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META-ANALYSIS OF MARINE NUTRIENT-ENRICHMENT EXPERIMENTS: VARIATION IN THE MAGNITUDE OF NUTRIENT LIMITATION

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Abstract. Nutrient bioassay experiments have been performed in many marine and estuarine environments around the world. Although protocols have been relatively uniform, these experiments have yielded mixed results, implicating nitrogen, phosphorus, silica, iron, and several other elements as factors limiting phytoplankton growth. Meta-analysis has the potential to explain much of this variation by exploring the relationship between the magnitude of limitation and various environmental characteristics. We quantified limitation with a simple metric, Δr, that estimates the change in the per unit growth rate of phytoplankton directly attributable to addition of a specific nutrient, such as nitrogen, iron, or phosphorus. Preliminary analyses indicated that experiments lasting $\leq$1 d exhibited time lags in the numerical response of phytoplankton to nutrient addition, while experiments lasting $>$7 d confounded nutrient limitation with processes such as increased grazing or depletion of other nutrients. Thus, we restricted the meta-analysis to results from 2–7 d experiments. These analyses showed that phosphorus enrichment usually had little impact on phytoplankton growth, while enrichments of nitrogen and iron increased phytoplankton growth by 0.1–0.3 d^{-1}. Nutrient limitation due to N, P, and Fe varied significantly among sites. Nitrogen limitation was greatest in nearshore, nutrient-polluted, and temperate environments (where most experiments have been performed), while phosphorus and iron limitation were strongest in open ocean, unpolluted, and tropical ecosystems, or those receiving pollutants with high N:P ratios. Because phosphorus-enrichment studies have been most often performed in relatively polluted coastal waters, the possible role of phosphorus in limiting primary production in unpolluted oceanic systems may have been underestimated. Examining heterogeneity of responses of different systems to experiments is a valuable application of meta-analysis and can facilitate the development of new ecological insights.

Key words: bioassay; coastal and estuarine studies; iron; marine studies; meta-analysis; nitrogen; nutrient-enrichment experiments; nutrient limitation; oceanic systems, pollution; phosphorus; phytoplankton.

INTRODUCTION

Marine primary production is of major ecological and economic importance. Phytoplankton production fuels the production of higher trophic levels, which supply more than 90×10^9 kg of food to the world economy each year (FAO 1993). Marine ecosystems (including estuarine, coastal, and marine habitats) are also essential in the global carbon budget, storing 50 times more inorganic carbon than the earth's atmosphere, suggesting that marine primary production may play a global climatological role (Ritschard 1992, Mackenzie et al. 1993). Consequently, understanding the factors that limit or regulate phytoplankton production in the sea is of vital global interest. One of the most important factors potentially limiting primary production is the availability of nutrients.

The identity of the nutrient that limits primary production has been inferred from geochemical budgets, ratios of elements dissolved in seawater or contained in phytoplankton-sized particles, and experimental nutrient additions. These approaches have yielded a diversity of answers. For example, geochemical budgets suggest that inorganic phosphorus (P) should be in shorter supply than nitrogen (N), and therefore limiting (Meybeck 1982), because atmospheric N_2 can be fixed (Redfield 1958, Vitousek and Howarth 1991). In contrast, ratios of dissolved inorganic nitrogen (N) and phosphorus (P) are often lower than average intracellular N:P ratios of marine organisms (Redfield 1934, Redfield et al. 1963), suggesting that nitrogen should limit marine primary production (e.g., Boynton et al. 1982). Such assessments provide only indirect evidence about the nutrients that limit phytoplankton growth, and offer little insight into the magnitude or severity of nutrient limitation.

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Experimental manipulation of inorganic nutrients constitutes a more direct approach to assessing both the quality and strength of nutrient limitation in phytoplankton assemblages, and such experiments have been a mainstay of marine research for more than 50 yr. Although these experiments are sometimes criticized because of the small scales on which they often are conducted (Hecky and Kilham 1988), experimental additions performed at small scales have been validated by large-scale addition experiments in both lakes and oceans (Schindler 1978, Granéli and Sundback 1985, D'Elia et al. 1986, Elser et al. 1990, Oviatt et al. 1995, Taylor et al. 1995).

Although most nutrient-addition experiments in oceans have been directed toward the study of nitrogen (Capone and Carpenter 1982), it is clear that nitrogen is not always limiting in the ocean. Enrichments of phosphorus, iron, and silica have also been shown to induce significant increases in marine primary producers in at least some experiments (Boynton et al. 1982, Martin and Fitzwater 1988, Martin et al. 1994). The variation in results of experimental studies suggests two new approaches. First, it may be more profitable to estimate the magnitude of limitation than the presence or absence of it, for example, by quantifying how much the ambient growth rate of phytoplankton is depressed below the potential growth rate under nutrient-saturated conditions (e.g., Hecky and Kilham 1988, Osenberg and Mittelbach 1996). This is a question with a quantitative answer that can be extracted from a number of available studies that have been conducted in a broad variety of marine habitats and at different times. This meta-analytic approach obviates problems inherent in using the frequency of occurrence of statistically significant effects of nutrient enrichment as a de facto criterion of the importance of limitation by a given nutrient, as used implicitly in expert reviews or explicitly in vote-counting syntheses (e.g., see Gurevitch et al. 1992).

A second approach that might be fruitful would be to directly examine the variation in results and seek a framework to explain the variation of results rather than seeking a single global answer (e.g., ''is it iron, or is it nitrogen''). Indeed, the exploration of variation in effects is one of the most valuable applications of meta-analysis. For example, a meta-analysis of nutrient limitation in freshwaters (thought to be primarily P limited) uncovered a surprisingly important role of N (Elser et al. 1990). Furthermore, clarifying sources of variation in nutrient limitation has important implications for both applied and basic research. For example, the current paradigm in ocean sciences remains focused on nitrogen limitation (Jacques and Tréguer 1986, Barnes and Hughes 1988, Valiela 1995). If nitrogen is not the principal element limiting primary production in all (or even most) locales, then scientific resources, and considerable remedial action, might be misdirected. Meta-analysis could help to interpret the overall conclusions that can be drawn from a set of disparate experiments, by revealing patterns, offering hypotheses to explain variation in effects, and pointing to conceptual and empirical holes in the current level of understanding.

