Responses of periphyton to artificial nutrient enrichment in freshwater kettle ponds of Cape Cod National Seashore

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Abstract

Nutrient enrichment bioassays, in conjunction with sampling and analysis of surface water quality, were conducted in freshwater lakes (kettle ponds) of Cape Cod National Seashore (Massachusetts, USA) to ascertain the importance of nitrogen and phosphorus in regulating the growth of periphyton. Agar-based nutrient diffusing substrata (NDS) were suspended 0.5 m below the water surface in each pond in July and August 2005. Algal biomass developing on NDS after ~3 weeks of exposure in each month was determined by quantifying chlorophyll *a* + phaeophyton pigments. In both July and August, strong responses to additions of N+P and N were observed in the majority of ponds, while additions of P had no stimulatory effect. These responses correspond well with low atomic ratios (1-17) of dissolved inorganic nitrogen (DIN) to total phosphorus (TP) in ambient surface waters. The results suggest that conditions in the kettle ponds may develop whereby nitrogen is the primary limiting nutrient to periphyton growth. While this may be a seasonal phenomenon, it has implications for nutrient management in individual lakes and within the larger watershed.

Introduction

Scattered throughout the interior of Cape Cod National Seashore (CCNS) (Massachusetts, USA) are numerous freshwater lakes and ponds of glacial origin. Collectively known as “kettle ponds”, due to their roughly circular shapes, these waterbodies were created by melting blocks of ice left behind on the outwash plain nearly

18,000 years ago. Highly valued as aesthetic, cultural, and ecological resources, much attention is focused on these ponds from the standpoint of preservation and management.

Periphyton, comprised of mainly of epiphyton (algae attached to aquatic plants), is conspicuous within in the littoral areas of CCNS ponds. Ecologically, periphyton plays an important role in nutrient cycling and biological productivity in aquatic systems, linking a number of bottom-up and top-down processes (Loeb et al., 1983; McCollum et al., 1998; Kiffney & Richardson, 2001; Dodds, 2003). As such, periphyton has often been used as an indicator of water quality and ecological functioning in both freshwater and marine environments (McCormick & Stevenson, 1998; Lemmens, 2003; Azim et al., 2005).

A pilot study examining how periphyton might be incorporated into kettle pond monitoring at CCNS revealed that growth on artificial substrates was positively correlated with dissolved inorganic nitrogen (DIN) concentrations in surface waters (Smith, 2004). This result was noteworthy given that P was assumed to be the primary limiting nutrient in these waterbodies (Soukup, 1977; Martin et al., 1993; Portnoy et al., 2001). However, this assumption had not been confirmed using experimental methods such as nutrient enrichment bioassays. By and large, it was rooted in the conventional wisdom of P limitation in freshwater systems or in water quality data from which one can calculate molar ratios of total N:total P (TN:TP). However, an increasing number of studies have documented stimulatory effects of N in lakes, ponds, and rivers, suggesting that N limitation may be more common than previously thought (Elser et al., 1990; Axler & Reuter, 1996; Guildford & Hecky, 2000; Rodusky et al., 2001; Camacho et al., 2003; Davies et al., 2004).

Predicating algal responses to N and P based on ambient concentrations of the total nutrient pools can be problematic, partly because of differences in bioavailability. Although dissolved inorganic forms are easily assimilated, organic N is bound by direct carbon bonds, rendering it much less available than organic P, which is bound indirectly through ester linkages. While much of the latter can be accessed through the activity of phosphatase enzymes, a large fraction of organic N is unusable. Thus, the ratio of DIN:TP may be a better index of algal nutrient limitation than TN:TP (Brand, 2001; Maberly et al., 2002; Matthews et al., 2002; Moraska-Lafrançois et al., 2003; Dzialowski et al., 2005).

In CCNS kettle ponds, atomic DIN:TP values of surface waters can fall below the Redfield ratio of 16, particularly during periods of thermal stratification in the summer (K. Lee, unpublished data), suggesting the potential for N limitation to occur. To investigate how N and P influence the growth of periphyton communities during this time of year, nutrient diffusing substrata (NDS) were used. In this type of in situ bioassay, algae colonize and grow on artificial substrates that leach nutrients (Fairchild et al., 1985; Ambrose et al., 2004). In July and August of 2005, NDS arrays were deployed in a total of 12 CCNS ponds for a period of ~ 3 weeks, after which periphyton growth responses were quantified. Surface water samples also were collected and analyzed for a suite of chemical constituents in accordance with an ongoing water quality monitoring program.

