An empirical evaluation of the nutrient-color paradigm for lakes

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Abstract

We analyzed a diverse set of 1,646 north-temperate lakes to evaluate the nutrient-color paradigm that integrates total phosphorus (TP) and colored dissolved organic carbon to define lake trophic status. Our objectives were to quantify the combined influence of TP and color (Col) on lake trophic status, to determine if TP and Col had similar relationships with hydrogeomorphic (HGM) variables, and to examine how TP and Col affected the balance of heterotrophic and autotrophic processes. For the latter we examined the Col to chlorophyll *a* ratio (Col : Chl *a*), an index of allochthonous contributions of carbon to pelagic consumers, and deviations of lake $p\mathrm{CO_2}$ from atmospheric, an index of net heterotrophy. Both Col and TP had strong effects on Chl *a* (positive) and Secchi transparency (negative), suggesting that ignoring Col would lead to misinterpretation of these widely used trophic status indicators. Lakes with high TP and Col tended to be shallower with large catchment to lake area ratios. Negative correlations with water retention time (WRT) were stronger for Col than for TP. Both TP and Col were related to forest and wetland land cover, although the direction of the relationships were opposite. Only 29% of the lakes had relatively high allochthony according to their Col : Chl *a* ratios; these were predominately high color, oligotrophic or mesotrophic lakes with short WRT. Over 90% of a subset of 682 lakes were net heterotrophic, with $p\mathrm{CO_2}$ exceeding atmospheric levels. The positive relationship between $p\mathrm{CO_2}$ and Col : Chl *a* suggests that only in very heterotrophic systems was the transfer of allochthonous carbon to pelagic consumers appreciable. Our results provide strong empirical support for the nutrient-color paradigm and highlight its importance both for management applications and for expanding our understanding of how lakes are influenced by terrestrial subsidies of carbon and nutrients.

The distinction among oligotrophic, eutrophic, mixotrophic, and dystrophic lakes has been a rudiment of limnology since the early 20th century. However, because of the prominence of eutrophication as a water-quality problem, limnological research and lake management during the latter half of the 20th century largely defined lake trophic status in terms of autotrophy along the oligotrophic to eutrophic gradient defined by nutrients (e.g., phosphorus and nitrogen). More recently, the importance of external or allochthonous subsidies of carbon to lake food webs has been recognized (Cole et al. 2007) as many, if not most lakes are considered to be net heterotrophic (Cole et al. 2000; Duarte and Prairie 2005). Williamson et al. (1999) reintroduced a paradigm for interpreting trophic processes in lake ecosystems (c.f., Rodhe 1969; Likens 1972) that is conceptualized by the well-studied nutrient axis defined by total phosphorus (TP) and an orthogonal axis defined by the amount of colored dissolved organic carbon (CDOC; Fig. 1). Williamson et al. (1999) argue that integration of these two axes into the nutrient-color paradigm more completely defines lake trophic status in terms of autotrophic and heterotrophic processes and better predicts how lake ecosystems might respond to environmental stressors such as climate change, ultraviolet (UV) radiation, acid deposition and eutrophication.

While the strong positive relationship between algal biomass and TP is a cornerstone of limnology, CDOC can alter lake physics, biogeochemistry, and food webs in ways that influence this relationship. For example, CDOC, through its strong light-attenuation properties, limits light penetration into the water column, thus limiting light available for aquatic plants and phytoplankton and reducing lake mixing depths (Jones 1992; Fee et al. 1996; Houser 2006). Additionally, the humic substances that comprise CDOC provide carbon and energy for heterotrophic production, interact chemically with other solutes such as metals, and may have other biochemical properties such as induction of stress response reactions in aquatic organisms (Jones 1992; Williamson et al. 1999; Steinberg et al. 2006). These multiple effects interact in ways that generate differences in nutrient cycling, lake metabolism, and resilience among lakes representing the extremes of oligotrophy, dystrophy, mixotrophy, and eutrophy (del Giorgio and Peters 1994; Cole et al. 2000; Beisner et al. 2003). Studies explicitly designed to explore the interactions between these two axes have found that both TP and CDOC influence algal biomass and productivity, water clarity, bacterial production, and the degree of heterotrophy in lakes (Carpenter et al. 1998; Nürnberg and Shaw 1999; Hanson et al. 2003).

Even though the TP and CDOC axes are depicted as orthogonal gradients in the nutrient-color paradigm, both are supplied to lakes by external sources and are subsequently influenced by internal lake processes. Although there have been studies relating hydrogeomorphic (HGM) features to TP or to DOC, few studies have compared the two sets of relationships together. For example, when evaluated alone, among-lake heterogeneity in TP and DOC concentrations has been related to HGM
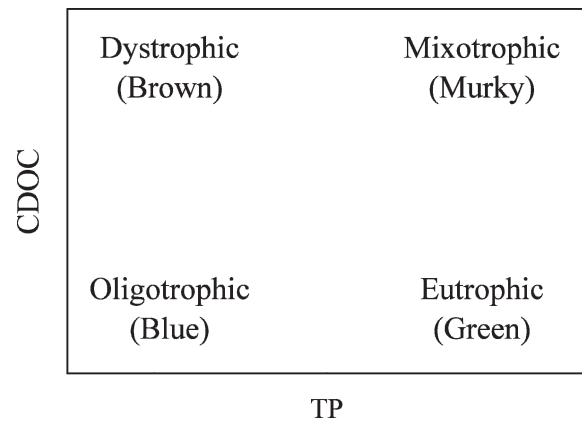

Fig. 1. The nutrient-color paradigm conceptual model redrawn from Williamson et al. (1999). TP is total phosphorus; CDOC is colored dissolved organic carbon.

features such as catchment area, water retention time, topography, land cover, geology, and soils (Dillon and Rigler 1974; Rasmussen et al. 1989; Gergel et al. 1999), lake area (Fee et al. 1996; Xenopoulos et al. 2003), and lake depth (Håkanson 1996) and to in-lake processes such as sedimentation and photolysis (Molot and Dillon 1997a). An improved understanding of the relative importance of HGM features in predicting both TP and CDOC will help elucidate the importance of terrestrial subsidies to lake foodwebs and lake metabolism (Duarte and Prairie 2005; Cole et al. 2007).

