Effects of whole-lake manipulations of nutrient loading and food web structure on planktonic respiration

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Abstract: We assessed planktonic respiration in whole-lake manipulations of nutrient loading and food web structure in three manipulated and one unmanipulated lake over 7 years. The manipulations created strong contrasts in zooplankton body size across a series of nutrient loads. Large-bodied zooplankton were suppressed by planktivorous fish in one lake, while in the other two manipulated lakes, large-bodied zooplankton dominated community biomass. Nutrients were added as inorganic N and P. Nutrient loads ranged from background to conditions resembling eutrophic lakes. Planktonic respiration was measured weekly in each lake by dark bottle oxygen consumption. Respiration was low when lakes were not fertilized (average 8.5 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$) and was correlated with differences in dissolved organic carbon among the lakes. Respiration increased with nutrient addition to a mean range of 12–25 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$; however, respiration differed among lakes at the same nutrient loading. Further, respiration was independent of dissolved organic carbon in the fertilized lakes. Differences in the intensity of zooplankton grazing as determined by food web structure strongly regulated primary and bacterial production across the range of nutrient loads. Consequently, respiration was positively related to primary production, phytoplankton biomass, and bacterial production and inversely related to the average size of crustacean zooplankton.

Résumé : Nous avons évalué sur une période de sept ans la respiration planctonique et la structure du réseau alimentaire dans trois lacs où nous avons manipulé globalement la charge de nutriants, ainsi que dans un lac laissé intact. Les manipulations ont entraîné des variations importantes de la taille corporelle des zooplanctontes sous diverses charges de nutriants. Le zooplancton de grande taille a été supprimé par les poissons planctivores dans l'un des lacs, tandis que, dans les deux autres lacs manipulés, le zooplancton de grande taille constituait la plus grande partie de la biomasse de la communauté. Les nutriants ajoutés étaient de l'azote et du phosphore inorganiques. Les charges de nutriants variaient depuis des concentrations de fond naturelles jusqu'à des concentrations correspondant à peu près à celles des lacs eutrophes. On a mesuré hebdomadairement la respiration planctonique dans chaque lac en quantifiant la consommation d'oxygène dans des bouteilles qui bloquent la lumière. La respiration était faible dans les lacs non fertilisés (moyenne de 8,5 μmol $O_2 \cdot L^{-1} \cdot jour^{-1}$), et était corrélée avec les différences dans les quantités de carbone organique dissous (COD) entre les lacs. Le taux de respiration s'accroissait quand on ajoutait des nutriants pour atteindre des valeurs moyennes de 12 à 25 μmol $O_2 \cdot L^{1} \cdot jour^{1}$; cependant, il différait d'un lac à l'autre pour une même charge de nutriants. De plus, la respiration était indépendante du COD dans les lacs fertilisés. Les différences dans l'intensité du broutage par le zooplancton reflétées par la structure du réseau alimentaire régulaient fortement la production primaire et bactérienne sous les diverses charges de nutriants. Ainsi, le taux de respiration variait en raison directe de la production primaire, de la biomasse de phytoplancton et de la production bactérienne, et en raison inverse de la taille moyenne des crustacés du zooplancton.

[Traduit par la Rédaction]

Introduction

Respiration is the endpoint of ecosystem metabolism representing the consumption and degradation of both autotrophically produced and allochthonous carbon. Respiration, therefore, represents an integrative measure of carbon utilization through both autotrophic and heterotrophic pathways in aquatic ecosystems and thus is a metric of system activity. In lakes, it is well known that respiration tends to increase with primary production, nutrient loading, and general measures of lake trophic condition (del Giorgio and Peters 1994). Similar patterns apply to other aquatic ecosystems (Duarte and Agustí 1998). Respiration also tends to be higher in dystrophic lakes rich in dissolved organic carbon (DOC) where microorganisms degrade allochthonous carbon from terrestrial and wetland inputs in addition to autochthonous sources (Salonen et al. 1983; Tranvik 1992). Respiration also increases with temperature, reflecting the strong coupling between metabolism and temperature in organisms (Peters 1983). Beyond these few general patterns, however, the regulation of respiration in lakes is poorly known, particularly in relation to current models that integrate the effects of food web structure and nutrients on phytoplankton primary production (e.g., Carpenter et al. 1996).

Planktonic respiration is the oxidation of organic matter

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by the entire community. In practice, crustacean zooplankton are too large and variable to include in the measurement of planktonic respiration (see Methods). Thus, planktonic respiration, as measured, is primarily the result of the metabolic activities of microorganisms (i.e., phytoplankton, bacteria, protozoans) as well as the smallest metazoans such as rotifers. The biomass and metabolism of these constituents are related to the loading of limiting nutrients such as N and P. Variation in planktonic communities, however, at a given level of nutrient input is related to a variety of factors including the type and size of zooplankton grazers. In lakes dominated by large-bodied species of *Daphnia* (>1 mm in length), intensive grazing on phytoplankton and heterotrophic microbes often leads to lower standing stocks (Carpenter et al. 1996; Pace et al. 1998). The presence or absence of large-bodied *Daphnia* is, in turn, dictated by the degree of size-selective fish predation. Lake food web structure, therefore, might have strong effects on respiration by promoting or suppressing large-bodied zooplankton that graze effectively on both phytoplankton and bacteria.

Another factor that may be independent of food web effects and of greater significance in determining respiration is the loading and consumption of DOC. Dystrophic lakes, while generally low in productivity, tend to have relatively high respiration rates, often in excess of primary production (Salonen et al. 1983). Further, the continuous degradation and consumption of DOC stabilizes metabolism in aquatic ecosystems (Wetzel 1992) so that variation in respiration may be distinct from the highly dynamic processes of pelagic primary production and grazing noted above that reflect unstable interactions of phytoplankton and zooplankton. Thus, respiration is generally considered less variable than primary production (Wetzel 1995).

We examined factors related to the variability in respiration in the context of whole-lake food web and nutrient loading manipulations. These experiments allowed us to test if respiration varied among lakes of differing food web structure receiving a range of nutrient loads. The experimental lakes also varied in DOC concentration (as described below). This allowed us to examine if differences among lakes in DOC concentrations led to differences in respiration that were greater than responses to food web manipulations or nutrient loading.

Methods

Study site description

Manipulations were carried out between 1991 and 1997 in a set of lakes located at the University of Notre Dame Environmental Research Center (46°13′ N, 89°32′ W) near Land O' Lakes, Wisconsin. Paul and Peter lakes are adjacent systems separated by a dike and have been extensively described in prior publications (Carpenter and Kitchell 1993). A second lake, Long Lake, was divided during May 1991 using two rubber curtains drawn across narrow points in the lake. The basins at each end of the lake used in this studied will be referred as East Long and West Long lakes. The western curtain was removed in September 1996 to assess possible effects on winter fish survival. We assume that this change had no effect on comparisons of West Long Lake for 1997 with earlier years. Morphometric and hydrologic properties of East Long and West Long lakes are extensively described elsewhere (Christensen et al. 1996; Cole and Pace 1998). All the lakes are small (<4 ha), steep-sided kettle systems that are relatively deep (maximum depths >10 m, means depths 4–6 m).