Materials and methods

Pond characteristics

Selection of the ponds included in this study (Fig. 1) was based mainly on their importance to resource management. Those selected are the largest and/or most heavily used by both residents and visitors to the area and there have been anecdotal accounts of increasing periphyton biomass in many of these ponds. In general, they are acidic, softwater systems with surface waters characterized by low pH (4.7-7.0), alkalinity (< 80 μEq L^{-1}), and conductivity (< 160 μS cm^{-1}) (Portnoy et al., 2001). April Secchi depths exhibit nearly a fivefold range of variation between 3.5 and 16 m (Portnoy et al., 2001). Secchi depths are influenced primarily by phytoplankton biomass as there is little to no coloration of these waters (Portnoy et al., 2001). Surface water total P (TP) concentrations are characteristic of oligotrophic to eutrophic lakes (< 50 μg L^{-1}) (Heiskary & Walker, 1988) whereas total N (TN) typically falls within the oligotrophic range (> 400 μg L^{-1}) (Nürnberg, 2001; USEPA, 2001).

In most of ponds, periphyton is conspicuous component of the littoral zone where it grows mainly on emergent and submerged macrophytes. Analyses of communities harvested from periphyton samplers in 2004 showed that *Mougeotia* species (filamentous green algae) comprised the vast majority of algal biomass (Smith, 2004). The amount of available attachment area in each pond, and thus the amount of periphyton, varies with aquatic plant biomass, which itself is linked to pond trophic status and successional stage. While no direct quantification has been done, the biomass of periphyton relative to phytoplankton in the ponds appears to be relatively high, as is frequently the case in acidic waters (Turner et al., 1995; Winkler, 1997; Vinebrooke et al., 2002).

Water chemistry

In August 2006, water samples from all study ponds were collected and analyzed as part of an ongoing monitoring effort. In situ pH and specific conductivity was measured using a YSI Incorporated™ multi-parameter water quality probe (model 6820) lowered into the water column to a depth of 0.5m. For other constituents, water was pumped through tygon tubing that had been previously acid-cleaned and rinsed with distilled water. One unfiltered sample was pumped into a 250-ml HDPE bottle for determination of alkalinity by titration with $CaCO_3$ immediately upon returning from the field.

For dissolved inorganic nutrient analysis, triplicate samples were filtered through a 0.45-μm membrane filter. The first few milliliters were discharged to waste while the rest was directed into a pre-acidified (0.5 ml of 2N trace metal grade HCl) 50-ml sterile centrifuge tube and stored at 5˚C until analyzed. For total nutrients, 40 ml of unfiltered water was pumped directly from the pond into acid cleaned 50 ml Pyrex glass digestion tubes with polypropylene Teflon-lined caps. The samples were then transferred on ice to the laboratory for immediate processing.

Dissolved inorganic nutrient concentrations were determined according to methods originally developed by Hansen & Grasshoff (1983). Ammonium (NH_4), nitrate/nitrite (NO_x), and orthophosphate (PO_4) were quantified to the nearest 0.1 μmole L^{-1} by flow injection analysis on a Lachat 8000+ series FIA autoanalyzer (Lachat Instruments, Loveland, Colorado) (Diamond, 2000; Knepel & Borgen, 2000; Diamond, 2001). For TN and TP, samples were digested with persulfate oxidizing reagent at 15 psi for 45 min. (120°C) in a pressure cooker and analyzed simultaneously by flow-injection analysis as per Valderrama (1981). Method detection limits are 0.3 µmole L^{-1} for NH_4-N and TN and 0.1 µmole L^{-1} for NO_x, PO_4-P and TP. For samples with nutrient concentrations below these limits, the threshold values themselves were used to calculate DIN (NH_4 + NO_x) and various N:P ratios (DIN:DIP, DIN:TP, TN:TP).