We analyzed data from a diverse set of 1,646 north-temperate lakes to evaluate the robustness of the nutrient-color paradigm. We use TP as a measure of the nutrient axis and water color as the measure of the CDOC axis. Water color is a useful surrogate for CDOC as it represents the humic portion of DOC (Rasmussen et al. 1989; Molot and Dillon 1997b). Our three objectives were to: (1) quantify the combined effects of color and TP on chlorophyll *a* (Chl *a*) and water clarity, common metrics of lake trophic status, (2) determine if TP and color were related in similar ways to the HGM features that represent internal processing and external sources from the landscape, such as water retention time (WRT) and catchment area, and (3) examine how TP and color affect the balance of heterotrophic and autotrophic processes. We explored this latter objective using two measures of the importance of allochthonous carbon to lake ecosystems: (1) the Col:Chl *a* ratio, defined here as allochthony or the proportional subsidy of terrestrial carbon to pelagic consumers (Carpenter et al. 2005) and (2) the surface-water partial pressure of carbon dioxide (pCO_2) which reflects heterotrophy or the net balance between primary production and respiration. Our results have implications for management of lake nutrients and for expanding our understanding of fundamental limnological properties and relationships.

Methods

Study lakes—We assembled data on TP, color, lake trophic variables, lake HGM variables, and land cover for

1,646 north-temperate lakes in four lake-rich U.S. states: Maine, New Hampshire, Michigan, and Wisconsin. Data came from databases maintained by state agencies responsible for monitoring lakes under the Federal Clean Water Act, which requires standard procedures and quality assurance/quality control protocols. We included only lakes with surface area ≥ 0.01 km² and maximum depth ≥ 2 m. Water-quality data were from a single sampling date representing the most recent observation for each lake with coincident measurements of TP, color, Chl *a*, and Secchi transparency. Most lakes were sampled between 1990 and 2003, with the earliest samples collected in 1975. We restricted observations to those collected during the summer stratification period, July to September, to reduce seasonal effects. Because sampling effort varied among lakes, we avoided biasing analyses toward intensively sampled systems by including a single sampling date for each lake.

Trophic variables—Forty percent of the Chl *a* and 60% of TP, TN, and color samples were collected from discrete depths between 2 m and 5 m within the epilimnion using a water-bottle sampler. The remaining samples were collected with a tube sampler that integrated either the top 2 m of the water column or the entire mixed layer of the epilimnion. TP was measured using persulfate digestion and both manual and semi-automatic colorimetric methods. Trichromatic Chl *a* was determined using a spectrophotometer. Total nitrogen (TN) was determined using colorimetric methods with cadmium reduction for 1,138 lakes. Secchi disk transparency was measured with a standard black and white disk. For 579 lakes, a viewscope was used for Secchi measurements to limit interference from surface scattering of light. To eliminate differences related to this method, we developed a regression equation (Eq. 1) from 204 concurrent measurements of transparency with (SD_{scope}) and without (SD) the viewscope (L. C. Bacon unpubl. data). The regression equation, developed from natural-log–transformed Secchi transparency, had an $R^2_{adj} = 0.957$ and standard error (SE) of 1.0 m.

$$SD = 0.91 \times \left(SD_{scope}\right)^{1.01} \tag{1}$$

Color measurements were made with visual comparators in platinum cobalt units (PCU) also referred to as Hazen units (Cuthbert and del Giorgio 1992). This measure of color represents humic fractions of DOC, with which it is generally well-correlated (Rasmussen et al. 1989). True color was determined from filtered samples for 437 lakes. Color for the remaining 1,209 lakes was measured as apparent color on unfiltered samples introducing a positive bias due to interference from suspended particles. Using side-by-side comparison data from 328 lake samples (L. C. Bacon unpubl. data) we developed a regression equation ($R^2_{adj} = 0.956$, SE = 4.4 PCU) to convert apparent color to true color (Eq. 2), which was used in all statistical analyses.

$$Color_{true} = 0.827 \times Color_{Apparent} \tag{2}$$

We calculated the color to Chl *a* ratio (Col : Chl *a*) for each lake, which Carpenter et al. (2005) calculated as a ratio derived from spectrophotometric measurement of color at 440 nm (color$_{400}$). In order to compare our ratios calculated using color measured in PCU units, we applied a correction factor derived from Eq. 2 in Cuthbert and del Giorgio (1992) of 17.6 to convert color$_{440}$ to color$_{PCU}$ to allow comparison with the Col : Chl *a* ratios reported by Carpenter et al. (2005) and Pace et al. (2007). Based on whole-lake additions of ^{13}C to five small lakes, Pace et al. (2007) found that lakes with Col : Chl *a* ratios >5.3 (or 0.3 when calculated using color$_{440}$) received allochthonous subsidies that comprised ~20% of the carbon used by pelagic consumers. We estimated pCO$_2$ using alkalinity, laboratory pH, lake water temperature, and conductivity measurements that were available for 682 lakes in our dataset using equations described in Prairie et al. (2002). Lakes with pCO$_2$ exceeding atmospheric concentrations (>37.5 Pa) were considered to be net heterotrophic (Cole et al. 2000). We eliminated one extremely low value, identified as an influential outlier, from further statistical analysis.

Hydrogeomorphic features—Our HGM data included measures of lake and catchment morphometry, water retention time, and land cover. We compiled maximum depth (Z_{max}), mean depth (Z_{bar}) and total catchment area (CA) from existing state databases, and we quantified lake surface area (LA) from state geographic information systems (GIS) data at 1 : 24,000 resolution. We used Eq. 3 to estimate the WRT of each lake.

$$WRT = (LA \times Z_{bar}) \times (CA \times runoff)^{-1} \tag{3}$$

Runoff was averaged within a lake's 500-m buffer from a GIS coverage of the mean annual runoff from 1951 to 1980 (http://water.usgs.gov/GIS/metadata/usgswrd/XML/runoff.xml#stdorder).

Forest and wetland land-cover percentages were determined within an equidistant 500-m buffer around each lake perimeter using GIS. Land cover data were from the 1992 National Land Cover Dataset (http://landcover.usgs.gov/natllandcover.php). Although the 500-m buffer represents only the local land cover, catchment boundaries were not available for our study lakes and an analysis of 461 lakes from our database showed that the percentages of wetland and forest in the 500-m buffer were each highly correlated with the corresponding percentages measured in the entire catchment ($r = 0.64$ and $r = 0.86$, respectively; $p < 0.001$; P. A. Soranno unpubl. data). Gergel et al. (1999) also found that land cover percentages in the 500-m buffer performed similarly to whole catchment measures of wetlands in predicting DOC in a set of Wisconsin lakes.

Data analysis—To address our first objective, we used both continuous (regression) and discrete analysis of variance (ANOVA) statistical approaches. First, we quantified the individual and combined influence of TP and color on Chl *a* and Secchi transparency by developing regression models with TP or color alone and both TP and color as predictor variables. We calculated variance inflation factors (VIF) and condition indices (CI) to assess potential collinearity between TP and color. All variables

were $\log_{10}$-transformed prior to analysis. For the ANOVA approach, we classified lakes into six trophic classes defined by TP and color to further examine patterns in Chl *a* and Secchi transparency across these two gradients. Our TP classes were defined by the widely accepted TP ranges of ≤ 10 (oligotrophic), >10 to ≤ 30 (mesotrophic), and >30 (eutrophic) μg L^{-1} (Wetzel 2001). This classification includes intermediate values of TP for mesotrophic lakes that are not explicitly represented in Fig. 1. The threshold defining low- (≤ 20 PCU) and high-color (>20 PCU) classes was based on our interpretation of data in Williamson et al. (1999) using equations in Cuthbert and del Giorgio (1992) to convert spectrophotometrically derived color data to their PCU equivalents. The 20 PCU value for color was also used by Nürnberg and Shaw (1999) as a lower limit for high-color lakes.