Paul Lake served as an unmanipulated reference system. Paul Lake has a single fish species, largemouth bass (*Micropterus salmoides*). In 1991, fish were removed from Peter Lake and the system was restocked with minnows consisting of a mixed assemblage composed primarily of golden shiner (*Notemigonus crysoleucas*), northern redbelly dace (*Phoxinus eos*), and fathead minnow (*Pimephalus promelas*). Peter Lake was further stocked as needed throughout the experiment to sustain high rates of planktivory, and large piscivorous fish were excluded (Schindler et al. 1997). In West Long Lake, the resident populations of piscivorous fish (*M. salmoides* and smallmouth bass (*Micropoterus dolomieu*)) were maintained and enhanced with periodic stocking. After the curtain addition in East Long, the lake became darkly stained and DOC concentrations increased (Christensen et al. 1996). Fish abundance declined subsequently and both piscivory and planktivory were low in this system throughout the experiment (J.F. Kitchell, unpublished data).

Beginning in 1993 the three treatment lakes (Peter, East Long, and West Long lakes) were amended weekly with N and P at an N:P ratio >30 by atoms (Carpenter et al. 1998). We manipulated nutrient loading so that loading varied among years but was similar among lakes within a given year with one exception. In 1995, a higher load was added to East Long Lake (Fig. 1). Natural loading was estimated from the Vollenweider equation:

$$(1) \qquad L_P = [P]q_s(1 + q_s/z)^{0.5}$$

where L_P is P loading to the lake (milligrams per square metre per year), [P] is the volume-weighted average lake P concentration (milligrams per cubic metre) based on weekly profiles of seven depths from late May to early September, q_s is the hydraulic load (metres per year) derived from Cole and Pace (1998), and z is the mean depth (metres) derived from morphometric maps of the lakes. Estimates based on this equation agreed well with estimates derived from P retention analyses using dated sediment cores (Houser 1998).

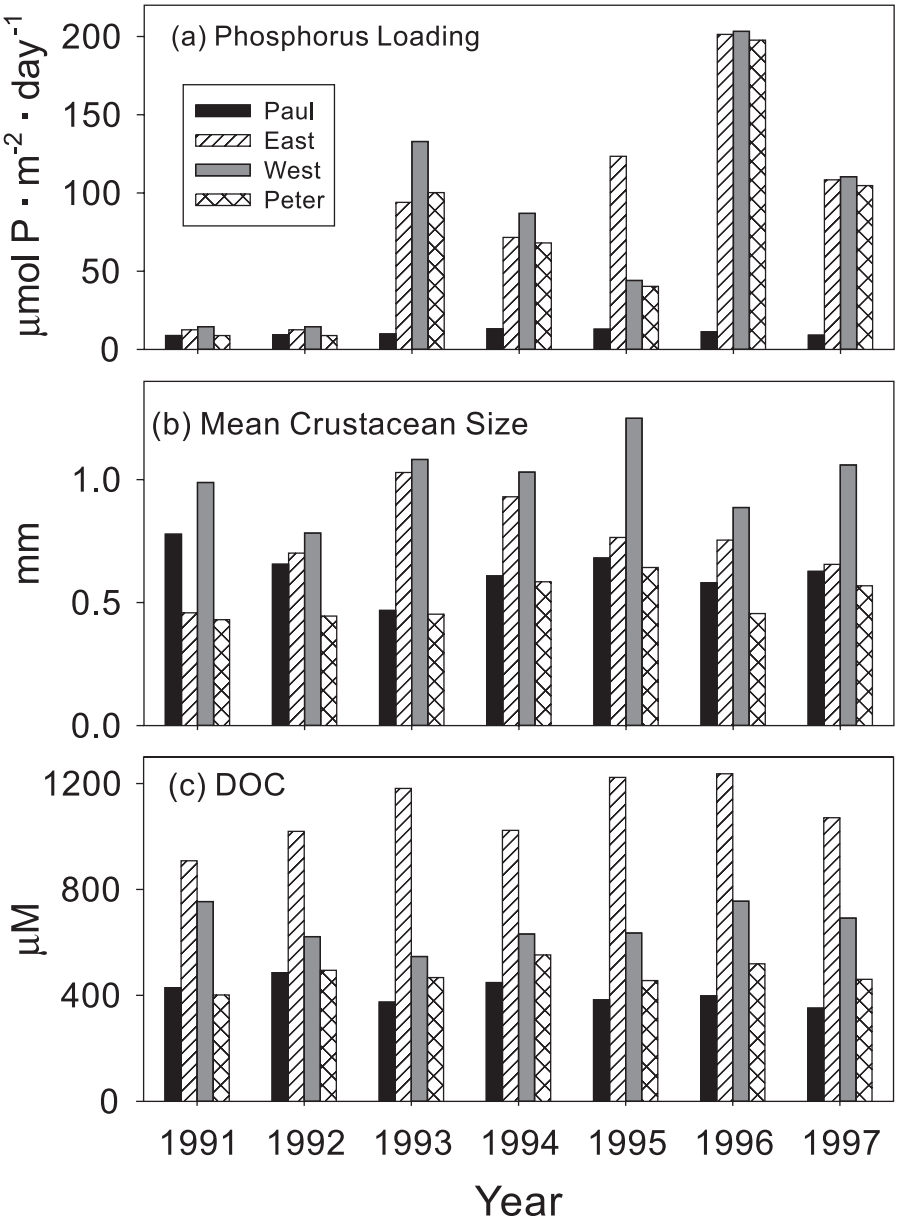
Study design

The manipulations established strong contrasts in three key parameters: nutrient loading, mean crustacean size, and DOC. Nutrient loading was measured by assaying nutrient addition solutions for P and N. We present nutrient loading as micromoles of P per square metre per day based on total P measurements of the nutrient solution because P was the limiting nutrient (see methods and justification in Carpenter et al. 1996). Nutrients were added weekly from June through August. Mean crustacean size was based on the biomass-weighted average size of crustacean zooplankton collected in weekly, calibrated vertical net hauls using an 80-μm-mesh net (Carpenter and Kitchell 1993). DOC was also measured weekly on a pooled epilimnetic sample (GF/F filtrate) using an Astro 2001 TOC analyzer with persulfate and ultraviolet oxidation (1991–1993) and a Shimadzu model 5050 high-temperature TOC analyzer (1994–1997). The two methods gave comparable results based on long-term observations of Paul Lake, and the change in methods is unrelated to the relationships derived below.