Nutrient-enrichment bioassays

To construct the NDS, the ends of 50 ml centrifuge tubes were sawed off and re-capped with circular disks (2.5 cm diameter, 0.5 cm thick, 70 µm pore size) of porous polyethylene – a material that is commonly used as an artificial substrate for periphyton (USGS, 1997; Downing, 2005). The tubes were then filled with nutrient-agar mixtures and capped at the end so that nutrients could only diffuse out through the polyethylene disks. Nutrient treatments were prepared as follows: nitrogen (0.5 M $NaNO_3$ in 2% agar), phosphorus (0.05 M Na_2HPO_4 in 2% agar), nitrogen and phosphorus (0.5 M $NaNO_3$ + 0.05 M Na_2HPO_4 in 2% agar) and control (2% agar only). These concentrations are identical or similar to those of other studies in freshwater systems (Biggs & Lowe, 1994; Higley et al., 2001; Pillsbury et al., 2002; Henry & Fisher, 2003). For each pond, twelve tubes (3 replicate tubes for each treatment) were randomly fitted into holes drilled 5 cm apart along 90-cm sections of 3.2-cm diameter PVC pipe, which were suspended in the water column (0.5 m depth) by a float attached to a permanent buoy (Fig. 2).

Given that large numbers of people walk and swim along the shorelines, the NDS arrays (one per pond) were fastened to buoys marking the deepest point in each pond in an attempt to prevent human interference with the experiments. Although not its usual habitat, periphyton can rapidly become established here when a suitable substrate is provided as evidenced by luxuriant growth on permanent buoys and anchor lines. The bioassays ran in 10 ponds for 26 days in July and 22 days in August. This duration was based upon previous work indicating that periphyton required 3+ weeks to become well developed on artificial substrates (Smith, 2004). The two bioassay runs had seven ponds in common (see list in Table 2). In August, Great (Wellfleet) and Gull ponds were substituted for Slough and Horseleech ponds in order to increase the total number of sites assayed for nutrient limitation. Unfortunately, the entire NDS array in Herring pond was lost in August.

At the end of the deployment periods, the tubes were collected and transported back to the laboratory in an ice chest. There, the disks were removed and placed in clean centrifuge tubes. To determine relative amounts of photoautotrophic biomass on the disks, chlorophyll *a* and phaeophytin were extracted in 15 ml of 90% acetone for 24 hrs. at 4°C in darkness. A fraction of the supernatant (3 ml) was pipetted into a cuvette and characterized spectrophotometrically on a Jenway™ 6305 UV/VIS Spectrophotometer. Relative amounts of these photosynthetic pigments (Chl+Ph) were determined by absorbance at 664 and 750 nm and at 665 and 750 after acidification with 0.1 N HCl.

Concentrations of both were then calculated as μg of pigment cm^{-2} of substrate based on standard formulae (APHA, 1998).

Statistical analysis

All data were log (X+1) transformed to improve normality and heteroscedasticity and subjected to one-way ANOVA ($\alpha=0.05$) to detect significant differences among treatment means (Statistica ver. 6.1). Specific means were then compared to each other using Tukey's Honest Significant Difference tests.

Results

Water chemistry

Alkalinity was ubiquitously low, ranging between -0.5 (Duck pond) and 4.3 mg $CaCO_3$ L^{-1} (Herring pond). Duck, Dyer, Great (Wellfleet), Long, Slough, and Spectacle ponds all had negative values, which indicates that there is zero buffering capacity in these ponds with an excess of strong acids that further depress the pH (Table 1). Among all ponds, alkalinity averaged 0.7 mg$CaCO_3$ L^{-1}. pH ranged between 5.1 (Duck, Dyer, and Slough ponds) and 7.1 (Herring pond) with a mean value of 5.9. With the exception of Great pond (Truro), which had a conductivity of 207 μS cm^{-1}, all ponds had extremely soft water, with values < 200 µS cm^{-1}. Dyer pond had the lowest conductivity at 93 µS cm^{-1}. Secchi depths showed a threefold level of variation, ranging between 3.0 (Herring pond) and 9.0 m (Great pond in Wellfleet).

