The light : nutrient ratio in lakes: a test of hypothesized trends in bacterial nutrient limitation

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Abstract
The light-to-nutrient hypothesis explores how the balance between energy (as light energy) and nutrients (as total phosphorus) shapes aquatic ecosystem structure and process. The balance of energy and nutrients is thought to regulate ecosystem structure and process such that, in a “high” light-to-nutrient environment, bacteria would probably be driven towards phosphorus (P) limitation, whereas, in a “low” light-to-nutrient environment, bacteria would be driven towards carbon (C) limitation. We assessed the growth limitation of bacteria in two reservoirs of the southern U.S.A. using a mortality-corrected dilution-growth approach. We compared the frequency of P and C growth limitation with the intralake variation in the light-to-nutrient environment. As a metric of the light-to-nutrient environment, we used the ratio of the mean light in the surface mixed layer (\(I_m\)) to the total phosphorus concentration (\(TP\)). In each lake, bacterial growth was more often P-limited when the \(I_m:TP\) ratio was above the median ratio than below. We believe our data provide the first evidence supporting this aspect of the light-to-nutrient hypothesis.

Keywords
Bacteria, lakes, light : nutrient ratio, nutrient limitation.

INTRODUCTION
The light-to-nutrient hypothesis (Sterner et al. 1997) suggests that the balance of energy entering an aquatic system (as light) and the availability of nutrients (as total phosphorus) regulates the elemental composition of autotrophs [the carbon : phosphorus (C : P) ratio], with attendant consequences affecting ecosystem structure and process. The light : nutrient ratio may act as a metric against which several ecosystem properties may be mapped, including herbivore gross growth efficiency, herbivore community composition and rate of consumer P recycling. One of the attractive features of the light-to-nutrient hypothesis is the emphasis on resource-driven effects on communities. These effects extend from bacteria through metazoan consumers, and thereby place aquatic bacteria into a broad, comprehensive view of aquatic system dynamics.

Sterner et al. (1997) hypothesized that the balance of energy and nutrients would regulate ecosystem structure and process such that, in a “high” light-to-nutrient environment, bacteria would probably be driven towards P limitation, as abundant light energy coupled with low nutrient availability and high competition for nutrients would shift autotrophs away from biomass production and promote the exudation of labile carbon (C) (Obernosterer & Herndl 1995). Similarly, in a “low” light-to-nutrient environment, nutrient conditions would promote autotroph biomass production rather than C excretion and drive the bacteria towards C limitation.

During a multiyear study of nutrient dynamics in two reservoirs of the southern U.S.A., we collected data that allowed us to test this aspect of the light-to-nutrient hypothesis. Specifically, we examined the frequency of bacterial growth limitation by P and C under both “high” and “low” light-to-nutrient environments.

MATERIALS AND METHODS
The two reservoirs in this study, Eagle Mountain Lake (EML) and Joe Pool Lake (JPL), are located in the north Texas region of the southern U.S.A. EML (32°35’N, 97°00’W, filled in 1932) has a surface area of 3638 ha and
a mean depth of 6.2 m. The lake is mesotrophic and stoichiometric indices of nutrient deficiency (Guildford & Hecky 2000) characterize the system as moderately P-limited (Table 1). Peak algal abundance occurs in late summer with abundant filamentous cyanobacteria. Additional information about EML may be found in Sterner & Grover (1998). JPL (32°35'N, 97°0'W) was impounded in 1986 and has a surface area of 3620 ha and a mean depth of 7.2 m. The lake is also mesotrophic and can be characterized stoichiometrically as extremely P-deficient (Table 1).

Peak algal abundance occurs in mid-summer (mixed-species bloom of the diatom *Aulacoseira granulata* and several cyanobacteria). Additional information about this lake may be found in Sterner (1994).

Each lake was sampled between March 1998 and December 1999 at a single station near the deepest part. The lakes were sampled about every 2 weeks when water temperature was above 16 °C and monthly at other times. Photosynthetically active radiation was measured at the surface (as non-reflective radiation) and at 1 m intervals (Li-Cor model LI-185B coupled to a spherical sensor). Depth profiles of temperature and dissolved oxygen were taken (YSI model 33) and, from these, the depth of the surface mixed layer was determined. Both lakes stratified only occasionally and weakly during the period of sampling. When lakes were not stratified, the bottom was taken as the limit of mixing.