In this paper, we present a meta-analysis of results from nutrient-enrichment-experiments performed in marine habitats around the world. Our objective is to look for patterns in the magnitude of nutrient limitation in marine phytoplankton assemblages to highlight the application of meta-analysis. The analysis is not meant to be exhaustive, but a first step to illustrate the utility of meta-analysis and the importance of conceptual steps required in its application (a future analysis of an expanded data set examining co-limitation and other facets of this question will be presented elsewhere). To this end, we take a three-tiered approach: (1) we provide a conceptual, quantifiable definition of nutrient limitation; (2) we conduct a preliminary analysis to assess the confounding influence of experiment duration; and (3) based on these considerations, we restrict our primary meta-analysis to the most appropriate data and seek to explain the variation in limitation observed among different studies. We limit this paper's focus to variation among marine habitats that differ in the degree of anthropogenic pollution or geographic locale. In so doing, we hope to take a first step in the explanation of this variation and provide a means for prediction.

Methods and Analyses

The available data

We began our analysis by searching the literature for experiments that assessed nutrient limitation. All of the studies listed in Downing (1997) were included, to which we added observations from an extensive literature review of marine journals (details available from J. A. Downing). Below, we describe the standard experimental protocol of these studies. We then provide an explicit discussion of our conceptual and operational definition of limitation. Based on this definition, time-scale considerations, and other criteria, we restricted the studies included in the meta-analysis. Because this refinement of the question and evaluation of time-scale issues are both important parts of ecological meta-analysis (Osenberg et al. 1997, 1999), and because selection criteria can affect the outcome of a meta-analysis (Englund et al. 1999), we specifically highlight the details of this approach below (see *The quantification of nutrient limitation: . . .* and *Time-scale considerations*).

The typical experiment found in this literature was initiated by taking a large water sample (usually many liters) from a given marine sampling station, usually near the surface in the photic zone. This large sample was usually sieved to remove macrozooplankton grazers that might mask phytoplankton responses to nutrient additions (microzooplankton cannot be removed by sieving). The water sample was divided into subsamples, some of which received a dose of various dis-

solved, inorganic nutrients; others were left unamended to serve as controls. The most common experiment dosed some subsamples with nitrogen as NO_3, and others with phosphorus as PO_4. Other nutrients commonly added included iron and silica. The subsamples were then incubated in situ or under simulated conditions of ambient temperature and light. During or after the incubation period, samples were withdrawn from the incubation vessels and phytoplankton biomass estimated, usually as chlorophyll *a* concentration, but sometimes as light absorbance, phytoplankton biovolume, or cell numbers. The influence of the various nutrient additions was typically assessed from comparisons of estimated phytoplankton biomass in unenriched control samples to that found in the enriched samples. The published comparisons were usually in the form of a statistical test or a graphical comparison.

The quantification of nutrient limitation: choosing an effect-size metric

At first glance, many of the results of published nutrient-addition experiments appear to be lacking in comparability. Experimental results have been expressed as plots of biomass over time (often without further analysis), as ratios of treatment to control biomass, as differences in biomass between treatment and control, as cell growth rates in treatment and control, as differences in the slopes of temporal trends in biomass accumulation, as differences in photosynthetic rates, etc. Some metrics of effect size, such as *d,* borrowed from the statistical literature on meta-analysis (e.g., Gurevitch et al. 1992), would only be calculable in a minority of these experiments, would be difficult to interpret biologically, and could give misleading answers (Osenberg et al. 1997). Nevertheless, with careful attention to the conceptual definition of nutrient limitation, many measures reported in published experiments can be converted to a single, biologically meaningful measure of nutrient limitation that is comparable across studies.

A conceptual definition of limitation requires explicit consideration of how population growth responds to the augmentation of a growth-limiting factor (Hecky and Kilham 1988). Here, we define nutrient limitation as ''the change in the per unit (per gram, per unit carbon, or per chlorophyll *a*) growth rate of an algal assemblage following the addition of surplus nutrients'' (Osenberg and Mittelbach 1996). Hence, if $N_{x,E}$ and $N_{x,C}$ are respectively the amount of algae in the Experimental (nutrient addition) treatment and the Control (ambient) at the start ($x = 0$) and end ($x = t$) of the experiment, then the degree of limitation, Δr, can be estimated as:

$$\Delta r = r_E - r_C = \frac{dN_E}{N_E dt} - \frac{dN_C}{N_C dt} = \frac{\ln\left(\frac{N_{t,E}}{N_{0,E}}\right) - \ln\left(\frac{N_{t,C}}{N_{0,C}}\right)}{t}. \quad (1)$$

Because control and enriched treatments typically begin with the same amount of algae (i.e., $N_{0,E} = N_{0,C}$), Eq. 1 often reduces to

$$\Delta r = \frac{\ln\left(\frac{N_{t,E}}{N_{t,C}}\right)}{t}. \quad (2)$$

As conceptualized, Δr should efficiently measure the magnitude of nutrient limitation of the extant algal assemblage. This growth-based definition of limitation is not the only one possible (although it is also the one suggested by Hecky and Kilham 1988), but is most appropriate for the type of data reported in most nutrient-addition experiments, which tend to focus on phytoplankton biomass over relatively short-term experiments (e.g., several days).

Our definition of limitation assumes that nutrients were added in excess. The quantity of added nutrients varied among studies, but nutrients were usually added in great excess relative to the range of concentrations observed in the studied system, i.e., often exceeding the observed range seen across the range of marine ecosystems (Downing 1997). Consequently, we feel it is reasonable to assume that these nutrient-addition experiments measure how far the phytoplankton were from nutrient-saturated growth, as required by our model. If nutrients were not provided above saturating levels, then our estimates of limitation may be biased low.