Due to an error in acidifying two of the three samples collected for inorganic nutrients, only one replicate could be analyzed. Among these samples, concentrations of NH_4-N were very low as all ponds had values ≤ 0.3 µmole L^{-1} (Table 1). NO_x-N, which accounted for most of the total DIN, was much higher with values ranging between 0.7 (Great pond - Wellfleet) and 1.8 µmole L^{-1} (Duck pond). However, total DIN was still generally low, ranging between 0.8 µmole L^{-1} (Spectacle pond) and 1.8 µmole L^{-1} (Duck pond). TN concentrations were between 3 and 20 times higher than DIN and considerably more variable among ponds. DIN as a proportion of TN ranged between 4.5% and 40%. With the exception of Herring pond, concentrations of PO_4-P were very similar among most ponds (~ 0.1 µmole L^{-1}). PO_4-P constituted a much higher proportion of total pool, ranging between 33% to 100% of TP.

Atomic DIN:TP ratios in surface waters ranged between 1 (Herring pond) and 18 (Great pond, Truro) with an average of 8 among all ponds, a value well below the Redfield ratio of 16. DIN:DIP showed similar variability but many values were two to three times higher. TN:TP ratios were nearly an order of magnitude higher than DIN:TP ratios (Table 1). While chlorophyll *a* or direct measurements of phytoplankton biomass in the water column were not quantified, Secchi depth (which is closely related to phytoplankton biomass) was significantly correlated with TN concentration ($F=7.6$, $p = 0.02$, $R^2=0.43$). No other water chemistry variable showed any statistically significant relationship with Secchi depth.

Nutrient-enrichment bioassays

In every pond, N+P additions had the largest effect on periphyton growth, yielding Chl+Ph concentrations that were 4.2 and 6.3 times that of the controls in July and August, respectively. Statistically, N+P values were higher than the controls in all ponds in July and in all but one (Long pond) in August (Fig. 3, Table 2). In addition, N+P treatments often resulted in Chl+Ph concentrations that were significantly higher than both N or P treatments. This occurred in 7 ponds in July and 2 ponds in August.

While responses to N alone were usually lower than those to N+P, they were generally much higher than controls. On average, Chl+Ph concentrations in N treatments were 2.3 and 5.6 times higher than controls in July and August, respectively. Significant responses to N treatments relative to controls were observed in 6 ponds in July and 8 ponds in August (Fig. 3, Table 2). In August, differences between the N+P and N treatments were much smaller, and often statistically indistinguishable, compared to July. Of the ponds that were assayed twice, only Duck, Dyer, and Ryder ponds exhibited significant N responses in both July and August. This occurred in Great (Truro), Long, and Spectacle ponds in July only and in Snow pond in August only.

In contrast to the N+P and N treatments, P enrichment yielded no significant increases in Chl+Ph in either July or August. Similarly, Chl+Ph in the controls were very low and quite variable among ponds. Herring and Snow ponds had the highest control values in July and August, respectively, while Duck and Dyer ponds had the lowest. This pattern relates well to the trophic status of these individual ponds as they lie at opposite ends of the trophic spectrum (Portnoy et al., 2001; Roman et al., 2001). However, when concentrations of the various nutrient species (NO_x, NH_4, TN, TP, PO_4) were plotted against Chl+Ph concentrations in the NDS controls for all ponds, no significant relationships were observed.

Microscopic examination of periphyton samples scraped from the PVC pipe that held the NDS tubes indicated that *Mougeotia* spp. were the dominant algae - an identification that was confirmed by an outside laboratory (Water's Edge Scientific, Ltd, Baraboo, WI). Although this is a different substrate, it is likely that *Mougeotia* spp. constituted the majority of algal biomass on the polyethyelene disks as well.

Discussion

This study provides compelling evidence that N plays an important role in regulating periphyton growth in CCNS kettle ponds. P enrichment alone had no stimulatory effect in any of the assays. However, the magnitude of responses to N+P treatments indicates that P can quickly become limiting once N deficiency has been alleviated. Based on the number of assays in which N+P treatments produced more algal biomass than N treatments, co-limitation by N and P may be a common occurrence in many ponds. Notwithstanding, predictions about nutrient limitation of periphyton based on surface water TN:TP ratios (and their proximity to the Redfield ratio) would have been inaccurate. This may partly be explained by the fact that numerous species of algae, including many chlorophytes (the division to which *Mougeotia* spp. belong), have higher optimal N:P requirements and may therefore experience N limitation at ratios higher than

16 (Tilman et al., 1986; Geider & Roche, 2001; Klausmeier et al., 2004). In contrast, DIN:TP ratios showed much better agreement with periphyton responses to nutrient enrichments.