We quantified differences among the TP and color classes using multiple comparison tests derived from a 2-way ANOVA model with TP and color classes as fixed effects and Chl *a* and Secchi transparency as response variables. We used a post hoc multiple comparison procedure with Bonferroni *t*-tests, which control the probability of Type I error, to identify significant differences among the six classes defined by TP and color.

To address our second objective, determining which HGM features best explained among-lake variation in TP and color, we used a similar combination of approaches. First, we used a multiple regression approach with TP or color as the response variable and HGM features as the predictor variables. We ran two sets of models with different sets of predictor variables: (1) a morphometry and land cover model (Z_{max}, LA, CA, CA:LA, % forest, and % wetland), and (2) a WRT and land cover model (WRT, % forest, and % wetland). We ran the second model because we were specifically interested in quantifying differences in the influence of WRT on TP and color. Because Z_{max} and Z_{bar} were strongly collinear ($r = 0.903$, $p < 0.0001$) and we had fewer estimates of Z_{bar}, we used Z_{max}

as the measure of lake depth in these models. For each set of regression models, we identified the best predictor set using Akaike's information criterion (*AIC*) and R^2. Predictor variables were removed if collinearity, indicated by either VIF or CI were high. Neither of these diagnostics exceeded 2.0 in the final models we present.

We then used a multiple-comparison approach to quantify class differences in HGM features (Z_{max}, LA, CA, CA:LA, and WRT) and land cover variables (% forest and % wetland). Our intent in this analysis was not to attribute causation by TP and color but to examine differences in HGM features among the trophic classes. Prior to analysis, all variables were natural-log–transformed except for land cover variables which were arcsine-square-root–transformed to meet statistical assumptions of homogeneity of variance and linearity of responses. SYSTAT v11 (Systat Software) was used for all statistical analyses except the regression analyses which were done using SAS/STAT v9.1 (SAS Institute).

Results

Distribution of lakes across the TP-color space—The 1,646 lakes in our database covered a wide range of trophic state, from 1 to 3 orders of magnitude in TP, color, Chl *a*, and Secchi transparency (Table 1). Distributions of TP and color (Fig. 2A) were skewed towards lower values with medians of 10 μg L^{-1} and 14 PCU, respectively (Table 1). Overall, our lake dataset contained twice as many low-color as high-color lakes with oligotrophic (51%) and mesotrophic (43%) lakes most common and eutrophic lakes rare (6%; Table 2). A result of the positive correlation between color and TP ($r = 0.413$; $p < 0.0001$) was that dystrophic lakes with low-TP and high color or eutrophic lakes with high TP and very low color (≤ 5 PCU) were relatively uncommon (Fig. 2A). Similar patterns were apparent in TP–color plots in Nürnberg and Shaw (1999).

Table 1. The median, range, and sample size (*n*) for variables used in analyses.

| Variable	Abbreviation	Units	*n*	Median	Range
Trophic variables					
Total phosphorus	TP	μg L^{-1}	1,646	10	1–765
Color	Col	PCU	1,646	14	0.8–193
Chlorophyll *a*	Chl *a*	μg L^{-1}	1,646	4	0.1–158
Secchi transparency	Sec	m	1,602	3.5	0.2–13.5
Total nitrogen	TN	μg L^{-1}	1,138	400	66–3,820
Total nitrogen to total phosphorus	TN:TP	ratio	1,138	34	1–1,030
Color to chlorophyll	Col:Chl *a*	ratio	1,646	3	0.1–76
Partial pressure of CO_2	$p CO_2$	Pa	682	82	0.3–939
Hydrogeomorphic features					
Max. depth	Z_{max}	m	1,646	9	2–96
Mean depth	Z_{bar}	m	1,627	4	1–33
Lake surface area	LA	km²	1,646	48	0.01–305.4
Catchment area	CA	km²	1,597	7.25	0.09–9,736
Catchment area to lake area	CA:LA	ratio	1,597	14	1–7,839
Water retention time	WRT	yr	1,574	0.5	0.001–37
% Wetland	% WET	percent	1,646	5	0–80
% Forest	% FOR	percent	1,646	0.5	4–100
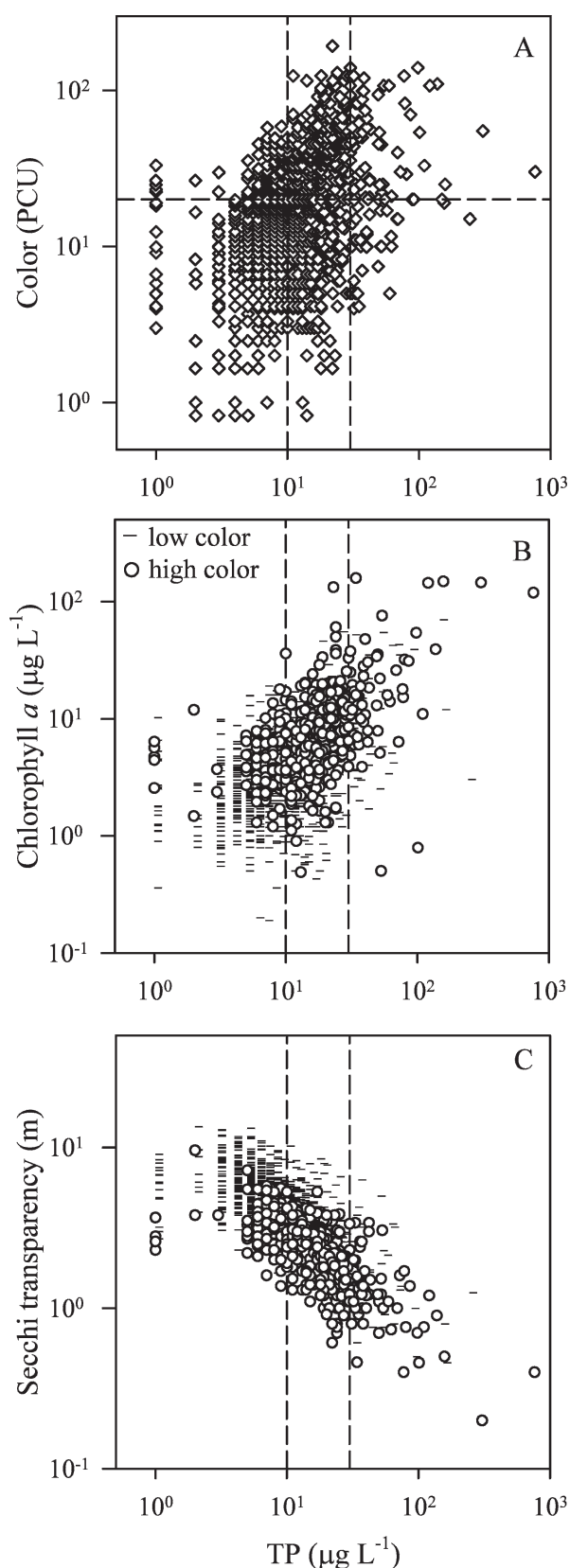
Fig. 2. Plots of (A) color, (B) chlorophyll *a*, and (C) Secchi transparency versus TP. All axes are plotted on a log$_{10}$ scale. Vertical dashed lines mark TP of 10 and 30 μg L^{-1} corresponding

