their small size, kettle morphometry, and presumably slower through-put rate could contribute to importance of this mechanism in seepage lakes. Mid-summer oxygen data for P-limited drainage lakes do not show development of anoxic depths. The occurrence of N + P limitation at one drainage lake (Duck) was attributed to geomorphometry (A_0 = 137 ha, Z_{max} = 3 m) which may favor sediment-dwelling denitrifying bacteria and/or epibenthic algae in this shallow system (Axler & Reuter, 1996; Bukaveckas & Shaw, 1998). Littoral wetlands can also influence pelagic nutrient concentrations by exporting particulate phosphorus (Wetzel, 1983; Meili, 1992), and concentrations of both dissolved and particulate phosphorus were correlated with epilimnetic DOC concentrations in 12 Adirondack lakes (SRP: r^2 = 0.74, P = 0.0003; TP: r^2 = 0.47, P = 0.0139; Saunders, 1992). However, in brownwater lakes, inorganic phosphorus is likely to be adsorbed by organic matter, and less available to autotrophs without capacity for heterotrophic uptake.

Differential remineralization and recycling by heterotrophs have been shown to alter relative N and P availability in pelagic food webs (Elser *et al.*, 1988), but our experiments and field data suggest that zooplankton assemblage structure and quality of nutrient limitation are not related in Adirondack lakes (Fig. 5). When large cladoceran species are abundant in oligotrophic systems, they can increase seston N/P ratios and stimulate changes in phytoplankton assemblage structure (Elser *et al.*, 1988; Sterner *et al.*, 1992; Elser *et al.*, 1995b). However, zooplankton of both drainage and seepage lakes of this study were predominantly small cladocerans and copepods, which generally release waste at lower N/

P ratios (Anderson & Hessen, 1991; Sterner *et al.*, 1992). The smaller N/P ratios of rotifers and protozoans make it unlikely that they could mediate these interlake differences in N and P availability (Sterner & Hessen, 1994; Elser *et al.*, 1996). Growth of pelagic bacteria has the potential to shift available nutrient ratios, but high C/N ratios and oligotrophic conditions do not favor nutrient excretion (Tezuka, 1990). N limitation is associated with presence of cyanobacteria, but analysis of 1990 assemblages had low or moderate biomass of coccoid blue-green species at Deer and Pitchfork, and heterocystous blue-green species only at Rat Pond (Saunders, 1992).

Patterns of grazer regulation

Removal of crustacean grazers stimulated chlorophyll increase primarily in clearwater drainage lakes. Grazer response was related to *in situ* zooplankton density in drainage lakes, but there was no clear pattern between zooplankton assemblage structure and grazer response at these sites (Figs .4 and 5). Bukaveckas & Shaw (1998) had similar results for experiments in their larger set of drainage lakes, where grazing response was related to *in situ* zooplankton density by a tangential model ($r^2 = 0.84$). It is possible that variation in phytoplankton assemblage structure explains variation not due to differences in zooplankton biomass, but we lack such data for drainage lake sites. In contrast, grazer removal stimulated significant chlorophyll *a* increase in only 1 of 16 seepage lake experiments, even when in-lake crustacean densities were as high or higher than in drainage lakes (Table 5). Light availability explained about one-third of the variation in response to grazer removal in each lake group, but differences in light availability do not explain why responses to crustacean grazer removal were so much weaker in seepage lake experiments (Figs. 2 & 4).

Zooplankton species composition and individual size may alter relative community grazing pressure, but average individual biomass was comparable between these drainage and seepage lakes. Larger cladoceran species are generally more efficient and voracious individual grazers than herbivorous copepods (Wetzel, 1983; p. 429), but percentage cladoceran biomass was greater in seepage lakes. We do not suggest that grazing is unimportant to phytoplankton of seepage lakes, only that the grazing impact of crustacean zooplankton is relatively low, given that phytoplankton had comparable growth rates in seepage and drainage lakes (shown by nutrient addition experiments).