Dark bottle respiration

Oxygen consumption in dark bottles was measured weekly over the period from late May to early September. For each lake, four 300-mL BOD bottles were filled to measure initial oxygen concentration. A second set of four dark bottles was filled and immediately returned to the lake. Samples were taken from the surface mixed layer (~1 m) using a peristaltic pump, taking care that all bottles were well flushed and free of air prior to closure. Samples were regularly collected at the same time each week (13:00–15:00) to minimize bias that might be related to diel patterns of respira-

Fig. 1. Mean conditions in the reference lake, Paul Lake, and three treatment lakes, East Long, West Long, and Peter lakes, from 1991 to 1997. Nutrients were added beginning in 1993 with each lake receiving a similar load within each year except in 1995 when a higher load was added to East Long Lake relative to Peter and West Long lakes.



tion. Dark bottles were collected after 24 h of in situ incubation at the depth of sampling. Oxygen concentrations in initial and final samples were determined using the Winkler method. Titrations were performed on 100-mL aliquots that were weighed on an analytical balance to assure accurate volume determinations. Endpoints were determined colorometrically. The analytical limit for detecting a difference between two samples was a 1.6 µmol $O_2 \cdot L^{-1} \cdot day^{-1}$ change. As described further below, mean oxygen consumption in the lakes ranged from 7 to 25 µmol $O_2 \cdot L^{-1} \cdot day^{-1}$ and was generally well above the detection limit.

Related limnological variables

Chlorophyll *a* was measured weekly at a series of fixed light depths (100, 50, 25, 5, and 1% of surface irradiance). Samples were filtered, frozen, and later extracted and chlorophyll determined fluorometrically using previously described methods (Carpenter et al. 1996). Chlorophyll for the mixed layer was calculated to provide mean volumetric chlorophyll concentrations for the depth zone corresponding to the respiration measurements.

Primary productivity was estimated based on ^{14}C uptake and a model relating daily surface irradiance, temperature, depth, dissolved inorganic C, and chlorophyll to vertical patterns of C fixation (Carpenter et al. 1998). Daily modeled estimates were averaged to obtain weekly values for the depth zone corresponding to the respiration measurements.

Bacterial abundance and production were measured weekly in samples taken from the mixed layer. Abundance was measured using the acridine orange direct count method. For 1991–1995, [^{3}H]leucine incorporation into protein was measured using the method of Kirchman (1993). In 1996, we adopted the microcentrifuge method of Smith and Azam (1992) to measure [^{3}H]leucine incorporation. In laboratory comparisons, we found that the

microcentrifuge method measured values similar to the original method (data not shown) but with improved precision.

Data analysis

The analysis of the manipulations considers four lakes over 7 years for a total of 28 "lake-years." Fifteen to 17 weekly values for the mixed layer were averaged to establish the lake-year means. These means provide the basis for comparing respiration among the lakes. In one analysis below (respiration and DOC), we also include nine additional means for Paul and Peter lakes and a third nearby lake, Tuesday Lake. For these three lakes, respiration and DOC were also measured weekly over the same seasonal period during the years 1988–1990 with the same methods (see Pace 1993). We treat each lake-year as an independent observation in our statistical analysis.

Occasionally, we measured negative values of respiration (i.e., final oxygen in the dark bottles exceeded initial oxygen measured at the first sampling). Such negative values were rare (15 of 450 observations) and probably resulted from a combination of measurement error and times when in situ respiration was low, making it difficult to distinguish against background variability. We present these values in reporting time series below but exclude them from the estimation of means and statistical analysis. Removing the negative values introduces a positive bias to the means, but this effect was small. Means including negative values were on average 93% of the means calculated when negative values were removed.

Results

Experimental contrasts

There were strong contrasts among the three treatment lakes in response to the nutrient and food web manipulations (Fig. 1). Phosphorus loading in the treatment lakes varied from 40 to 200 μmol $P \cdot m^{-2} \cdot day^{-1}$ in comparison with natural loading rates of about 9–15 μmol $P \cdot m^{-2} \cdot day^{-1}$ in the unmanipulated lake as well as the treatment lakes prior to enrichment (Fig. 1*a*). In each year except 1995 when East Long Lake received a higher loading, we established similar loading rates in the three enriched systems (Fig. 1*a*). Water residence times in these lakes were also similar based on direct measurements (Cole and Pace 1998); thus, P loading served as a comparative metric for the manipulations and was well correlated with epilimnetic total P concentrations ($r = 0.9$).

In the manipulated lakes, mean crustacean size was largest in the lake with piscivores (West Long Lake) and smallest in the lake with planktivorous minnows (Peter Lake). Average size differed by 0.3–0.6 mm between these two systems (Fig. 1*b*). Crustacean size was more variable in East Long Lake (Fig. 1*b*). In the first 2 years of the experiment, smaller zooplankton were most important in East Long Lake. *Daphnia* spp. (*D. pulex* and *D. rosea*) became abundant in the later part of 1992 and eventually dominated the zooplankton biomass in East Long Lake for much of the period between 1993 and 1997. Crustacean size in the reference lake, Paul Lake, varied among years, reflecting population oscillations in the most important cladocerans (*Daphnia* spp. and *Holopedium gibberum*) (Post et al. 1997).

Separating Long Lake with curtains also created strong contrasts among the lakes in DOC (Fig. 1*c*). After 1991, mean DOC in East Long Lake was >1000 μM, and the lake was darkly stained. DOC in West Long Lake was intermediate, with means ranging from 550 to 760 μM. Peter Lake was lowest in DOC among the manipulated lakes, with seasonal means of 400–550 μM, and had concentrations similar to those in Paul Lake (Fig. 1*c*).

Respiration time series

Respiration increased in both magnitude and variability in all the experimental lakes with nutrient additions in 1993 (Fig. 2). Shifts in respiration are most apparent when compared with the reference lake. For example, only three observations in Paul Lake exceeded 20 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$, whereas numerous observations in East Long (26), West Long (18), and Peter (44) lakes exceeded this value (Fig. 3). The greatest increase in respiration was observed in Peter Lake, the lake with the smallest zooplankton and lowest DOC (Figs. 2 and 3). Average respiration in this lake exceeded 20 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$ in both 1996 and 1997 whereas mean respiration never exceed 20 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$ in the other two fertilized lakes or 10 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$ in the reference system.