Precipitation, or lack thereof, may have affected nutrient responses during the bioassay period. Although not directly measured, atmospheric deposition of N was low since July and August were much drier than normal. Consequently, there would have been little overland flow or flushing of groundwater N into the ponds. The previous summer was much wetter, with 6.1 cm more rainfall during these months (approximately 150% higher than in 2005) (NADP, 2005). This may explain the higher DIN:TP ratios in August of that year (range of 1 to 30 with an average of 20) although several ponds had still values near or below the Redfield ratio.

Seasonal N limitation in lakes that develop a strong thermocline has been observed by others (Rodusky et al., 2001; Matthews et al., 2002; Davies et al., 2004). Moreover, it appears that a subset of waterbodies exhibiting N limitation share conditions of acidic or circumneutral pH, low alkalinity, and oligotrophy (Wolfe et al., 2001, Matthews et al., 2002; Moraska-Lafrançois et al., 2003; Nydick et al., 2004; Bergström et al., 2005). While we hesitate to extrapolate our results to phytoplankton, CCNS ponds share the aforementioned characteristics and DIN:TP ratios suggest that N limitation could occur within the upper part of the water column. To assess how both allochthonous and autochthonous sources of N could influence these and other primary producers, a better understanding of N cycling (particularly inputs and losses from the system), vertical profiles of nutrient concentrations, and response thresholds is necessary. How algal species composition may be altered by N enrichment also is critical to understanding ecological impacts in a broader sense. Regardless of these current gaps in our knowledge, the bioassays suggest that N may have greater impact in freshwater ponds of CCNS than previously thought. Protection and management of these ponds should therefore target N, as well as P.

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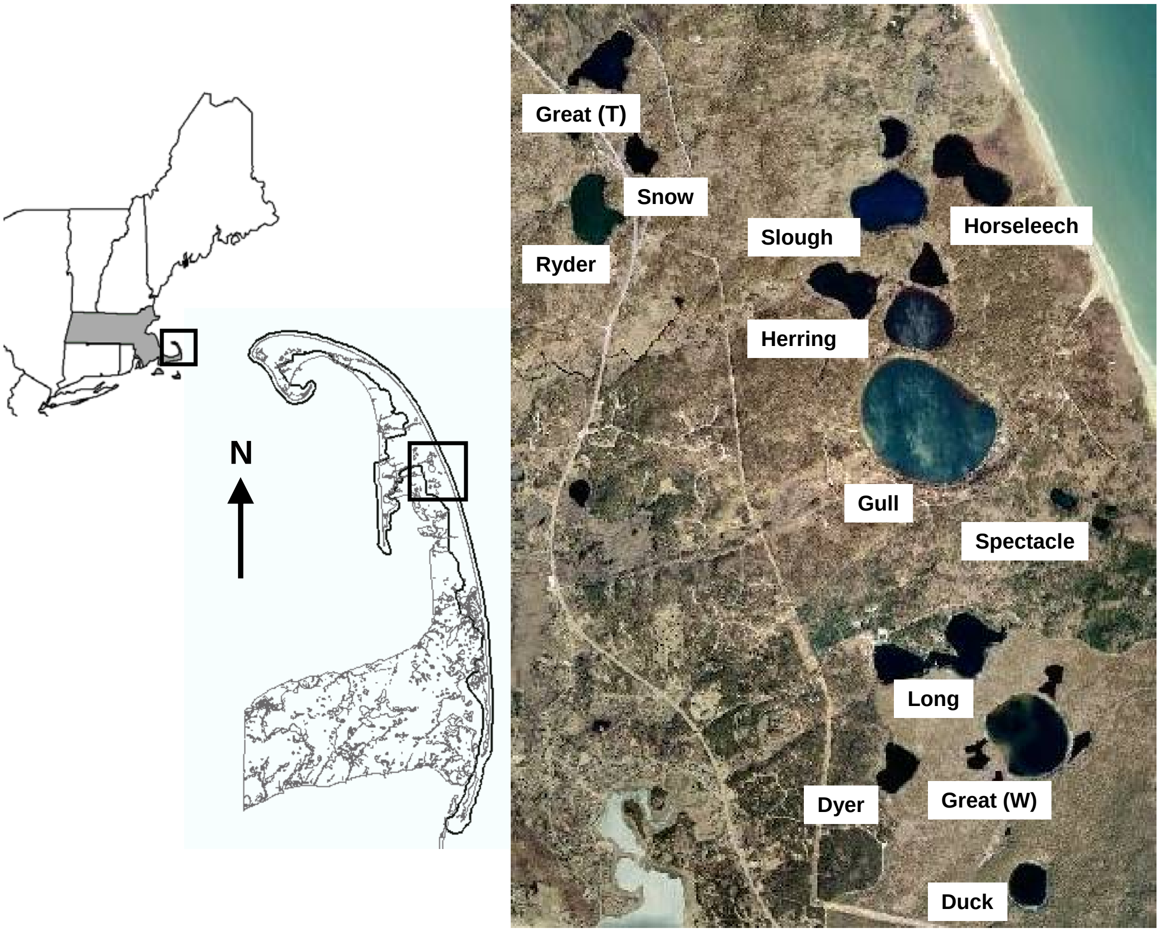
Figure legend