As an index of energy supply to the system, we used the mean irradiance in the surface mixed layer, $I_m (\mu E/m^2/s)$, where

$$I_m = \left[ \left( \frac{\alpha}{I_0} \right) I_0 (1 - e^{-Kz_{mix}}) / KZ_{mix} \right]$$

and $z$ is the irradiance entering the water column (the intercept of a plot of $\ln I_\circ$ against depth, where $I_\circ$ is the irradiance at depth $\circ$), $I_0$ is the non-reflective surface irradiance, $I_m$ is the surface irradiance expected for the sampling period, $K$ is the attenuation coefficient and $Z_{mix}$ is the depth of the mixed layer. Sterner et al. (1997) recommended the scaling of the surface irradiance out of this index when comparing lakes over broad geographical areas; however, we retain it here as it is an important component of the seasonal variation in the radiant energy supplied to a given lake.

Water samples were taken with a 6 L Van Dorn bottle at discrete depths near the top, bottom and middle of the mixed layer, screened through 153 μm Nitex and combined in 20 L polyethylene carboys to create a pooled mixed layer (PML) sample. Three such pooled samples were taken during each sampling, thereby creating true triplicate samples.

As an index of nutrient supply to the plankton, we used the total phosphorus ($TP, \mu m$), which includes particulate (seston) and dissolved forms of P (Menzel & Corwin 1965).

The effect of nutrients on the growth of bacteria was assessed using a mortality-corrected dilution-growth approach (Landry & Hassett 1982). Details of the methods may be found in Chrzanowski et al. 1995. Briefly, equal portions of each PML sample were combined and diluted 1 : 9 (whole : filtered) with filtered lake water. The filtered lake water was prepared from equal portions of each PML sample that had been combined and filtered (0.2 μm). Samples of bulk diluted water were taken for the determination of the initial bacterial abundance. Diluted water (500 mL) was dispensed to 700 mL clear polycarbonate bottles. Nutrient spikes, consisting of nitrogen (N), phosphorus (P) and carbon (C), were added in a factorial design. N was added (as NH₄Cl) to 50 μM above ambient, P was added (as NaH₂PO₄) to 10 μM above ambient and C was added (as equimolar portions of glucose and sodium acetate) to 83 μM above ambient, and controls received no supplements.

All dilutions were randomly placed on a rotary shaker held in a controlled environment room, where the irradiance (fluorescent lamps) was adjusted to equal $I_m$ at the time of sampling, the photoperiod was adjusted to 14 h : 10 h (light : dark) and the temperature was adjusted to equal the mean temperature of the mixed layer at sampling. Dilutions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Eagle Mountain Lake</th>
<th>Joe Pool Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Minimum</td>
</tr>
<tr>
<td>$I_m (\mu E/m^2/s)$</td>
<td>191.2</td>
<td>45.4</td>
</tr>
<tr>
<td>$TP (\mu m)$</td>
<td>1.75</td>
<td>1.13</td>
</tr>
<tr>
<td>C : N</td>
<td>12.9</td>
<td>8.0</td>
</tr>
<tr>
<td>C : P</td>
<td>204.3</td>
<td>50.6</td>
</tr>
<tr>
<td>N : P</td>
<td>16.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Bacteria (10⁶ cells/L)</td>
<td>4.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Chlorophyll (μg/L)</td>
<td>19.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>23.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

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were sampled after 24 h of incubation and the growth rates of bacteria were determined from the epifluorescence direct counts of formaldehyde (2% final concentration)-preserved samples (Porter & Feig 1980). We assumed no lag phase prior to exponential growth, and thus our growth rate estimates should be considered as conservative.

The impact of added nutrients on bacterial growth was analysed using analysis of variance (ANOVA) for factorial designs, with a separate analysis conducted for each sampling date. All treatments had three replicates. The significance of all main effects and interactions was tested ($P < 0.05$).

**RESULTS AND DISCUSSION**

Sterner et al. (1997) hypothesized that the balance of energy and nutrients would regulate ecosystem structure and process such that, under “high $I_m : TP$ conditions”, bacteria would probably be limited by $P$, as abundant light energy coupled with low nutrient availability would shift phytoplankton away from biomass production and promote carbon exudation. Similarly, under “low $I_m : TP$ conditions”, bacterioplankton would probably be limited by $C$, as $P$ availability would promote phytoplankton biomass production rather than $C$ excretion.