Relationship of respiration to nutrient loading and food web structure

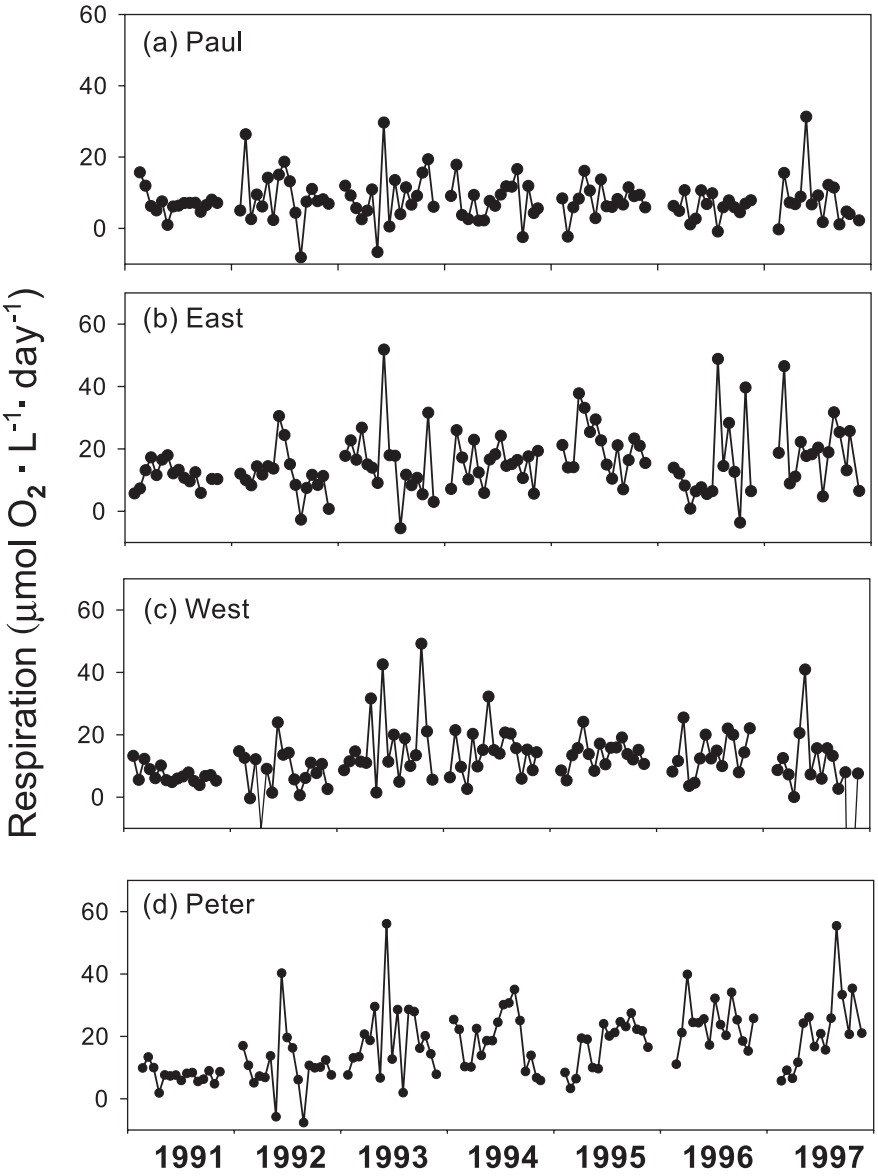
Respiration was not strictly correlated with nutrient loading. Instead, mean respiration increased rapidly with nutrient loading at low loading rates but slowly or not at all at higher loading (Fig. 4). An asympototic model based on an exponential rise to a maximum ($y = a(1 - e^{-bx})$) fit the data well for West Long and East Long lakes ($r^2 = 0.71$ and 0.70, respectively, $P < 0.02$ for both). This model was superior in each case to a linear regression. A power model ($y = ax^b$) provided the best fit to the Peter Lake data ($r^2 = 0.81$, $P < 0.01$), as in this case, respiration did not reach a maximum but continued to rise with nutrient loading (Fig. 4).

Part of the reason for the difference among lakes in respiration is related to zooplankton size structure, a sensitive indicator of food web structure (Carpenter and Kitchell 1993; Carpenter et al. 1996). Mean size was smallest in lakes dominated by planktivorous fish (Peter Lake) and largest in lakes dominated by piscivorous fish (West Long Lake). Crustacean zooplankton biomass, however, did not vary systematically among the lakes with one exception in 1993 when Peter Lake was almost entirely dominated by rotifers with few crustaceans. Otherwise, for the fertilization years 1994–1997, biomasses were similar, with mean ranges of 210–480, 260–400, and 200–420 μg dry weight$\cdot L^{-1}$ in East Long, West Long, and Peter lakes, respectively. When all the fertilized lake-years are considered, respiration varied inversely with mean crustacean length (Fig. 5) and was unrelated to zooplankton biomass (data not shown). Thus, when fertilized, West Long Lake with the largest zooplankton had the lowest respiration, while Peter Lake with the smallest zooplankton had the highest respiration. This result is consistent with the strong effects of large grazers on phytoplankton and bacteria in the fertilized lakes (Pace and Cole 1996; Carpenter et al. 1998).

Respiration and DOC

Respiration tended to increase with DOC in unfertilized lakes (Fig. 6*a*). This positive, significant ($P < 0.002$) correlation encompasses a fourfold range in respiration over a less than threefold range in DOC for lakes of very similar nutri-

Fig. 2. Time series of dark bottle respiration in (*a*) the reference lake, Paul Lake, and three treatment lakes, (*b*) East Long (larger grazers, high DOC), (*c*) West Long (large grazers, medium DOC), and (*d*) Peter lakes (small grazers, low DOC). Values are weekly observations from late May to early September in each year.



ent loading and primary productivity. While the relationship between DOC and respiration was noisy, it is consistent with prior observations of higher respiration in more DOC-rich lakes (Salonen et al. 1983; del Giorgio and Peters 1994). In contrast, respiration and DOC were not significantly correlated in the fertilized lakes (Fig. 6*b*).

Relationship with phytoplankton and bacteria

Respiration observed in the fertilized lakes covaried positively with chlorophyll *a* as well as with primary productivity. The strongest relationship was with primary production (Fig. 7*a*), although this was only marginally better than the relationship with chlorophyll. Note that the pattern is driven by the fertilized lakes (solid circles in Fig. 7*a*). The production to respiration ratios (*P*:*R*) averaged 1.0 and 2.1 for the unfertilized and fertilized lake-years, respectively.

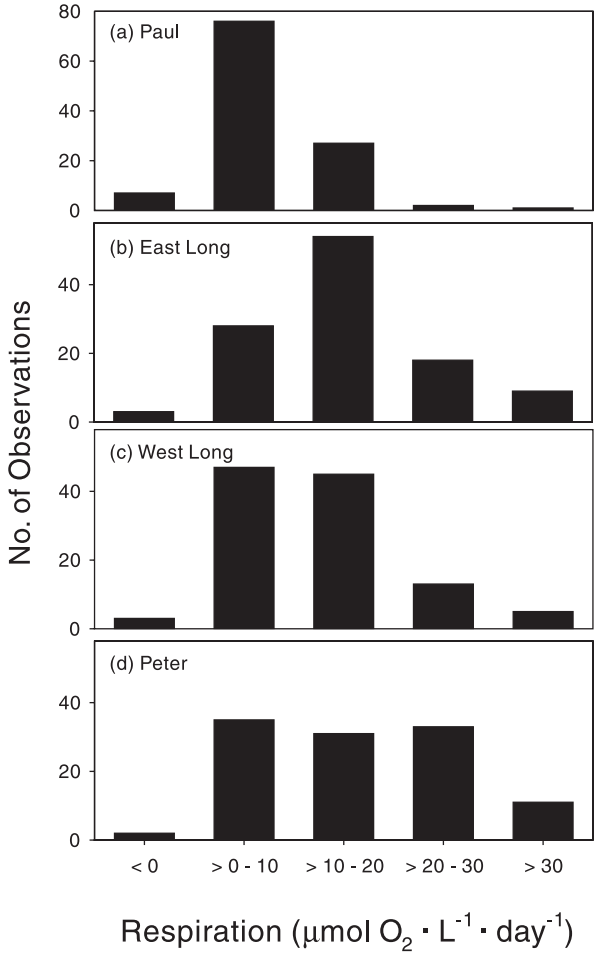
Respiration was also correlated with measures of bacterial abundance (r = 0.38, P = 0.05) and leucine incorporation (r = 0.77, P = 0.0001). The weak relationship with abundance but stronger correlation with leucine incorporation reflects the relatively small change in bacterial abundance (fivefold difference among means) relative to the large variation in leucine (30-fold difference among means). A regression between leucine and respiration (Fig. 7*b*) is clearly more variable than the pattern observed for primary production with large scatter at low rates of leucine incorporation (<5 nmol·L^{-1}·day^{-1}).