Effects of TP and color on trophic variables—In our study lakes, relationships between TP and Chl *a* and Secchi transparency (Figs. 2B,C) were similar to well-known limnological patterns. However, the slope of our TP–Chl *a* regression (Table 3) was lower than many reported in the literature (Brown et al. 2000; Håkanson and Boulion 2001), most likely due to the relatively small proportion of highly eutrophic lakes in our dataset. Not surprisingly, color had a negative relationship with Secchi transparency due to its light attenuation properties; the positive relationship between color and Chl *a* (Table 3) was unexpected. Regressions with color as the sole predictor had lower explanatory power than those with solely TP for both Chl *a* and Secchi transparency, whereas the best regression equations included both TP and color as predictors. The effects of TP and color on these measures of trophic status were not influenced by collinearity based on VIF and CI diagnostics.

The effect of color on these two trophic variables was such that within a TP class, the distributions of Secchi transparency and Chl *a* were nearly always significantly different between high- and low-color lakes (Figs. 3A, B). Secchi transparency was negatively and Chl *a* positively related to TP. However, lakes with low color had deeper Secchi transparencies and lower chlorophyll for a given TP concentration than lakes with high color. Although TN increased across the TP classes, it showed no relationship with color. In our study lakes N-limitation (TN : TP <10 by mass) was rare (2% of the lakes) and >90% of the lakes had TN : TP >17 suggesting P-limitation (Table 1).

Relationships of TP and color to hydrogeomorphology— Based on multiple regression analysis, TP and color had similar relationships with basin and catchment morphometry, but different relationships with land cover. For both TP and color, the best HGM and land cover model contained the predictors Z$_{max}$, CA : LA, % forest, and % wetland (Table 4). Standardized parameter estimates indicated a stronger negative relationship between TP and % forest than for % wetland (Table 4). In contrast, color was positively related to both land cover types and standardized parameter estimates were similar. For the WRT and land cover model, WRT was a much better predictor of color than TP (Table 4). While both TP and color exponentially declined with WRT (Fig. 4A,B) the relationship was stronger for color such that lakes exceeding 20 PCU were rare when WRT >1 yr (Fig. 4B). When WRT was included as a predictor, % wetland was no longer included in the TP model but became more important than % forest in the color model (Table 4).

Using multiple comparison tests, lakes in the TP–color classes differed in HGM characteristics (Fig. 5). Lakes in trophic classes with higher TP and color tended to be

←

to TP classes. The horizontal dashed lines at 20 PCU marks the division between low and high color (panel A). Lakes are coded by color class, a dash for low color and open circle for high color, in panels B and C.

Table 2.   Percent of study lakes ($n = 1,646$) in each trophic class defined by total phosphorus (TP) and color.

	Low color ≤20 platinum cobalt units (PCU)	High color >20 platinum cobalt units (PCU)	Total
Oligotrophic TP≤10 μg L^{-1}	41.0	9.7	50.7
Mesotrophic 10<TP≤30 μg L^{-1}	22.9	20.4	43.3
Eutrophic TP>30 μg L^{-1}	2.4	3.6	6.0
Total	66.3	33.7	

shallower, with larger catchment areas (data not shown) and CA : LA ratios (Figs. 5A,C). Lake area did not differ among lake classes (Fig. 5B). For WRT, stronger and more consistent differences occurred between high- and low-color classes than among TP classes (Fig. 5D). High-color lakes had a shorter WRT than low-color lakes within all TP classes. Further, the negative relationship between WRT and TP classes was apparent only for the high-color lakes; WRT was similar among TP classes when only low-color lakes were compared.

Weaker patterns were found when comparing land cover among the TP and color classes (Figs. 5E,F). Lakes with higher TP tended to have higher % wetland cover while color showed no apparent relationship with % wetland. Percent forest cover was highest for oligotrophic lakes, and was lowest by far in the low-color eutrophic lakes.

The Col : Chl a *ratio and lake metabolism*—The median value of the Col : Chl a ratio in our study lakes was 3.0 (Table 1) and 29% of the lakes had Col : Chl a >5.3, which corresponds to an estimated allochthonous carbon supply to consumers of $\sim$ 20% (Pace et al. 2007). These lakes were most common in oligotrophic and mesotrophic classes with high color and rare to absent in high- and low-color eutrophic lakes (Fig. 6A). Differences among color classes in Col : Chl a likely reflect the fact that color is the numerator of the Col : Chl a ratio making statistical comparisons between color classes inappropriate. However, within the color classes, there was an overall trend for lower Col : Chl a ratios with increasing TP. Similar to Pace et al. (2007), the Col : Chl a ratio was negatively related to

lake area in our dataset ($r = -0.115$; $p = 0.0012$) but was more highly correlated with WRT ($r = -0.337$; $p < 0.0001$). Dividing lakes into short (≤1 yr; $n = 1,085$ lakes) and long (>1 yr; $n = 490$ lakes) WRT, we found that within each TP class, lakes with longer WRT had significantly lower Col : Chl a ratios than lakes with shorter WRT (Fig. 6B). As for color, the divergence was most extreme in eutrophic lakes. This result suggests that WRT may control the Col : Chl a ratio through its mediation of TP and color.

Over 90% of the lakes in the 682-lake dataset had pCO_2 estimates >37.5 Pa indicating net heterotrophy. In addition, pCO_2 was positively correlated with the Col : Chl a ratio ($r = 0.200$; $p < 0.0001$; $n = 681$; for log$_{10}$-transformed variables; Fig. 7) as well as color ($r = 0.320$; $p < 0.0001$), and negatively related to lake area ($r = -0.262$; $p < 0.0001$) and WRT ($r = -0.238$; $p < 0.001$). Further, lakes with extremely low pCO_2 only occurred at Col : Chl a ratios <5.3.