Time-scale considerations

Because organismal responses occur on various time scales, critical consideration in the application of this metric to published data is the time scale of experimental observations (see a more theoretical development of this question in Osenberg et al. [1999]). To highlight the importance of time scale, we contrast two alternative metrics of effect size: Δr (Eq. 2) and the log response ratio (Hedges et al. 1999; the logarithm of the ratio of algal biomass in the two treatments, $\ln(N_{t,E}/N_{t,C})$). For example, at the start of a nutrient-addition experiment, the biomass of algae in the control and nutrient-addition treatment will diverge at a constant specific (i.e., per unit) rate. As a result, the log response ratio will increase linearly with time (Fig. 1A). The slope of this relationship will be equal to Δr. As feedbacks arise (e.g., due to numerical increases in micrograzers, or limitation by other nutrients), this slope will decline, and after some period of transient dynamics, biomass will eventually settle down to a constant value after the two treatments each reach new equilibria. This asymptote (as $t \rightarrow \infty$, see Fig. 1a) will be determined not only by nutrient limitation, but also by all other feedbacks and forms of density dependence that operate in the system, as well as the structure of the food web within the experimental vessels (Schaffer 1981, Bender et al. 1984). As a result, two studies with

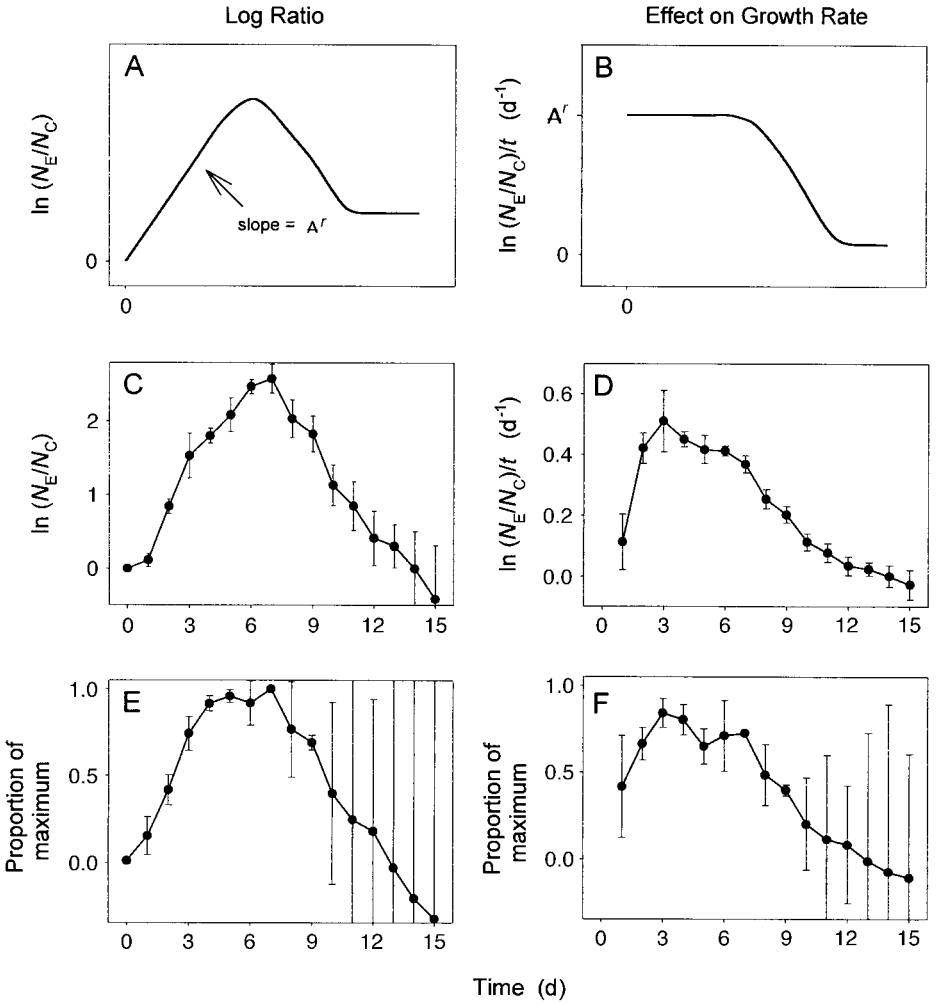

FIG. 1. The response of algal biomass to nutrient enrichment, expressed using the log ratio response ($\ln[N_{t,C}/N_{t,E}]$), and the effect on the per capita growth rate ($\Delta r = \ln[N_{t,C}/N_{t,E}]/t$). The top panels (A and B) give theoretical expectations, which at short time scales yield estimates of nutrient limitation of growth rate (the initial slope in A; the initial ceiling in B), but at longer time scales include system feedbacks that interfere with the estimation of limitation. The middle panels (C and D) give results obtained from data reported in Fig. 2 of D'Elia et al. (1986). The bottom panels (E and F) summarize results from seven studies, including Thomas et al. (1974: Fig. 3 [Sewage, All, All−Fe, All−Trace elements, All−Vitamins, All+Sewage] and Fig. 4 [N, Sewage, All, All−P, All−Si, All−Fe, All−Trace elements, All−Vitamins, All+Sewage]); D'Elia et al. (1986: Fig. 2 [ammonium addition] and Fig. 3 [NO_3^- addition]); Harrison et al. (1990: Fig. 2 [P, N+P]); and Hein and Riemann (1995: Fig. 1A [N+P addition]). In (E) and (F), all estimates of the log ratio and Δr were first standardized by dividing by the maximum that was observed in that time series. These standardized responses were then averaged across all time series. The bottom four panels (C–F) show means and 95% confidence intervals. Confidence intervals in (C) and (D) were estimated using the formula for variance of the log response ratio from Hedges et al. (1999). Sample sizes in (C) and (D) were $N_{t,C} = 5$, $N_{t,E} = 12$; in (E) and (F) the number of comparisons varied from 7 to 22 for days 1–6 and from 2 to 3 for days 7–15.

precisely the same dynamics would have radically different log ratios if they were of different durations but had not yet re-equilibrated (Osenberg et al. 1997, 1999).

In contrast to the log response ratio, Δr can provide more comparable results over short time scales, which are more appropriate to most nutrient-enrichment studies. For example, during the period that the two treatments diverge at a constant rate, Δr should have a constant value (ignoring initial time lags: Fig. 1B). This initial value of Δr provides a direct estimate of growth-rate limitation (Eq. 2; see also Osenberg and Mittelbach 1996, Osenberg et al. 1997, Laska and Wootton 1998). As time passes, however, Δr should eventually decline in magnitude toward zero, as the added nutrient is depleted below saturating concentrations, other nutrients become limiting, grazers increase in abundance, etc. Thus, studies used to estimate limitation of growth rates should be restricted to the early phase of divergence in which Δr is time invariant (i.e., during the flat portion of the curve in Fig. 1B).