Discussion

Evaluation of respiration measurements

In theory, oxygen consumption in dark bottles measures

Fig. 3. Histograms of respiration values from (*a*) the reference lake, Paul Lake, and the three treatment lakes, (*b*) East Long, (*c*) West Long, and (*d*) Peter lakes. Note the difference in the scale of the *y*-axis for Paul Lake where most respiration values fell in the range of >0–10 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$.



respiration of all enclosed autotrophs and heterotrophs as well as any chemical oxidation over the measurement interval. Chemical oxidation is significant in humic lakes (Miles and Brezonik 1981), but oxygen consumption by this process is usually much less than respiration. For example, Granéli et al. (1996) measured photooxidation and respiration in a series of oligotrophic Swedish lakes spanning a DOC range similar to that in the lakes considered in our study. Depth-integrated photooxidation for the epilimnion was with one exception 10% or less of respiration. Photooxidation was also relatively constant (~4 mmol $C \cdot m^{-2} \cdot day^{-1}$) in the Swedish lake study (Granéli et al. 1996). This value is 0.2–11% of the mean respiration values observed in the current study, suggesting that photooxidation, even if it occurred in the dark bottles due to accumulated oxidizing constituents, was relatively small compared with respiration.

A second issue regarding the measurement of respiration is accuracy and precision. We achieved reasonable precision among replicate oxygen samples. For example, in 1997, 113 of 120 sets of quadruplicate measurements (separate bottles) had a coefficient of variation <2%. Respiration (i.e., the difference in oxygen) is more variable with greater precision

Fig. 4. Relationship of P loading to mean respiration in the three treatment lakes. Squares, circles, and triangles are for Peter, East Long, and West Long lakes, respectively. Note that the diamond shows the mean and standard deviation of annual means in P loading and respiration in Paul Lake to indicate background variability in these rates.

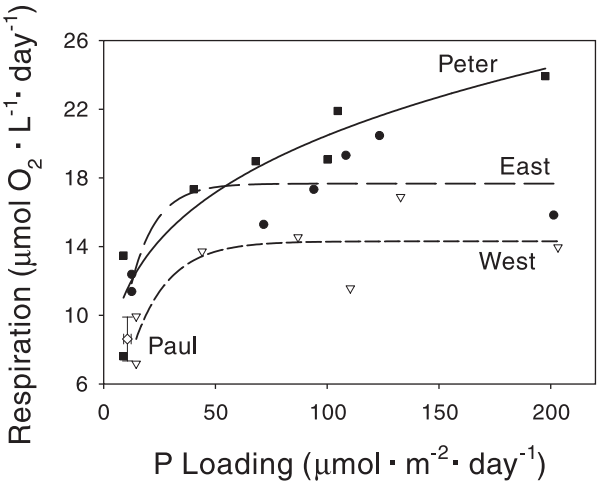
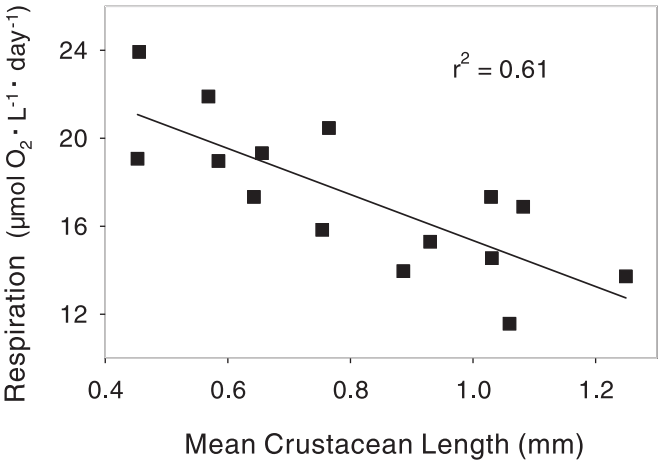


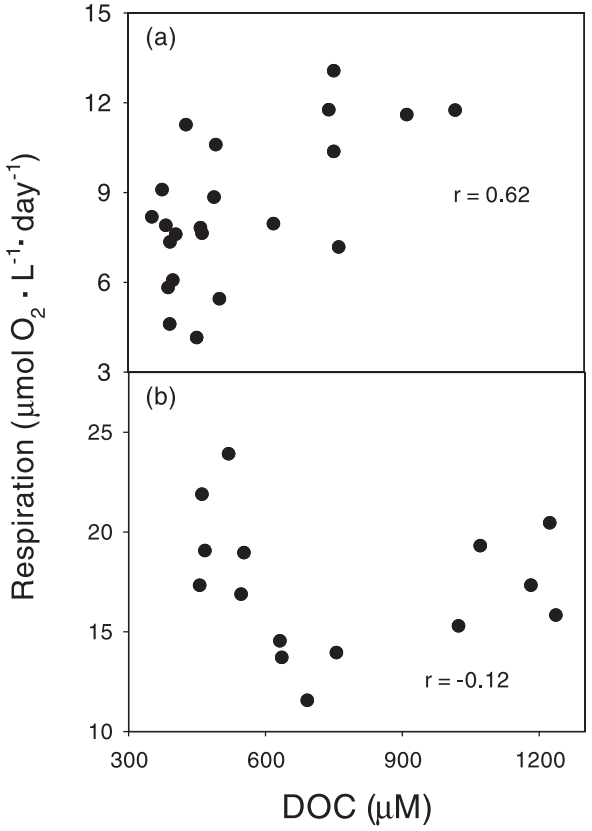
Fig. 5. Relationship of respiration to crustacean size for all fertilized lake-years, 1993–1997.



at higher rates. For example, in 1997, coefficients of variation estimated by propagation of error tended to be <20% (25 of 29 cases) at high respiration rates (>12.5 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$), but coefficients of variation were usually >20% when rates were low. Greater precision is possible by improving the measurement of titrant and endpoint detection (Granéli and Granéli 1991) and by refining the standard Winkler procedure (Carignan et al. 1998), but our precision was sufficient for the purposes of this study.