Discussion

Influence of TP and color on lake trophic variables—The influence of TP and color on measures of trophic status in our study lakes provides strong quantitative support for incorporating CDOC into the trophic paradigm (Rodhe 1969; Likens 1972; Williamson et al. 1999; Duarte and Prairie 2005). Because the effect of color on Chl a and Secchi transparency was independent of TP, ignoring color could cause serious misinterpretation of limnological data, particularly in attributing causes for lower Secchi trans-

Table 3.   Regression equations relating chlorophyll (Chl a) and Secchi transparency (Sec) to TP and color; all variables were log$_{10}$ transformed. The No. of observations was 1,646 for chlorophyll and 1,602 for Secchi. All model coefficients and F-statistics were significant at $p < 0.0001$. SE is the standard error around the slope estimates provided in the order in which the predictors appear in the regression equation.

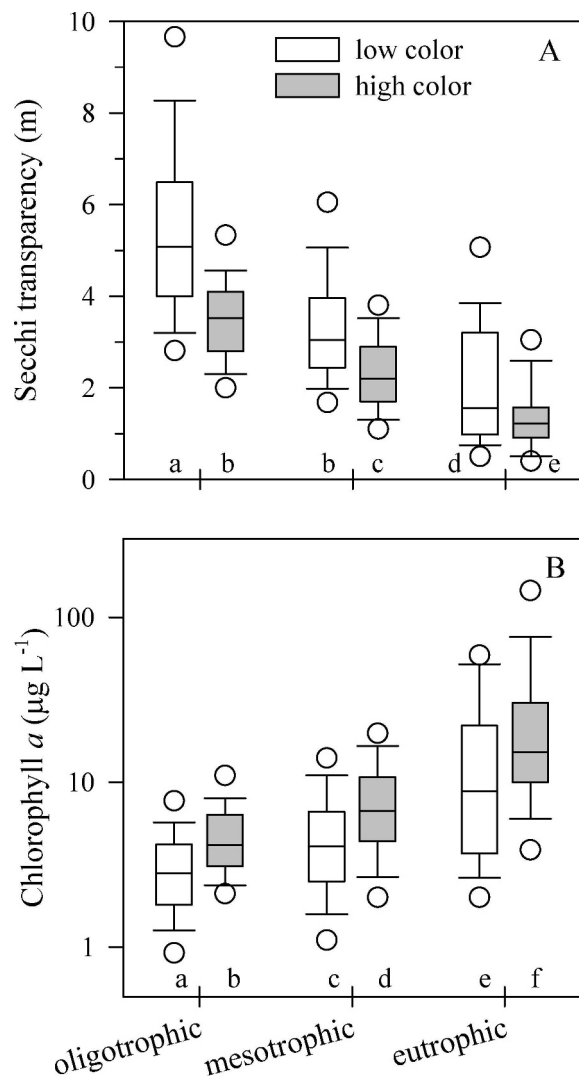| Response variable	Regression eq.	SE	R^2_{adj}
logChl a	0.60logTP+0.015	0.02	0.285
	0.46logCol+0.11	0.02	0.243
	0.44logTP+0.29logCol−0.16	0.02, 0.02	0.364
logSec	−0.56logTP+1.11	0.01	0.528
	−0.38logCol+0.97	0.01	0.372
	−0.44logTP−0.22logCol+1.23	0.01, 0.01	0.627

Fig. 3. Box-plots of (A) Secchi transparency, and (B) chlorophyll *a* within classes defined by TP (oligotrophic, mesotrophic and eutrophic) and color (open boxes are low color ≤20 PCU, grey boxes are high color >20 PCU). All *y*-axes are plotted on a $\log_{10}$ scale except for Secchi transparency. Box boundaries indicate the 25th and 75th percentiles; whiskers represent the 10th and 90th percentiles; the inner horizontal line is the median; circles indicate the 5th and 95th percentiles. Boxes marked with different letters are significantly different (*p* < 0.05) based on multiple comparison tests using all six classes.

lakes is the restriction of algal populations within a smaller volume of suitable photic zone habitat. A second explanation is that our sampling methods did not capture metalimnetic peaks in algal biomass, such as those found in mesotrophic lakes with poorly mixed water columns (Klausmeier and Litchman 2001). Chl *a* measured on an areal basis (per m^2 of lake surface area) should be the same in colored and clear lakes if these mechanisms were operational. However, Carpenter et al. (1998) found that areal measures of Chl *a* had lower values in lakes with higher CDOC, which they attributed to the effects of shading. Other explanations include: (1) the fact that many humic lakes have distinct algal assemblages, often dominated by high densities of motile flagellates such as cryptophytes (Jones 1992), (2) the selective attenuation of UV by humic substances that protects algae from photo-inhibition in the surface layers (Moeller 1994), and (3) higher Chl *a* content produced by individual phytoplankton cells in response to low light levels (Foy and Gibson 1982, 1993). Although our data and analyses cannot distinguish among these possible mechanisms, our results suggest that this is an interesting and relevant area for future research.

External and internal controls on TP and color—We found that lakes with higher TP and color were shallow, had short WRT, and had large CA : LA ratios. These relationships underscore the importance of *external* controls related to the strength of connections between a lake and catchment sources and *internal* controls that determine the fate of these two trophic drivers.

Although WRT is influenced by external hydrologic factors related to hydraulic loading rates, we use WRT as a measure of the relative influence of internal loss processes on TP and color. WRT is a key component of nutrient-loading models for TP in lakes and determines the importance of biological uptake and recycling of phosphorus (Smith 1998). WRT also influences the importance of internal processing of CDOC, which is degraded by photolysis in the presence of UV radiation and microbial breakdown (Jones 1992; Wetzel et al. 1995; Morris and Hargraves 1997) and largely lost to the atmosphere as CO_2 or to the sediments (Molot and Dillon 1997*a*). In our lakes, WRT was more closely related to color than to TP such that when WRT >1 yr, color rarely exceeded 20 PCU. One explanation for this result is that this strong control of color by WRT may be partly a function of our summer sampling period. For example, based on the lower ratios of color to DOC measured in Ontario lakes during summer low flow Molot and Dillon (1997*a*) concluded that photobleaching during the summer caused high loss rates of colored DOC; a 1.8-yr WRT was sufficient for removal of photosensitive components of DOC. The seasonal patterns in the color-to-DOC ratio suggest that during spring and autumn the relationship between color and WRT may be weaker as 'newer' and more colored DOC is supplied by runoff events. However there is other evidence that such a seasonal effect may be less important compared to temporal variability related to the frequency and intensity of precipitation events that supply 'new' DOC

parency solely to increased algal biomass stimulated by nutrient enrichment.