Given that it is clearly critical to determine the time

scale over which Δr can be applied (i. e., the experiment duration over which Δr is time invariant), we evaluated the behavior of Δr in a subset of appropriate studies, and then only extracted data for the meta-analysis from the appropriate time frame. Ideally, time-scale issues should be investigated for each study, but unfortunately, few time series were available from the literature (most investigators only sample algal biomass at the end of the experiments). Therefore, we had to estimate the appropriate time frame from a subset of studies and then apply this assessment to the entire collection. We examined how Δr and, for comparison, the log ratio ($\ln(N_{t,E}/N_{t,C})$) changed through time for the only study (D'Elia et al. 1986) that sampled algal biomass over at least 10 d, found nutrient limitation in some treatment, and reported variances in $N_{t,C}$ and $N_{t,E}$ (necessary for estimating error in the estimate of Δr; see Hedges et al. 1999). We also examined the general trend over time by using all available studies that reported time series and found nutrient limitation (seven papers, yielding 22 different comparisons). To put data from these studies on the same scale (studies varied in the magnitude of limitation), we first adjusted each day's response by the maximum response observed over the sampled time period, and then averaged across the studies for each day of the experiments.

Data from D'Elia et al. (1986), as well as the aggregated data set from all seven papers with time-series data, demonstrated three notable features (Fig. 1d and f): (1) Δr, as expected, appeared to converge toward 0 at relatively long time scales ($\geq$12 d); (2) At shorter time scales, on the order of 2–7 d, Δr was relatively independent of time, while the log ratio was strongly time dependent; and (3) There was an initial ($\leq$1 d) lag in algal response (i.e., Δr at day 1 is less than Δr on days 2–7), which may reflect a physiological lag between nutrient uptake and conversion to new biomass. Given that algal division rates under good conditions are on the order of once per day, an initial 1-d lag is not surprising. Despite this slight lag, the Experimental and Control treatments diverged from one another at an approximately exponential rate for the first 2–7 d (Fig. 1C and E, i.e., except for the inflection at day 1, the log ratio showed an approximately linear increase up to day 5 or so, which led to the relative constancy in Δr over the same time period—from roughly day 2 through day 7). After ~7 d the direct effects of nutrient limitation on algal growth was counteracted by other factors (e.g., numerical responses of micro-grazers), which caused Δr to decline. This analysis suggests that studies lasting $\leq$1 d are too short to quantify limitation (due to the time lag in the numerical response of algae), and studies lasting more than ~7 d are too long (due to feedbacks involving other components of the system).

The restricted data set

Based on the preliminary analyses of time scale, we restricted the final meta-analysis to estimates of Δr derived from reports of phytoplankton biomass in enriched and control treatments collected 2–7 d after nutrient addition. When data were reported for multiple sample dates within this 6-d period, an average Δr was calculated for the study. Unfortunately, this restriction on duration meant that almost half of the available experiments (48%) could not be used because they either lasted $\leq$1 d, or they were not sampled during the first week of a long-term enrichment experiment. This severe selection criterion is justified given our conceptual definition of limitation and the errors that could result from inclusion of studies that did not measure nutrient limitation as defined here (e.g., see Fig. 1).

We also excluded studies that reported data that could not easily be incorporated into our quantitative definition of limitation (Eqs. 1 and 2). For example, some studies provided estimates of total plankton production rates (e.g., ^{14}C assimilation, oxygen evolution, expressed at the level of the entire assemblage), after several days of incubation with nutrients, rather than biomasses or abundances of cells. These studies give biased (over-) estimates of limitation because they ''double count'' the effect of nutrient limitation. Total production reflects both the enhancement of per cell (or per unit biomass) production rates stimulated by nutrient addition, as well as the increased biomass of phytoplankton in the vessel, which had accumulated over the course of the experiment. Rates of change in production rates will therefore be greater than rates of change in biomass, so incorporating both sources into a quantitative estimate would essentially ''double count'' the effect of nutrient limitation.

Many studies also included treatments that consisted of the addition of multiple nutrients, most commonly N plus P. The most extreme form of this experiment (often termed ''nutrient-deletion'' experiments) consisted of the addition of a vast suite of nutrients and then the sequential removal of single nutrients from this enrichment solution. These deletion experiments assessed the response of the phytoplankton assemblage to the omission of a particular nutrient, when all other nutrients were present in excess (e.g., Paerl and Bowles 1987). We excluded all mixed nutrient results because they did not measure the magnitude of nutrient stress due to a single nutrient—they altered the background nutrient environment in addition to the availability of the target nutrient. Furthermore, although mixed algal assemblages can show co-limitation, whereas single-species systems should not (as argued in Hecky and Kilham 1988), we were most interested in the amount of limitation imposed by single nutrients, especially nitrogen, phosphorus, silicon or iron (for which we found the most data). Experiments examining co-limitation of algal assemblages will be analyzed in a future, more extensive manuscript.

Most frequently, the natural phytoplankton community was incubated in the collected sea water, but some researchers (18% of studies) filtered out the nat-

ural algal community, replacing it with a cultured algal species. In this case, the decision whether to include such studies is not obvious and meta-analysts may diverge on how they proceed (Englund et al. 1999). We chose to include these experiments (because they were always performed using indigenous, dominant algae) and examine their influence on the data set empirically. The results of these studies did not yield estimates of Δr that differed significantly overall from those obtained using only natural algal assemblages (resampling test; $P = 0.78$).

After restricting our data set to studies as defined above, we were left with data compiled from 16 different papers (Ryther and Dunstan 1971, Vince and Valiela 1973, Thómas et al. 1974, Granéli and Sundbäck 1985, Martin and Fitzwater 1985, D'Elia et al. 1986, Granéli 1987, Bonin et al. 1989, Le Rouzic and Bertru 1989, Granéli et al. 1990, Harrison et al. 1990, Pederson and Borum 1991, Rudek et al. 1991, Martin et al. 1994, Hein and Riemann 1995, Taylor et al. 1995), which provided 303 different comparisons of nutrient-addition and control treatments. For data availability see the Appendix. The large number of comparisons per paper exists because single papers usually provided data from multiple experiments (e.g., multiple sites and multiple times) or from multiple treatments (e.g., separate N, P, Fe, and Si additions in the same experiment). Therefore, our estimates are not wholly independent. The degree of non-independence is difficult to assess, however, and is likely to vary depending on the specific source of non-independence (e.g., two estimates from different sites, but reported in the same paper may well be less dependent on one another than two estimates from the same locale but different times and reported in different papers). At this point, the data set is too sparse to permit a rigorous evaluation of non-independence, so we raise this primarily as a cautionary note (e.g., see Gurevitch and Hedges 1999).