Fig.1. Map showing Massashusetts (left), outer Cape Cod with CCNS boundary (middle), and the specific kettle ponds that were assayed (right).

Fig. 2. Photograph of NDS array in Ryder pond (July 2005).

Fig.3. Chlorophyll *a* and phaeophytin (Chl+Ph) by pond, treatment, and month of bioassay (bars represent standard error of the mean; means that are statistically equal share a common letter).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Duck

Figure 1.

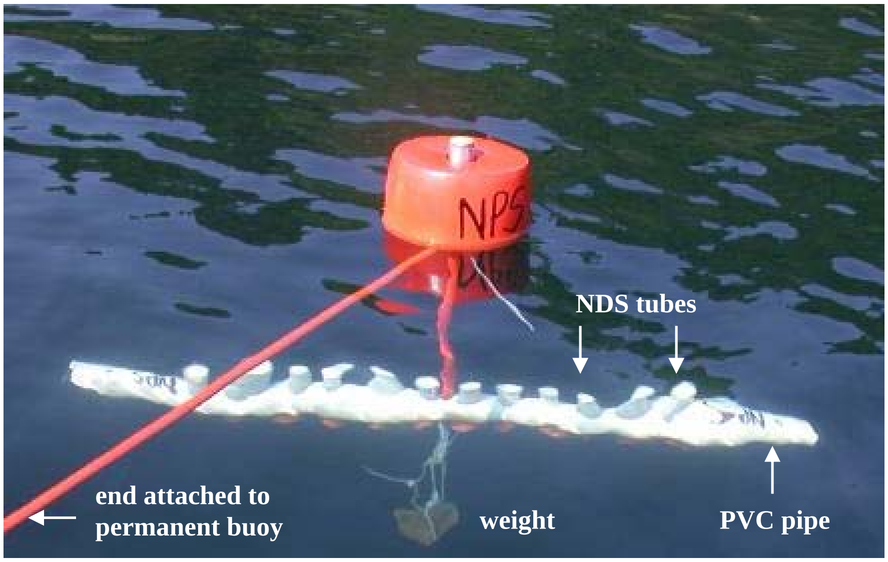
NDS tubes
end attached to permanent buoy
weight
PVC pipe
NPS

Figure 2.