In our study lakes, within a given TP class, those with higher color had lower Secchi transparency and higher Chl *a*, a pattern also observed by Nürnberg and Shaw (1999). Several alternative mechanisms can explain this positive relationship between color and Chl *a*. Humic substances have large effects on the vertical distribution of light and thermal stratification so that for lakes of comparable size, colored lakes may have shallower euphotic zones than noncolored lakes due to increased attenuation of light (Snucins and Gunn 2000; Houser 2006). Therefore, one explanation for higher Chl *a* concentrations in colored

Table 4. Multiple regression results for TP and color as predicted by hydrogeomorphic (HGM) features. Two regression models for each response variable are displayed: (a) the model using lake and catchment morphometry and land cover variables, and (b) the model that included only WRT and land cover variables. Values are standardized parameter estimates for each predictor variable with significance levels represented by asterisks (* $p < 0.05$; ** $p < 0.001$). $n =$ No. of observations. See Table 1 for abbreviations of predictor variables.

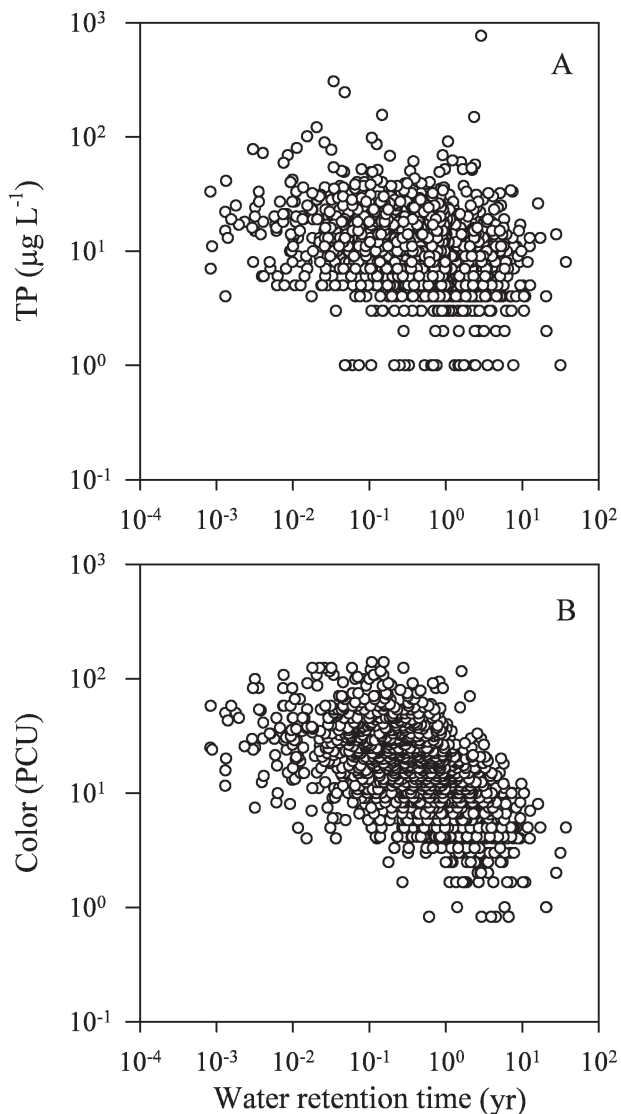|	(a) morphometry and land cover model		(b) WRT and land cover model	
	TP	Color	TP	Color
WRT	—	—	−0.28 **	−0.55 **
Z_{max}	−0.44 **	−0.41 **	—	—
CA : LA	0.07 *	0.34 **	—	—
%FOR	−0.40 **	0.13 **	−0.36 **	0.10 **
%WET	−0.06 *	0.14 **	Ns	0.16 **
N	1,597	1,597	1,575	1,575
Adj R^2	0.346	0.355	0.208	0.316

Fig. 4. (A) TP and (B) color against WRT (retention time in yr) plotted on $\log_{10}$ scale.

to lakes even during the summer months (Pace and Cole 2002). While we were unable to control for seasonal or climatic effects on color, our results, combined with observations that CO_2 (Sobek et al. 2003) and both DOC and color (Rasmussen et al. 1989; Gergel et al. 1999) have negative relationships with WRT, suggest that WRT plays a key role in carbon as well as phosphorus cycling in lakes.

For external controls, we found that important predictor variables in our models confirmed previous studies for both TP (Dillon and Rigler 1974; Håkanson 1996) and DOC or water color (e.g., Rasmussen et al. 1989; Kortelainen 1993; Gergel et al. 1999; Canham et al. 2004). We found that TP and color had similar relationships with the HGM features catchment area and lake depth, but they had divergent relationships with % forest and % wetland. In the 500-m buffer around our study lakes, % forest was negatively correlated with human-influenced land cover defined as the sum of % agriculture and % urban development ($r = -0.83$). Therefore, we expected increasing TP with decreasing % forest. Because TP is also exported from forested catchments (Dillon and Rigler 1974), the negative relationship between TP and % forest indicates that TP export from human-influenced landscapes overwhelms that from forests and wetlands. The positive relationship between color and % wetlands and % forest in our lakes has been observed in other studies that focused on predictors of DOC in lakes (Kortelainen 1993; Xenopoulos et al. 2003; Canham et al. 2004). However, % wetlands did not explain much variation in either TP or color in our lakes, which is consistent with Gergel et al. (1999) and Xenopoulos et al. (2003) who both found that multiple regressions predicting lake DOC from wetland cover and other HGM features had low predictive power ($R^2 = 0.27$–0.38). Other results from Gergel et al. (1999) suggest that aggregating land cover at spatial extents other than the 500-m buffer used here may improve on these relationships. Unfortunately we did not have the data to explore this interesting question further.