A more difficult issue is the effect of bottle enclosure on microbial dynamics that might cause estimates to be inaccurate. The time during the day–night cycle when samples were collected might influence bacterial respiration rates, as these organisms consume released photosynthate and dissoved organic matter photolytic products. We did not attempt to measure the variability in these processes or of respiration within the 24-h cycle, but any error should have

Fig. 6. Mean DOC and respiration in the (*a*) unfertilized and (*b*) fertilized lakes for all lakes and years. Included in the data set for the unfertilized lakes are 3 years of annual mean respiration in nearby Tuesday Lake measured using the same methods.



been systematic, as we collected our samples at the same time of day throughout the study. It is also possible that the 24-h dark incubations underestimated respiration because photolysis of DOC, which can be significant to bacterial metabolism (Moran and Zepp 1997), was blocked in the dark bottles.

Previous studies, however, have supported the accuracy of the dark bottle method and found estimates compatible with other measures of ecosystem processes in both oligotrophic and eutrophic waters (e.g., Williams 1981; Schwaerter et al. 1988; Smith and Kemp 1995). Further, independent estimates of respiration by phytoplankton and bacteria agree reasonably well with the dark bottle measurements (see next section). We therefore conclude that the respiration method was sufficient for detecting responses of the lakes to the manipulations.

Relative importance of autotrophs and heterotrophs

We can assess the relative significance of the various groups of planktonic microorganisms and metazoans in determining the rates of respiration based on standing stock data. Protozoan and rotifer abundances were measured from 1991 to 1994 (Pace et al. 1998), while bacterial densities and chlorophyll were measured throughout the entire study. Using 1994 as an example, average phytoplankton biomass in the enriched lakes was on the order of 600–1200 µg C·L^{-1} assuming a C to chlorophyll ratio of 60. Average bacterial

Fig. 7. Relationships of (*a*) primary production and (*b*) leucine incorporation to respiration. Open circles are unfertilized lake-years and solid circles are fertilized lake-years.

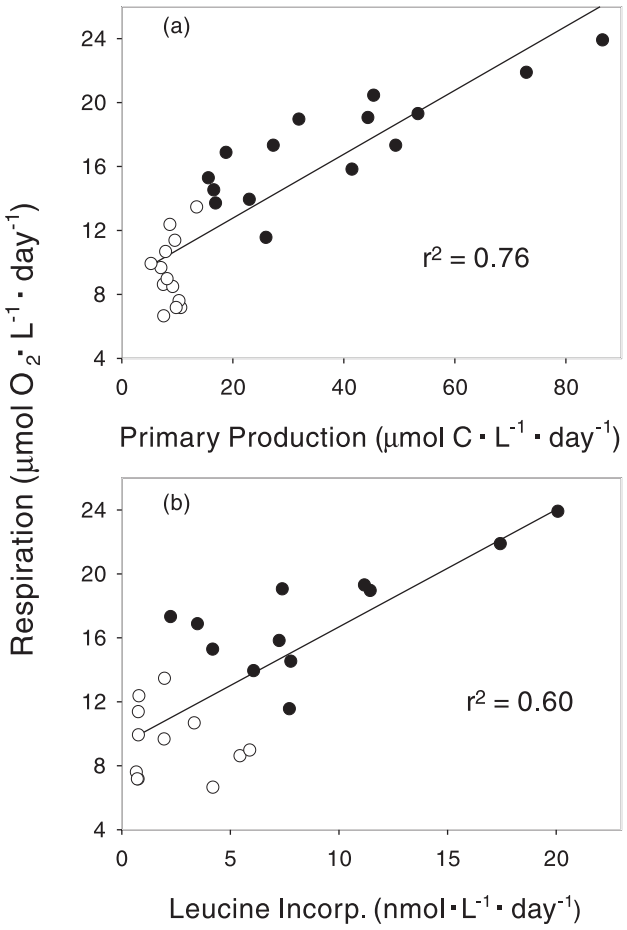
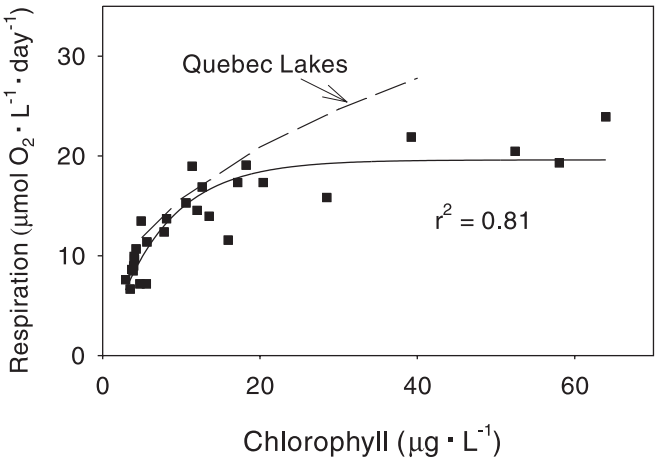


Fig. 8. Respiration and chlorophyll for Paul, Peter, East Long, and West Long lakes annual means from 1991 through 1997. The pattern observed for 20 Quebec Lakes by del Giorgio and Peters (1994) is also plotted.



biomass ranged from 60 to 180 µg C·L^{-1} assuming cell C contents in the range of 8–20 fg (Lee and Fuhrman 1987; Pace 1993). Average heterotrophic flagellate, ciliate, and

Table 1. Estimated rates of bacterial and phytoplankton respiration and their sum (= total) compared with measured rates based on means for 1997.

Lake	Bacterial respiration	Phytoplankton respiration	Total	Measured	Ratio of total to measured	Ratio of bacterial respiration to total
Paul	8.3	1.2	9.5	10.6	0.90	0.87
East Long	11.8	17.4	29.2	22.8	1.28	0.40
West Long	9.7	4.8	14.5	13.8	1.05	0.67
Peter	14.8	11.8	26.6	25.8	1.03	0.56

Note: See text for assumptions. All units are $\mu mol\ C \cdot L^{-1} \cdot day^{-1}$.

rotifer biomasses were 7–10, 9–44, and 6–47 $\mu g\ C \cdot L^{-1}$ based on average individual weights that approximated the size of the dominant components of each community (i.e., flagellates were small ($<30\ \mu m^3$), ciliates were mostly small oligotrichs (20–40 μm in size), and rotifers were primarily small species; see Pace et al. 1998 for further discussion). While these estimates are rough, the differences are sufficiently large to indicate that phytoplankton and bacteria were likely the main contributors to respiration in the bottles and that respiration by other heterotrophs was relatively minor.