Analytical procedures

Experiments were performed in a wide variety of locations, spanning latitudes from 5° S to 60° N, in heavily polluted areas (e.g., New York City Harbor) to pristine open oceans (e.g., Atlantic and Pacific oceans), in estuaries, bays and harbors, coastal zones, sounds and nearly landlocked seas, shelf zones, and oceanic waters. We therefore coded each study based on the environment in which the study was conducted. We defined six levels of pollution based on descriptions of sites presented by the authors of the studies. ''Very polluted'' sites were those that were indicated as directly receiving polluted effluent, ''moderate pollution'' was indicated if study sites were adjacent to, but not directly within heavily polluted waters, ''light pollution'' was indicated if sites were between polluted and unpolluted waters, ''unpolluted'' waters were those where authors indicated that there was no obvious source of nutrient pollution, whereas ''pristine'' waters were distinguished as those where authors specifically indicated that analyses showed a lack of nutrient pollution. We categorized these sites as ''high N:P'' when the authors indicated the habitat received polluted waters with N:P consistently above the ratio most frequently required by phytoplankton (N:P = 16, as atoms). We then investigated variation in the response to nutrient enrichment among these different types of environments.

Tests for significant differences in responses among treatment types and 95% confidence intervals of effect sizes were made by using resampling methods and unweighted analyses (Adams et al. 1997) programmed in Meta Win 1.00 (Rosenberg et al. 1997). Although a mixed model using unequal weights would be more desirable (see Gurevitch and Hedges [1999] for further discussion of model types), a weighted analysis cannot be made without estimates of within-study sampling variability, which were not available from most of the studies. The 95% confidence intervals were generated from 5000 resampling iterations using equal weights for each study (i.e., assuming that all variances of Δr were approximately equal; J. Gurevitch, *personal communication*). This assumption is undoubtedly not strictly accurate, but does not bias estimated group means, although it probably inflates the sizes of estimated confidence intervals (Gurevitch and Hedges 1999).

Results and Discussion

Phytoplankton response to nutrient enrichment

Overall general effects of nutrient enrichment.—Additions of nitrogen or iron elicited similarly large average magnitudes of response in phytoplankton specific growth rates when data from all zones and levels of pollution were considered together (Fig. 2). The average responses to these two nutrients can also be expressed in terms of relative doubling times: on average, the nutrient-addition treatment achieved twice the algal biomass as the control in 3.3 d for nitrogen and 4.1 d for iron. Silicate also stimulated phytoplankton growth (Fig. 2), but doubling times in enriched treatments averaged nearly 10 d. In contrast, addition of phosphorus did not, on average, stimulate phytoplankton growth (Fig. 2). These results confirm the prevailing view among marine scientists that N and Fe are the two most important limiting nutrients in marine environments. Note, however, that these results give average responses, which do not address potential variation in nutrient limitation among different habitats.

Variation in limitation among different environments.—Comparisons were classified based on the amount and form of nutrient pollution present at the study site. The environmental conditions of available studies are strongly biased, with ~60–70% of all comparisons coming from studies conducted in waters we classified as ''very polluted.'' Approximately 30% of the comparisons were from moderately polluted sites,

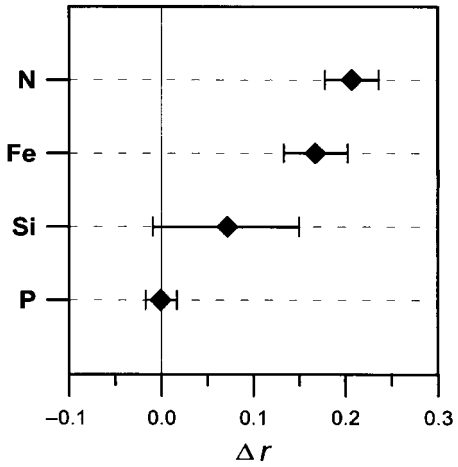

FIG. 2. Effects of nutrient addition on phytoplankton growth, as measured by Δr, the change in per unit (per gram; per unit carbon, or per chlorophyll *a*) growth rate of an algal assemblage following the addition of surplus nutrients. Results give responses for each nutrient added singly to phytoplankton assemblages and show, using an unweighted analysis with resampling procedures, that nutrients varied in their effect on phytoplankton growth ($P = 0.002$). Error bars represent 95% confidence intervals of Δr based on the resampling procedures with 5000 iterations. N denotes experiments enriching with nitrogen, P denotes phosphorus addition, Si denotes silicate addition, and Fe denotes iron addition. The means are based on 148 (N), 114 (P), 35 (Fe), and 6 (Si) experiments.

and only ~5% were from sites that were pristine or relatively unlikely to have been polluted. In contrast, most of the ocean waters on the globe receive little nutrient pollution and would be classed as ''unpolluted'' or ''pristine'' based on our classification scheme. Experiments to assess limitation by specific nutrients also have not been performed uniformly in all nutrient environments (Fig. 3). For example, >75% of the experimental nitrogen enrichments have been performed in very polluted or moderately polluted waters, while >90% of the experiments on iron limitation have been performed in ''pristine'' habitats. This biased distribution of studies has important effects on the results of the overall meta-analysis (i.e., Fig. 2).

The magnitude of response to nutrient enrichment varied significantly among marine environments (Fig. 4). Phosphorus limitation was high (nearly as high as the average effects of nitrogen and iron limitation) in pristine, unpolluted waters (probably high in N:P; Downing 1997) and those polluted with nutrients with a high N:P ratio (higher than the average ratio in plankton tissue). Further, experiments performed on waters taken from geographic areas unlikely to receive nutrient pollution (e.g., unenclosed coastal zones and those in open oceans) show that responses to P addition were significantly positive and differed significantly from the negligible responses to P enrichment in estuaries and enclosed bays ($P = 0.011$; Fig. 5). Since much of the world's oceans are relatively free of nutrient pollution and are far from coastal zones, phosphorus may play a greater role in nutrient limitation than is currently believed.