Our results show that the external controls on the amount of TP and DOC exported from the catchment combined with internal controls related to WRT produce heterogeneity in TP and color among lakes in the
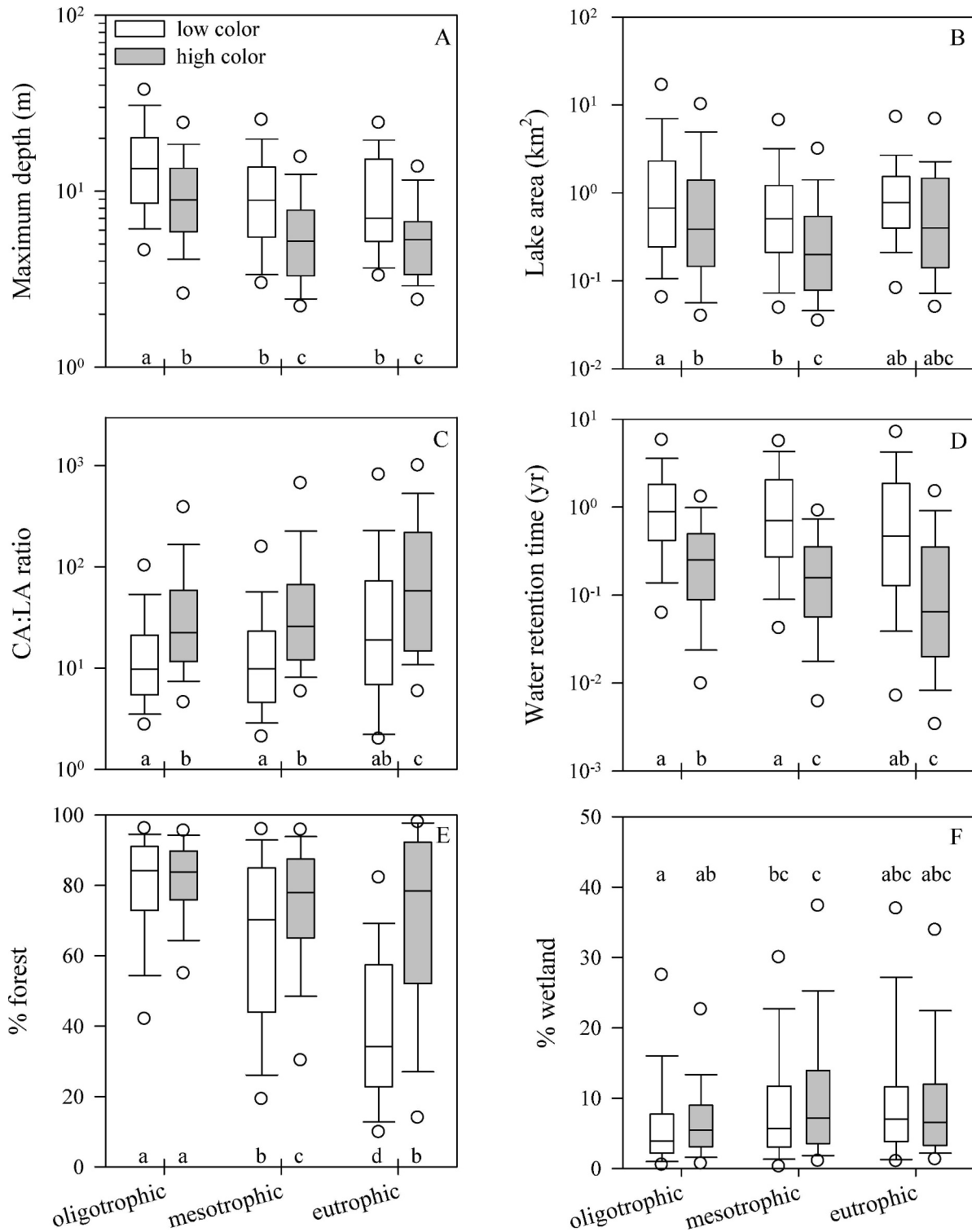
Fig. 5. Box-plots of (A) maximum depth, (B) lake area, (C) CA : LA, (D) WRT, (E) % forest, and (F) % wetlands within TP classes (oligotrophic, mesotrophic, and eutrophic) and color classes (open boxes are low color ≤20 PCU; grey boxes are high color >20 PCU). All y-axes are plotted on a $\log_{10}$ scale except for % forest and % wetland. Box boundaries indicate the 25th and 75th percentiles; whiskers the 10th and 90th percentiles; the inner horizontal line is the median; circles indicate the 5th and 95th percentiles. Boxes marked with different letters are significantly different ($p < 0.05$) based on multiple comparison tests using all six classes.
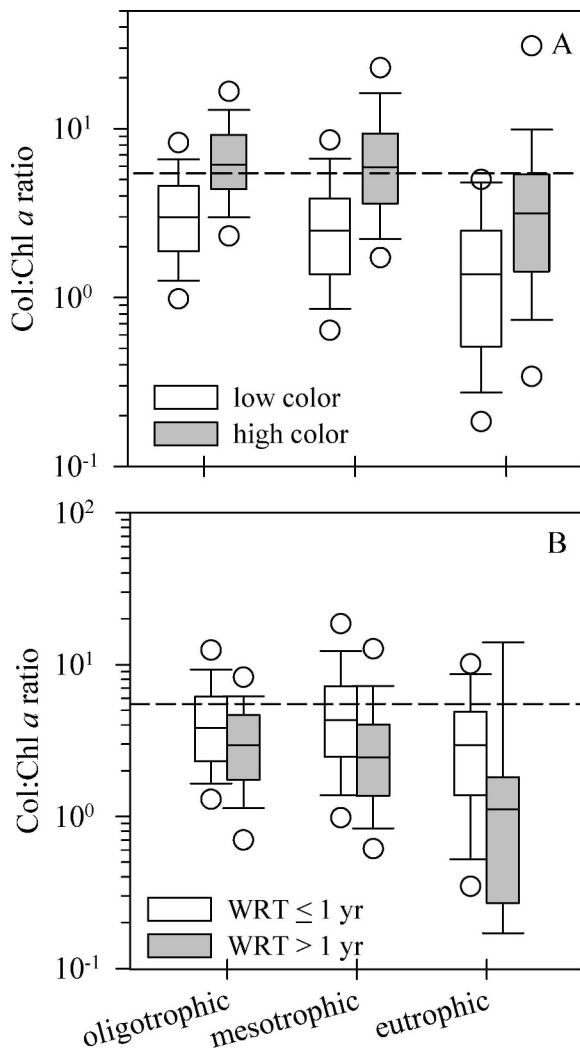
Fig. 7.   Plot of $p\mathrm{CO_2}$ and Col : Chl a ratio for 682 lakes. The regression line is shown by the solid line. The atmospheric concentration of $p\mathrm{CO_2} = 37.5$ Pa is shown as the dashed line. The arrow on the *x*-axis indicates the value of Col : Chl $a = 5.3$ corresponding to an estimate of 20% supply of allochthonous carbon to lake food webs in Pace et al. (2007).

Fig. 6.   Box-plots of (A) Col : Chl a ratio (on $\log_{10}$ scale) for trophic classes defined by TP (oligotrophic, mesotrophic, and eutrophic) and color (open boxes are low color ≤20 PCU, grey boxes are high color >20 PCU); and (B) Col : Chl a ratio (on a $\log_{10}$ scale) for trophic classes defined by TP (oligotrophic, mesotrophic, and eutrophic) and WRT (open boxes are lakes with short WRT ≤1 yr, grey boxes are lakes with long WRT >1 yr). Boxes represent the median with 25th and 75th percentiles; whiskers represent the 10th and 90th percentiles; circles represent the 5th and 95th percentiles. Boxes marked with different letters are significantly different ($p < 0.05$) based on multiple comparison tests. The horizontal dashed line at Col : Chl a equal to 5.3 corresponds to an estimate of 20% supply of allochthonous carbon to lake food webs determined by Pace et al. (2007).

landscape. However, the rarity of lakes with low TP and high color or high TP and low color emphasizes some commonalities in the pathways supplying these terrestrially derived solutes to lakes and the mechanisms influencing in-lake concentrations.