In developing the concept of the light : nutrient ratio and how it might regulate ecosystem structure and process, Sterner et al. (1997) focused primarily on interlake differences and only loosely specified the conditions that delimit “high $I_m : TP$” or “low $I_m : TP$”. However, the $I_m : TP$ ratio also varies seasonally within lakes. Irradiance reaches a seasonal maximum during mid-summer at the time when $TP$ in the mixed layer typically approaches a seasonal minimum. Here, rather than addressing interlake differences in $I_m : TP$, we address the light-to-nutrient hypothesis relative to bacterial nutrient limitation by considering intralake variability in $I_m : TP$ and seasonal nutrient regulation of bacterial growth. To establish “high $I_m : TP$” or “low $I_m : TP$” conditions, we calculated the median $I_m : TP$ for each lake and considered values above the median to be “high” and those below the median to be “low”. This separation imposes a set of stringent conditions on the separation criteria, as intermediate $I_m : TP$ conditions, where nutrient limitation may be lessened or not easily predicted, are not considered separately.

Figure 1 portrays the variation in $I_m : TP$ in EML and JPL. The letters above each data point associate the light-to-nutrient conditions with carbon (C) or phosphorus (P) growth limitation of bacteria, determined from dilution-growth experiments. In this analysis, we excluded cases in

![Figure 1](image.png)

**Figure 1** Relationship between the light-to-nutrient environment ($I_m : TP$) and nutrient limitation of bacteria in Eagle Mountain Lake (top) and Joe Pool Lake (bottom). Letters C and P represent C and P limitation, respectively. Single element nutrient limitation was not detected where letters are absent.
which the addition of both C and P stimulated growth interactively, making the identification of the main effects of C and P ambiguous. \( I_{m}:TP \) in each lake had a strong seasonal signature: the ratio was high in the summer months and low during the winter months. The median \( I_{m}:TP \) for each lake is shown as a horizontal line.

We calculated the frequency of P limitation and C limitation under low \( I_{m}:TP \) and high \( I_{m}:TP \) conditions (Fig. 2). In both lakes, there was a greater frequency of P limitation under high \( I_{m}:TP \) conditions than under low \( I_{m}:TP \) conditions (chi-squared test, \( P < 0.01 \)). Similarly, in both lakes, C limitation was more frequently encountered under low \( I_{m}:TP \) conditions than under high \( I_{m}:TP \) conditions; however, the data could not be separated statistically. We consider this latter result somewhat surprising, not because we were unable to demonstrate a statistically higher frequency of C limitation under low \( I_{m}:TP \) conditions, but because the signal existed at all.

EML and JPL are reservoirs with distinct recharge characteristics. During the warmer parts of the year, the lake levels fall due to decreased rainfall, and increased evaporation and water use. The lakes recharge during the wet, cool winters. Typically, lake levels fluctuate by about a metre between mid-summer and mid-winter. During the cooler season, the lakes are typically turbid as increased flow from the watersheds suspends sediments and brings nutrients into the systems (Sterner 1994). It seems likely that allochthonous inputs of labile carbon would also occur at this time, and so detecting any nutrient limitation during periods of recharge assumes some importance.

Sterner et al. (1997) developed the idea of the light-to-nutrient hypothesis using lakes situated on the Canadian Shield and one of the Laurentian Great Lakes. They pointed out that some of the ecosystem processes predicted by the light-to-nutrient hypothesis might be modified by systemspecific characteristics, such as, for example, systems whose dissolved organic carbon dynamics are driven by allochthonous inputs. Clearly, EML and JPL fall within this description during the winter recharge. Data collected during the high \( I_{m}:TP \) conditions typical of the summer clearly support the concepts proposed by the light-to-nutrient hypothesis. Furthermore, EML and JPL are quite different from each other with respect to age and nutrient conditions and, collectively, the two lakes are quite different from the lakes of the north temperate zone for which the light-to-nutrient hypothesis was developed. Despite these differences, bacteria in both EML and JPL appeared to respond similarly to changes in the light-to-nutrient conditions, and trends in nutrient limitation are in line with those predicted by the hypothesis.

When considered as a whole, we believe that this is the first test of some of the concepts of the light-to-nutrient hypothesis and that our data support the hypothesized trends in ecosystem processes relative to bacterial nutrient limitation.

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REFERENCES


BIOSKETCH

Thomas Chrzanowski’s research focuses upon the microbial ecology of freshwater and estuarine systems. He is particularly interested in determining how nutrient availability structures microbial food webs.

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