Herbivory increased as a function of crustacean zooplankton density at drainage lakes, but rates were much lower in seepage lakes with similar *in situ* zooplankton density (Bukaveckas & Shaw, 1998; Fig. 4). The importance of crustacean regulation in drainage and seepage lakes may differ due to general differences in food web structure. Allochthonous carbon and enhanced bacterial production are also important to support of food webs in brownwater lakes (Tranvik, 1988; Hessen, 1992; Meili, 1992; Salonen *et al.*, 1992a,b). Salonen & Hammar (1986) demonstrated that filter-feeding cladocera depended on heterotrophic flagellates and bacteria for food in brownwater lakes, and their impact on phytoplankton would be 'diluted' where heterotrophic prey were abundant. At a monthly or seasonal time scale, one might expect such heterotrophic 'subsidies' to support greater crustacean biomass. This need not be the case, as enhanced heterotrophic production often adds limits on zooplankton populations, e.g. reduced habitat (anoxia) and increased abundance of invertebrate predators.

Experimental considerations

Our 48-h measurements of relative grazer regulation assume zero change in algal biomass due to nongrazer factors, and these probably are less precise measures than those for nutrient limitation. Results from MDEZ treatments showed that our assumption of zero growth is less of a problem than recreating true control conditions for grazer-removal manipulations. Significant 48-h chlorophyll decrease in one set of cubitainers incubated with MDEZ plankton might be the result of natural assemblage decline, or even a reduction in significant nutrient regeneration by macrozooplankton. However, experimental removal of grazers did not cause us to overestimate potential nutrient limitation, because we excluded macrozooplankton from both nutrient treatments and their control. The 48-h incubation allowed for response time lags due to uptake processes, while minimizing experimental effects (Lean & Pick, 1981). Coefficients of variance (CV) for replicate samples averaged $6.0 \pm 6.2\%$ for drainage lake and $6.2 \pm 5.0\%$ for

seepage lake experiments. Relatively high (15–30%) CV's probably did occlude statistical detection ($P \geqslant 0.05$) of moderate (1.25–1.5 X) nutrient responses in three experiments (Fox 28 Jul 90; Kanacto 2 Jul 90; Moss 22 Jul 90).

Increase in chlorophyll may not represent an increase in cell biomass. Low light levels can cause an increase in chlorophyll per cell, though chlorophyll per cell will decrease if cells are nitrogen limited (Geider & Osborne, 1992; p. 176). If nitrogen is added, chlorophyll will increase almost immediately, but there may not be a corresponding response in cell growth or division. This would affect measurements of grazer regulation relative to nutrient control of algal growth; in low-light systems, we might over-estimate the impact of macrozooplankton on algal biomass or cell number. If microzooplankton remaining in cubitainers are important grazers, we might be underestimating nutrient responses in these systems, as well as underestimating the importance of grazing.

Summary and Conclusions

Our data showed that regulation of epilimnetic phytoplankton biomass differs substantially between drainage and seepage Adirondack lakes. Nutrient addition experiments showed that drainage lake phytoplankton were often P limited, while seepage lake phytoplankton were generally limited by N or N + P. Grazer-removal experiments found phytoplankton biomass was more strongly influenced by crustacean zooplankton in drainage lakes. These experiments thus suggest a relationship between lake hydrology and food web function with respect to phytoplankton. Lake hydrologic regime is correlated with general physical and chemical differences that include size, morphometry, connection to the drainage basin, dependence on precipitation, presence/absence of shoreline wetlands, concentration of dissolved organic carbon compounds, and the presence or absence of an anoxic hypolimnion. In the context of other studies, especially those of humic-influenced lakes, we propose that food webs in these seepage lakes are different from those in drainage lakes due to a difference in the role of heterotrophic processes. Discussion of patterns in relative control of phytoplankton biomass by herbivory and lake trophic status would benefit from consideration of the effects of watershed–pelagic interactions, allochthonous carbon inputs, associated nutrient and light limitation, and heterotrophic production.

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