Nitrogen limitation tended to show the opposite pattern across the pollution gradient (Fig. 4), although limitation did not vary significantly among environments receiving differing degrees of nutrient pollution (Fig. 4; $P = 0.19$) or among habitats (e.g., Fig. 5; P

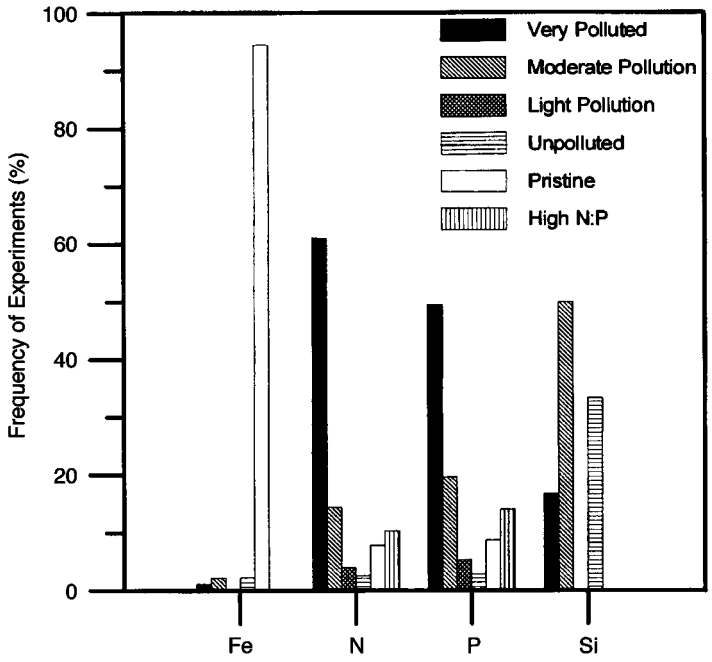

FIG. 3. Distribution of different kinds of nutrient-enrichment experiments performed in environments differing in probable degree of nutrient pollution. Total sample sizes are as in Fig. 2. Percentages are calculated over all experiments performed using each element; thus, e.g., ''silica'' experiments sum to 100% across all pollution categories.

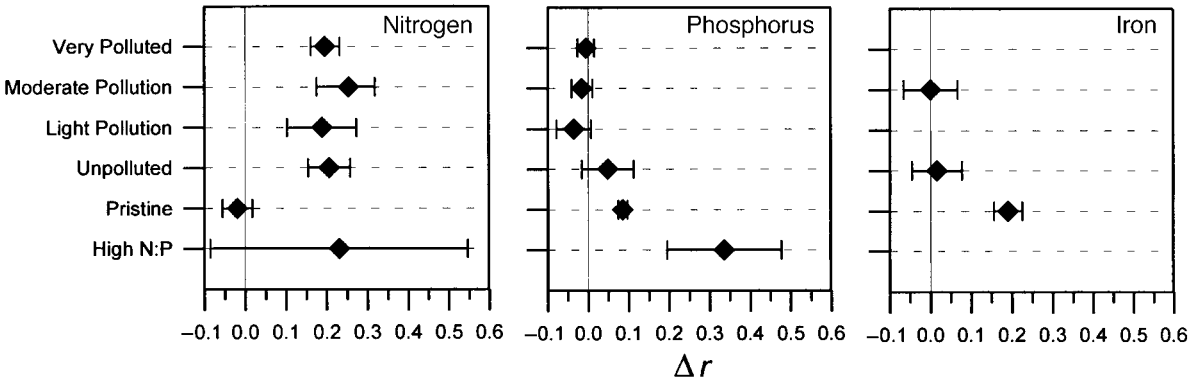

FIG. 4. Variation in nitrogen, phosphorus, and iron limitation among sites that differed in the degree of nutrient pollution. Pollution categories were assigned based on data presented in published reports (see *Methods and analyses: Analytical procedures*). Error bars give 95% confidence intervals of Δr from resampling procedures (5000 iterations). For N ($P = 0.19$), the means are based on 98 (very polluted), 37 (moderately polluted), 5 (lightly polluted), 4 (unpolluted), 2 (pristine), and 2 (high N:P) experiments; for P ($P = 0.0002$), the means are based on 64 (very polluted), 37 (moderately polluted), 5 (lightly polluted), 4 (unpolluted), 2 (pristine), and 2 (high N:P) experiments; and for Fe ($P = 0.0008$), the means are based on 2 (moderately polluted), 2 (unpolluted), and 30 (pristine) experiments. *P* values are probability estimates (from resampling tests) that experiments performed in different nutrient environments were sampled from identical distributions (e.g., with equal values of Δr).

= 0.16). Significant differences in nitrogen limitation among nutrient environments were somewhat masked by the highly variable responses found in waters that were extremely high in their N:P ratio (Fig. 4).

Although based on a much smaller sample size, the response to Fe enrichment also varied across habitats. Iron enrichment appeared to have little influence on phytoplankton growth in moderately polluted or unpolluted habitats, but yielded significantly ($P = 0.0008$) greater increases in growth in pristine habitats (Fig. 4). Likewise, whereas Fe had little impact on coastal phytoplankton, it increased algae growth significantly ($P = 0.0002$) in oceanic waters and at similar rates to those seen for N enrichment in inshore and polluted waters.