Estimates of bacterial and phytoplankton respiration support this inference as illustrated for 1997 (Table 1), a year of nutrient loading and strong food web contrasts in the experimental lakes (Fig. 1). Bacterial respiration was estimated from average leucine incorporation rates converted to C units assuming 1.546 kg $C \cdot mol^{-1}$ (Simon and Azam 1989), an isotope dilution factor of 2 (Pace and Cole 1994), and an empirical relationship between growth efficiency and C production (Roland and Cole 2000). Estimated growth efficiencies ranged from 10 to 25% and corresponded to efficiencies previously estimated for Paul and Peter lakes using a food web model (Vézina and Pace 1994). Algal respiration was estimated from average chlorophyll concentrations, an assumed maximum rate of light-saturated photosynthesis (P_{bmax}) of 4 mg $C \cdot mg\ Chl^{-1} \cdot h^{-1}$, and a respiration factor of 15% P_{bmax} (Cole et al. 1992). Dark bottle oxygen consumption was converted to C by assuming a respiratory quotient of 0.85.

The sum of bacterial and phytoplankton respiration (= "total" in Table 1) agreed well with measured respiration, especially considering the uncertainty of the factors used to make these estimates. The summed rate was sufficiently higher than the measured value in East Long Lake to suggest that phytoplankton respiration was probably overestimated in this lake. Bacterial respiration was a significant component of the summed respiration in all cases and was the dominant source of respiration in the unfertilzed reference lake, Paul Lake. Using this as a baseline, the calculations indicate that phytoplankton respiration increased most strongly with fertilization (4–15 times), while changes in bacterial respiration were more modest (less than two times). The magnitude of these differences is consistent with differences in bacterial and phytoplankton biomass and productivity observed among the lakes. Respiration increased primarily in response to changes in the phytoplankton observed in the experiments. A relative increase in phytoplankton respiration is consistent with the shift in correlations of DOC with respiration observed between the fertilized and unfertilized lakes (Fig. 6). Similar patterns with regard to the comparison of measured and estimated rates prevailed in other years (data not shown). Overall, nutrient loading stimulated phytoplankton growth and hence respiration to a greater degree than bacterial growth and respiration, as observed by Schwaerter et al. (1988) in enclosure experiments.

Response of respiration to the experimental manipulations

Nutrient loading alone, however, was insufficient to predict the patterns of respiration in the experimental lakes. Respiration was more closely related to phytoplankton as determined by the interactions of nutrients, food web structure, and DOC (Carpenter et al. 1998). A major difference among the lakes was the relative abundance of large-bodied species of *Daphnia*. Grazing by these animals limited phytoplankton biomass, primary productivity, and bacterial productivity. The asymptotes of the respiration versus P loading relationships for East Long and West Long lakes (Fig. 4) reflect the relative grazing pressure in these lakes over the course of the experiments. West had the largest zooplankton, consistently abundant *Daphnia*, and the lowest asymptote. The asympototic relationships should not, however, be overinterpreted. Other models could be fit to the respiration data for individual lakes, and there is uncertainty in the true form of the relationship, given the limited number of observations. Other factors such as the high levels of light extinction related to DOC, especially in East Long Lake (see Carpenter et al. 1996), may also have contributed to the observed patterns. Peter Lake contrasted with East Long and West Long lakes in having increased respiration across the gradient of P loading. Peter Lake also had the lowest grazing pressure, as the zooplankton community was primarily small-bodied species (Pace et al. 1998). The inverse relationship of crustacean mean size and respiration in the fertilized lakes (Fig. 5) supports the importance of grazing in determining differences among the lakes.

Despite some cautions noted above, the general results should extrapolate to other situations, given that our manipulations, while strong, fall within the range of conditions found in other lakes. For example, the P loads and mean crustacean zooplankton size are well within the variation observed in lakes globally (Carpenter et al. 1996). Further, crustacean zooplankton biomass in the fertilized lakes was in the range of 200–400 μg dry $weight \cdot L^{-1}$ and corresponded to expectations based on models derived from lake trophic gradient studies (Hanson and Peters 1984).

DOC was positively correlated with respiration in unfertilized lakes but not with the mean responses observed in the experimental lakes with nutrient additions. This suggests

that the lake manipulations increased respiration of autochthonous material (because of higher primary production) and diminished the *relative* importance of respiration of the allochthonous component of DOC.

Comparison with general empirical models of respiration

Across productivity gradients in lakes, epilimnetic respiration tends to increase more slowly than primary production (del Giorgio and Peters 1994; Duarte and Augusti 1998). We found a similar pattern plotting the data from this study as a function of mean chlorophyll concentration (Fig. 8).

We can compare our results directly with a relationship between chlorophyll and respiration derived for 20 southern Quebec lakes by del Giorgio and Peters (1994) using methods similar to ours. While data from our study cover a broader range, there is reasonable overlap between the two models with a tendency toward departure at higher chlorophyll concentrations, reflecting primarily an underlying difference in model functional form (power versus asymptotic) rather than strong differences in the data. For example, respiration averaged 27 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$ in the most productive Quebec lake at a chlorophyll concentration of 37 $\mu g \cdot L^{-1}$, which compares well with a value of 25 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$ observed in East Long Lake at a chlorophyll concentration of 39 $\mu g \cdot L^{-1}$. At chlorophyll concentrations above those observed in the Quebec lakes, we found little or no increase in the average rate of respiration.

Planktonic *P*:*R* values for our study lakes were higher than observed by del Giorgio and Peters (1994). Estimates of primary production for our lakes exceeded their measures, and this difference accounts for most of the discrepancy in *P*:*R*. It is not clear whether this difference reflects methodology or is related to some fundamental difference between the lakes. One potentially important difference was the relatively shallow mixed layers of our systems compared with the deeper mixed layers over which the estimates were integrated for the Quebec lakes.

The asymptotic pattern that we observed (Fig. 8) does not conform to the most general empirical model of respiration provided by Duarte and Agustí (1998) in their summary of studies of 82 lakes. The median respiration for their set of lakes was 25.6 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$ with a range of 0.8–1166 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$, in comparison with our median annual mean value of 13.7 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$ with a range of 6.6–23.9 μmol $O_2 \cdot L^{-1} \cdot day^{-1}$. Duarte and Agustí (1998) reported respiration increasing as a power function of production. Thus, the asymptotic relationship that we observed may not be general or may reflect only lake conditions with relatively high zooplankton grazing. A better understanding of the mechanisms underlying the pattern in Fig. 8 requires further study and a more comprehensive analysis of respiration in the water column and sediments of lakes.

Conclusions

Prior models of respiration have related this fundamental measure of ecosystem metabolism to seasonal dynamics of temperature and productivity (e.g., Howarth et al. 1992; Smith and Kemp 1995) and to variations among aquatic systems in productivity (Duarte and Agustí 1998). We also observed strong relationships between primary and bacterial production and respiration in the surface waters of the manipulated lakes. Phytoplankton and bacterial productivity, however, were partially uncoupled from nutrient loading in these lakes by differences in food web structure. The two manipulated lakes dominated by large-bodied zooplankton had lower respiration than the lake where high rates of planktivory limited the development of large-bodied zooplankton. Thus, trophic cascades arising from top predators affect oxygen and C metabolism within lakes, as also supported by analysis of primary production, gas exchange, and stable isotopes of C in our experiments (Schindler et al. 1997).