The nutrient-color paradigm and lake metabolism—The nutrient-color paradigm implies that the CDOC and TP axes reflect mechanisms and processes that favor heterotr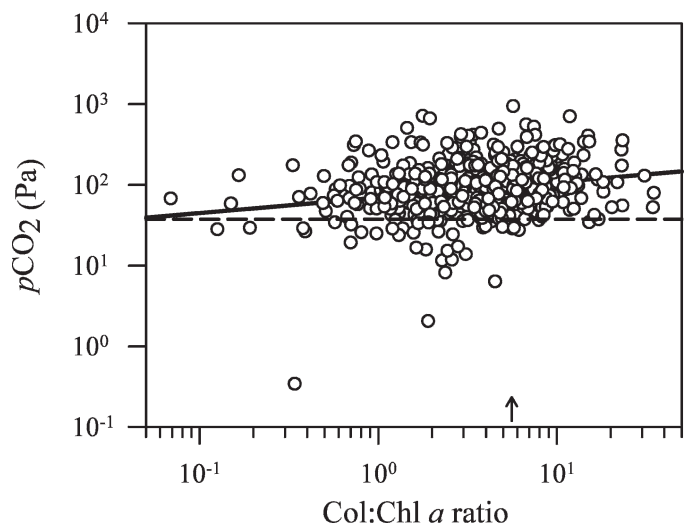ophy and autotrophy, respectively. Studies designed to examine patterns along gradients of nutrients and humic color or DOC using either direct measurements of heterotrophy as the excess of respiration (R) over gross primary productivity (GPP; Prairie et al. 2002; Hanson et al. 2003), inferred heterotrophy from measurements of $p\mathrm{CO_2}$ (Sobek et al. 2003), or estimated allochthony based on stable isotope measurements (Bade et al. 2007) support this understanding. Unproductive systems are considered net heterotrophic (Duarte and Prairie 2005) with the degree of heterotrophy related to DOC (Prairie et al. 2002; Hanson et al. 2003). Across gradients in DOC, higher DOC is associated with GPP≪R and lakes that are strongly heterotrophic (Cole et al. 2000; Prairie et al. 2002; Sobek et al. 2003). In contrast, when nutrients are high and DOC is low, lakes are more autotrophic (Cole et al. 2000; Hanson et al. 2003; Duarte and Prairie 2005). More difficult to address is the middle range of TP and CDOC where many lakes reside, including most of our study lakes.

In accordance with results from other studies (e.g., Cole et al. 2000), a majority of our study lakes were heterotrophic based on $p\mathrm{CO_2}$ saturation; however, the amount of terrestrial carbon that actually subsidizes lake food webs remains in question. We found a statistically significant increase in Col : Chl a with increasing $p\mathrm{CO_2}$ (Fig. 7), although the relationship was noisy. However, this suggests that as lake heterotrophy increases, the terrestrial carbon subsidy also increases. If we use Pace et al.'s (2007) value of 5.3 (in PCU units) corresponding to a contribution of 20% terrestrial carbon to pelagic consumers, with higher Col : Chl a indicating higher terrestrial contributions, then 71% of our study lakes had Col : Chl a ratios lower than 5.3. The lowest values were in low-color eutrophic lakes and those with ratios exceeding 5.3 were mainly high-color oligotrophic and mesotrophic lakes. These results suggest

that although heterotrophy is a common metabolic state for our study lakes, subsidies of terrestrial carbon to aquatic consumers may be important in only a subset of the lakes. An alternative explanation for the high variation associated with our pCO_2 data is that values were calculated using pH from samples that may have been held for up to a week before laboratory analysis and did not always have a closed headspace. As a result, the relationship between Col : Chl a and pCO_2 may be dampened, with both supersaturated and undersaturated values tending towards equilibrium with the laboratory atmosphere. With these caveats and the assumption that the Col : Chl a ratio reflects transfer of terrestrial carbon to pelagic consumers, as explored in Pace et al. (2007), we conclude that in this set of predominately heterotrophic lakes, the relative contribution of terrestrial carbon subsidies to pelagic consumers may be low and is influenced by HGM features related to lake water retention time and nutrient status.

It is important to note that the extrapolation of results from the small No. of lakes studied by Pace et al. (2007) to our more diverse set of lakes should be made with caution due to uncertainties in the effects of lake features like surface area and WRT. Our set of lakes covers a much wider range in nutrients and water color as well as a broad range of lake area. Pace et al. (2007) discussed the possible confounding effects of lake area on using the Col : Chl a ratio to estimate terrestrial subsidies to pelagic food webs. Because larger lakes have a lower perimeter-to-lake-area ratio than smaller lakes, areal loading rates of terrestrial carbon may also be lower. In our lakes, we found a negative relationship between the Col : Chl a ratio and lake area, although correlations with water retention time were stronger. The positive correlation between the Col : Chl a ratio and pCO_2 evident in Fig. 7 provides independent support for the use of the ratio as a measure of allochthony although the low correlation suggests there is much noise in the relationship. The observation that very few lakes with WRT exceeding 1 yr had appreciable Col : Chl a ratios supports the contention that WRT is a key mediator of the combined influence of nutrient enrichment and, possibly, terrestrial subsidies of carbon to lake trophic status.

Our results correspond to other studies reporting that lakes with high color, indicating greater inputs of allochthonous CDOC, should be more heterotrophic, and that autochthony should become dominant only in productive, high-TP lakes (Cole et al. 2000; Prairie et al. 2002; Duarte and Prairie 2005). In short, our data quantitatively confirm previous studies concluding that both TP and DOC are important drivers of lake metabolism and further emphasize an important role for modulation by HGM features that control external and internal processes.

We quantified a large influence of humic color on lake trophic properties, rivaling more well-understood relationships with TP. These patterns emerged because we studied a large and diverse set of north-temperate lakes that facilitated detection of patterns difficult to discern in lakes covering narrower ranges in TP and color and located within smaller geographic areas. In addition to being major drivers of lake trophic status, TP and color reflect a balance between internal loss processes and external connections between lakes and their catchments that are reflected in HGM features. We further showed how these relationships might provide additional insights into questions regarding lake metabolism and the importance of allochthonous subsidies of carbon to pelagic food webs. The nutrient-color paradigm is a powerful conceptualization of how linkages between terrestrial and aquatic systems, mediated by hydrogeomorphology, determine how terrestrial subsidies of TP and CDOC are expressed in lakes across diverse landscapes.

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