These responses to nutrient enrichment generally agree with biogeochemical hypotheses concerning differences in nutrient limitation among marine environments (Downing 1997). This analysis also reveals a very strong tendency for marine-nutrient bioassays to be performed primarily in polluted or land-impacted ecosystems that may not be representative of marine ecosytems as a whole. This meta-analysis thus suggests that inferences about the potential impact of nutrient enrichment on marine production or the global carbon cycle must be tempered with an understanding of the

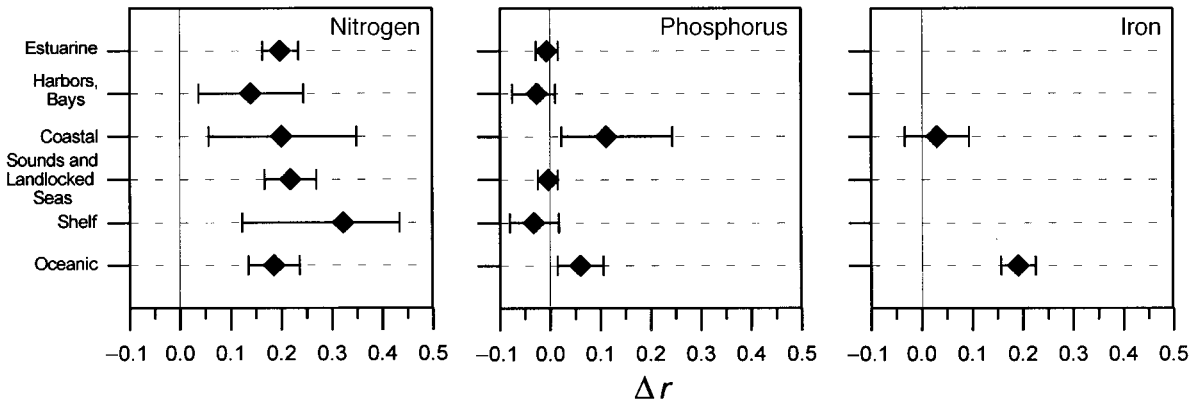

FIG. 5. Variation in nitrogen, phosphorus, and iron limitation among different marine habitats. Marine habitat classes were discerned from data and information presented in published reports. Error bars give 95% confidence intervals of Δr from resampling procedures (5000 iterations). For N ($P = 0.16$), the means are based on 92 estuarine, 10 harbors and bays, 8 coastal zones, 24 sounds and landlocked seas, 12 shelf zones, and 2 oceanic experiments; for P ($P = 0.011$), the means are based on 59 estuarine, 9 harbors and bays, 8 coastal zones, 24 sounds and landlocked seas, 12 shelf zones, and 2 oceanic experiments; and for Fe ($P = 0.0002$), the means are based on 3 coastal zones and 30 oceanic experiments. *P* values are probability estimates (from resampling tests) that experiments performed in different habitats were sampled from identical distributions (e.g., with equal values of Δr).

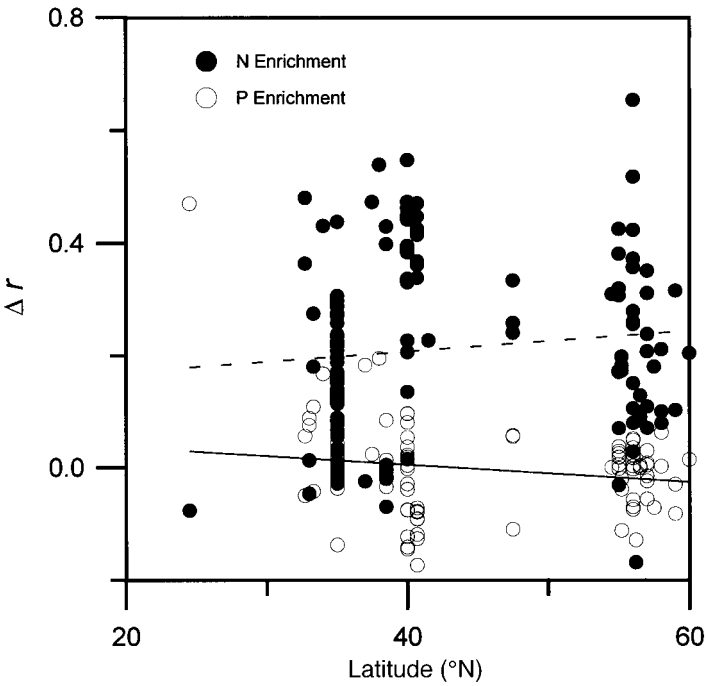

FIG. 6. Relationship between Δr in all nitrogen- and phosphorus-enrichment experiments and the latitude of the marine habitat from which water samples were taken. The dashed and solid lines represent (respectively) the least-squares regression relationship of the weak positive correlation between Δr and latitude in N-enrichment experiments ($r = 0.11$, $n = 147$, $P = 0.18$) and the relationship of the significant negative correlation between Δr and latitude in P-enrichment experiments ($r = -0.19$, $n = 114$, $P = 0.042$).

sensitivity of experimental results to the ambient nutrient environment.

Latitudinal patterns in experimental results.—It has been suggested by several authors that tropical marine ecosystems, especially those dominated by carbonate-rich sedimentary environments, are more frequently phosphorus limited than temperate ones (Smith 1984, Short et al. 1985, Fouqurean et al. 1993, Feller 1995). One might therefore hypothesize that Δr for P-enrichment experiments might be negatively correlated with latitude, that is, be highest in the low-latitude tropics, and lowest in the temperate zone. Conversely, Δr for N-enrichment experiments might be lower in experiments performed at low latitudes than in experiments performed at high latitudes. Although regression and correlation analyses of meta-analytical data are somewhat controversial when one cannot weight by appropriate variance estimates (Cooper and Hedges 1994, Hedges and Olkin 1985, Gurevitch and Hedges 1999), the correlations shown in Fig. 6 support this theory. There is a significant ($P = 0.042$) negative correlation between Δr and latitude in P-enrichment experiments, and a very weak positive relationship ($P = 0.18$) between Δr and latitude in N-enrichment experiments (Fig. 6). The correlations are probably weak due to the influences of a variety of other variables that we have not accounted for (such as seasonality and degree of pollution [Fig. 4]). It is encouraging, however, that any trend is seen in this preliminary analysis. Tests of hypotheses of this type would be extremely costly if they required newly collected primary data. Thus, meta-analysis, or comparative analyses, of existing data can greatly facilitate tests of important hypotheses that might not otherwise be feasible.

Conclusions

Meta-analytical methods offer powerful instruments for the interrogation of experiments performed under disparate conditions and can lead to the exploration of large-scale trends in responses reflecting differences in the functioning of ecosystems. Interpretation of the large number of analyses of marine nutrient limitation was facilitated by the choice of an appropriate metric reflecting the response of interest and the discovery of technical biases that can mask trends in experimental results.