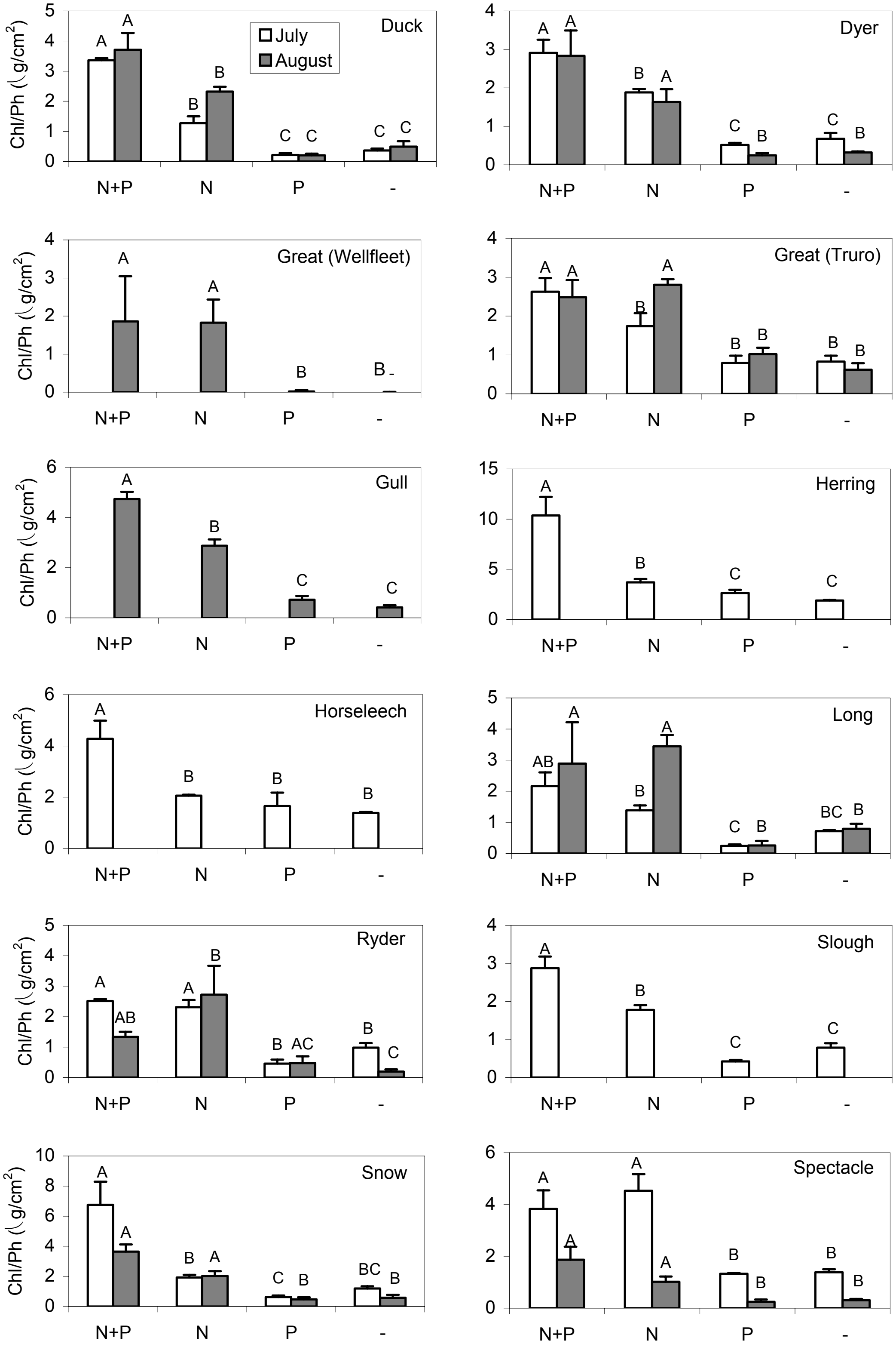
Chl/Ph (√g/cm^2)
July
August
N+P
N
P
-
Duck
0
1
2
3
4
5
A
A
B
B
C
C
C
C
Dyer
0
1
2
3
4
A
A
B
A
C
B
C
B
Great (Wellfleet)
A
A
B
B
Great (Truro)
A
A
B
A
B
B
B
B
Gull
0
2
4
6
A
B
C
C
Herring
0
5
10
15
A
B
C
C
Horseleech
A
B
B
B
Long
AB
A
B
A
C
B
BC
B
Ryder
A
AB
A
B
B
AC
B
C
Slough
A
B
C
C
Snow
0
2
4
6
8
10
A
A
B
A
C
B
BC
B
Spectacle
A
A
A
A
B
B
B
B

Figure 3.

Table 1. Water chemistry variables and atomic N:P ratios for surface water samples collected in August 2005 from the study ponds (n.d. = not detected; values in parenthesis next to TN and TP concentrations are standard errors of triplicate samples, "-" indicates standard error of zero).