Despite the importance of respiration in determining oxygen concentrations and as an integrating measure of ecosystem metabolism, there are no general models of respiration akin to those for primary production. This study suggests that such models must consider not only nutrient loading but also food web structure. In addition, in aquatic systems unperturbed by large nutrient inputs, DOC is a strong correlate of respiration. Respiration models must, therefore, account for key ecological interactions as well as littoral and land inputs of organic matter and the processes facilitating DOC degradation within lakes.

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References

Carignan, R., Blais, A.M., and Vis, C. 1998. Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method. Can. J. Fish. Aquat. Sci. **55**: 1078–1084.

Carpenter, S.R., and Kitchell, J.F. (*Editors*). 1993. The trophic cascade in lakes. Cambridge University Press New York.

Carpenter, S.R., Kitchell, J.F., Cottingham, K.L., Schindler, D.E., Christensen, D.L., Post, D.M., and Voichick, N. 1996. Chlorophyll variability, nutrient input and grazing: evidence from whole-lake experiments. Ecology, **77**: 725–735.

Carpenter, S.R., Cole, J.J., Kitchell, J.F., and Pace, M.L. 1998. Impact of dissolved organic carbon, phosphorus, and grazing on phytoplankton biomass and production in experimental lakes. Limnol. Oceanogr. **43**: 73–80.

Christensen, D.L., Carpenter, S.R., Cottingham, K.L., Knight, S.E., LeBouton, J.P., Schindler, D.E., Voichick, N., Cole, J.J., and Pace, M.L. 1996. Pelagic responses to changes in dissolved organic carbon following division of a seepage lake. Limnol. Oceanogr. **41**: 553–559.

Cole, J.J., and Pace, M.L. 1998. Hydrologic varaibility of small, northern Michigan lakes measured by the addition of tracers. Ecosystems, **1**: 310–320.

Cole, J.J., Caraco, N.F., and Peierls, B.L. 1992. Can phytoplankton maintain a positive carbon balance in a turbid, freshwater, tidal estuary? Limnol. Oceanogr. **37**: 1608–1617.

del Giorgio, P.A., and Peters, R.H. 1994. Patterns in planktonic *P*:*R* in lakes: influence of lake trophy and dissolved organic carbon. Limnol. Oceanogr. **39**: 772–788.

Duarte, C.M., and Agustí, S. 1998. The CO_2 balance of unproductive aquatic ecosystems. Science (Washington, D.C.), **281**: 234–236.

Granéli, W., and Granéli, E. 1991. Automatic potentiometric determination of dissolved oxygen. Mar. Biol. **108**: 341–348.

Granéli, W., Lindell, M., and Tranvik, L. 1996. Photo-oxidative production of dissolved organic carbon in lakes of different humic content. Limnol. Oceanogr. **41**: 698–706.

Hanson, J.M., and Peters, R.H. 1984. Empirical prediction of crustacean zooplankton biomass and profundal macrobenthos biomass in lakes. Can. J. Fish. Aquat. Sci. **41**: 439–445.

Houser, J.N. 1998. Food web structure and experimental enrichment: effects on phosphorus sedimentation and retention. M.Sc. thesis, University of Wisconsin-Madison, Madison, Wis.

Howarth, R.W., Marino, R., Garritt, R., and Sherman, D. 1992. Ecosystem respiration and organic carbon processing in a large, tidally influenced river: the Hudson River. Biogeochemistry, **16**: 83–102.

Kirchman, D.L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. *In* Handbook of methods in aquatic microbial ecolgy. *Edited by* P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole. Lewis, Boca Raton, Fla. pp. 509–512.

Lee, S., and Fuhrman, J.A. 1987. Relationships betwen biovolume and biomass of naturally derived marine bacterioplankton. Appl. Environ. Microbiol. **53**: 1298–1303.

Miles, C.J., and Brezonik, L. 1981. Oxygen consumption in humic-colored waters by a photochemical ferrous–ferric catalytic cycle. Environ. Sci. Technol. **15**: 1089–1095.

Moran, M.A., and Zepp, R.G. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol. Oceanogr. **42**: 1307–1316.

Pace, M.L. 1993. Heterotrophic microbial processes. *In* The trophic casade in lakes. *Edited by* S.R. Carpenter and J.F. Kitchell. Cambridge University Press, New York. pp. 252–277.

Pace, M.L., and Cole, J.J. 1994. Primary and bacterial production: are they coupled over depth? J. Plankton Res. **16**: 661–672.

Pace, M.L., and Cole, J.J. 1996. Regulation of bacteria by resources and predation tested in whole-lake experiments. Limnol. Oceanogr. **41**: 1448–1460.

Pace, M.L., Cole, J.J., and Carpenter, S.R. 1998. Trophic cascades and compensation: differential response of microzooplankton in whole-lake experients. Ecology, **79**: 138–152.

Peters, R.H. 1983. The ecological implications of body size. Cambridge University Press, New York.

Post, D.M., Carpenter, S.R., Christensen, D.L, Cottingham, K.L., Kitchell, J.F., Schindler, D.E., and Hodgson, J.H. 1997. Seasonal effects of variable recruitment of a dominant piscivore on pelagic food web structure. Limnol. Oceanogr. **42**: 722–729.

Roland, F., and Cole, J.J. 2000. Prediction of bacterial growth efficiency in a large turbid river. Verh. Int. Ver. Limnol. In press.

Salonen, K., Kononen, K., and Arvola, L. 1983. Respiration of plankton in two small, polyhumic lakes. Hydrobiologia, **101**: 65–70.

Schindler, D.E., Carpenter, S.R., Cole, J.J., Kitchell, J.F., and Pace, M.L. 1997. Influence of food web structure on carbon exhange between lakes and the atmosphere. Science (Washington, D.C.), **277**: 248–251.

Schwaerter, S., Søndergaard, M., Riemann, B., and Jensen, L.M. 1988. Respiration in eutrophic lakes: the contribution of bacterioplankton and bacterial growth yield. J. Plankton Res. **10**: 515–531.

Simon, M., and Azam, F. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. **51**: 201–213.

Smith, D.C., and Azam, F. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food Webs, **6**: 107–114.

Smith, E.M., and Kemp, W.M. 1995. Seasonal and regional variations in plankton community production and respiration for Chesapeake Bay. Mar. Ecol. Prog. Ser. **16**: 217–231.

Tranvik, L. 1992. Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. Hydrobiologia, **229**: 107–114.

Vézina, A.F., and Pace, M.L. 1994. An inverse model analysis of planktonic food webs in experimental lakes. Can. J. Fish. Aquat. Sci. **51**: 2034–2044.

Wetzel, R.G. 1992. Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems. Hydrobiologia, **229**: 181–198.

Wetzel, R.G. 1995. Death, detritus, and energy flow in aquatic ecosystems. Freshwater Biol. **33**: 83–89.

Williams, P.J.leB. 1981. Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. Oceanol. Acta, **4**: 359–364.