Pond	Area (ha)	Mean depth (m)	Max depth (m)	Aug Secchi (m)	Aquatic plant cover	pH	ALK (mg $CaCO_3$ L^{-1})	Cond (µS cm^{-1})	NH_4-N (µmoles L^{-1})	NOx-N (µmoles L^{-1})	DIN (µmoles L^{-1})	TN (µmoles L^{-1})		PO_4-P (µmoles L^{-1})	TP (µmoles L^{-1})		TN:TP	DIN:DIP	DIN:TP
Duck	5.1	7	19.1	6.1	low	5.1	-0.5	117	≤0.3	1.8	2.1	6	(0.9)	0.3	0.3	(-)	20	6	7
Dyer	4.8	5	11.2	7.5	low	5.1	-0.3	93	n.d.	1.0	1.3	10	(0.4)	0.1	0.1	(-)	100	10	13
Great (T)	7.0	4	13.2	7.3	high	6.6	0.5	207	≤0.3	1.5	1.8	14	(1.7)	0.1	0.1	(-)	140	17	18
Great (W)	17.8	6	17.6	9.0	medium	5.3	-0.2	126	≤0.3	0.7	1.0	7	(1.1)	0.1	0.3	(0.1)	23	7	3
Gull	44.0	10	19.8	7.5	medium	6.9	3.6	162	≤0.3	0.8	1.1	16	(0.7)	0.1	0.3	(0.1)	53	9	4
Herring	8.1	3	5.8	3.0	high	7.1	4.3	165	≤0.3	0.8	1.1	18	(1.0)	0.8	0.8	(0.1)	23	1	1
Horseleech	10.0	3	4.9	4.4	medium	7.0	0.9	193	≤0.3	0.8	1.1	15	(0.9)	0.1	0.2	(0.1)	75	10	6
Long	15.0	4	15.6	7.7	medium	5.0	-0.5	104	≤0.3	1.2	1.5	7	(1.3)	0.1	0.1	(-)	70	14	15
Ryder	8.3	7	15.3	5.5	medium	6.6	0.5	148	≤0.3	0.8	1.1	13	(1.2)	≤0.1	≤0.1	(-)	130	11	11
Slough	11.9	5	11.1	6.6	low	5.1	-0.3	142	≤0.3	1.4	1.7	4	(0.9)	≤0.1	≤0.1	(-)	40	17	17
Snow	2.3	4	8.2	3.2	high	6.1	0.4	102	≤0.3	0.7	1.0	22	(2.2)	≤0.1	0.2	(0.1)	110	10	5
Spectacle	0.5	3	7.7	5.1	medium	5.3	-0.2	149	≤0.3	0.8	1.1	13	(2.0)	≤0.1	0.2	(0.1)	65	11	5
Grand means	11.2	5.0	12.5	6.1		5.9	0.7	142	≤0.3	1.0	1.3	12		0.2	0.2		71	10	9

Table 2. Statistical summary of ANOVA F and p values and Tukey's test p values in comparisons of nutrient responses vs. controls ("-" indicates ponds not assayed; "X" indicates NDS array was lost).

	July					August				
Pond	ANOVA F	ANOVA p	p (N+P vs. control)	p (N vs. control)	p (P vs. control)	ANOVA F	ANOVA p	p (N+P vs. control)	p (N vs. control)	p (P vs. control)
Duck	86.6	<0.001	<0.001	0.002	0.584	44.4	<0.001	<0.001	0.002	0.601
Dyer	44.6	<0.001	<0.001	0.002	0.761	24.9	<0.001	0.001	0.008	0.967
Great T	11.0	0.003	0.007	0.096	0.998	18.6	0.001	0.002	0.001	0.355
Great-W	-	-	-	-	-	11.4	0.004	0.026	0.012	0.999
Gull	-	-	-	-	-	83.0	<0.001	0.000	<0.001	0.242
Herring	36.1	<0.001	<0.001	0.034	0.418	X	X	X	X	X
Horseleech	9.0	0.006	<0.001	0.475	0.971	-	-	-	-	-
Long	22.4	<0.001	<0.001	0.095	0.098	17.2	0.001	0.088	0.013	0.266
Ryder	38.9	<0.001	<0.001	0.003	0.049	11.1	0.003	0.048	0.004	0.788
Slough	62.3	<0.001	<0.001	0.010	0.174	-	-	-	-	-
Snow	38.3	<0.001	<0.001	0.300	0.276	29.3	<0.001	0.002	0.026	0.974
Spectacle	22.5	<0.001	<0.001	0.001	0.998	11.9	0.003	0.006	0.099	0.987