Our exploratory and illustrative analysis revealed several general lessons regarding the application of meta-analysis in ecology. For example, most of the available studies did not report or allow calculation of variances among replicates, either because there were no replicates, or because variances were not included in publications. In previous ecological meta-analyses, such data sets would have been discarded (e.g., Gurevitch et al. 1992, Curtis 1996). We were, nonetheless, able to gain valuable insights about the strength of nutrient limitation and its variation across systems because (1) we used a metric (i.e., Δr) that did not require estimates of variance, and (2) we did not mandate that effect sizes be weighted by the inverse of their within-study variances (e.g., see Gurevitch and Hedges 1999). Although the absence of weights might have led to some bias in our calculation of confidence intervals (Gurevitch and Hedges 1999) and a reduction in sta-

tistical power, we believe it is far better to obtain approximate answers to important questions, than to remain ignorant of the answers because the data might be judged less than ideal.

Finally, our analyses demonstrated the value in conducting preliminary analyses to ascertain the relevance of particular data (e.g., collected over different time scales) and in performing exploratory analyses that investigate possible sources of variation in effect size. Indeed, the variation in nutrient limitation among different marine habitats has conceptual implications that affect how we apply literature reviews to the global oceanic system. In particular, very few nutrient-enrichment bioassays could be found that examined nutrient limitation in unpolluted open oceans. As a result, the average estimates of nutrient limitation were heavily biased toward polluted coastal systems, which are often the most easily studied. This has probably led to bias in our inferences about the relative importance of various nutrients in limiting production in marine systems, and its impact on major issues such as marine resources and global carbon budgets. Our analyses not only suggested the nature of this bias, but also pointed out holes in the existing data that should help direct future primary investigations. Like many other important questions that ecologists test using experimental data, the salient questions to pose concerning nutrient limitation in marine habitats are clearly more interesting than asking ''is the ocean limited by N, or is it P?''

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Literature Cited

Adams, D. C., J. Gurevitch, and M. S. Rosenberg. 1997. Resampling tests for meta-analysis of ecological data. Ecology **78**:1277–1283.

Barnes, R. S. K., and R. N. Hughes. 1988. An introduction to marine ecology. Blackwell, Oxford, UK.

Bender, E. A., T. J. Case, and M. E. Gilpin. 1984. Perturbation experiments in community ecology: theory and practice. Ecology **65**:1–13

Bonin, D. J., M. C. Bonin, and T. Berman 1989. Mise en évidence expérimentale des facteurs nutritifs limitants de la production du micro-nanoplancton et de l'ultraplancton dans une eau cotière de la Méditerranée orientale (Haifa, Israel). Aquatic Sciences **51**:129–152.

Boynton, W. R., W. M. Kemp, and C. W. Keefe. 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. Pages 69–70 *in* V. S. Kennedy, editor. Estuarine comparisons. Academic Press, New York, New York, USA.

Capone, D., and E. J. Carpenter. 1982. Nitrogen fixation in the marine environment. Science **217**:1140–1142.

Cooper, H., and L. V. Hedges. 1994. The handbook of research synthesis. Russell Sage Foundation, New York, New York, USA.

Curtis, P. S. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. Plant, Cell and Environment **19**:127–137.

D'Elia, C. F., J. G. Sanders, and W. R. Boynton. 1986. Nutrient enrichment studies in a coastal plain estuary: phytoplankton growth in large-scale, continuous cultures. Canadian Journal of Fisheries and Aquatic Sciences **43**:397–406.

Downing, J. A. 1997. Marine nitrogen:phosphorus stoichiometry and the global N:P cycle. Biogeochemistry **37**:237–252.

Elser, J. J., E. R. Marzolf, and C. R. Goldman. 1990. Phosphorus and nitrogen limitation of phytoplankton in freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences **47**:1468–1477.

Englund, G., O. Sarnelle, and S. Cooper. 1999. The importance of data-selection criteria: meta-analyses of stream predation experiments. Ecology **80**:1132–1141.

FAO [Food and Agriculture Organization]. 1993. Fishery statistics yearbook. Volume 72. Rome, Italy.

Feller, I. C. 1995. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). Ecological Monographs **65**:477–505.

Fourqurean, J. W., R. D. Jones, and J. C. Zieman. 1993. Processes influencing water column nutrient characteristics and phosphorus limitation of phytoplankton biomass in Florida Bay, FL, USA: inferences from spatial distributions. Estuarine Coastal and Shelf Science **36**:295–314.

Granéli, E. 1987. Nutrient limitation of phytoplankton biomass in a brackish water bay highly influenced by river discharge. Estuarine, Coastal and Shelf Science **25**:555–565.

Granéli, E., and K. Sundbäck. 1985. The response of planktonic and microbenthic algal assemblage to nutrient enrichment in shallow coastal waters, southwest Sweden. Journal of Experimental Marine Biology and Ecology **85**: 253–268.

Granéli, E., K. Wallstrom, U. Larsson, W. Granéli, and R. Elmgren. 1990. Nutrient limitation of primary production in the Baltic Sea area. Ambio **19**:142–151.

Gurevitch, J., and L. V. Hedges. 1999. Statistical issues in ecological meta-analyses. Ecology **80**:1142–1149.

Gurevitch, J., L. L. Morrow, A. Wallace, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist **140**:539–572.

Harrison, P. J., M. H. Hu, Y. P. Yang, and X. Lu. 1990. Phosphate limitation in estuarine and coastal waters of China. Journal of Experimental Marine Biology and Ecology **140**:79–87.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidences of the effects of enrichment. Limnology and Oceanography **33**:796–822.

Hedges, L. V., J. Gurevitch, and P. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. Ecology **80**:1150–1156.

Hedges, L. V., and I. Olkin. 1985. Statistical methods for meta-analysis. Academic Press, New York, New York, USA.

Hein, M., and B. Riemann. 1995. Nutrient limitation of phytoplankton biomass or growth rate: an experimental approach using marine enclosures. Journal of Experimental Marine Biology and Ecology **188**:167–180.

Jacques, G., and P. Tréguer. 1986. Écosystèmes pélagiques marins. Masson, Paris, France.
Laska, M. S., and J. T. Wootton. 1998. Theoretical concepts and empirical approaches to measuring interaction strength. Ecology **79**:461–476.
Le Rouzic, B., and B. G. Bertru. 1989. Détermination par bioessai de la biodisponibilité des ressources azote et phosphore, dans les eaux du Golfe du Morbihan. Revue des Sciences de l'Eau **5**:97–111.
Mackenzie, F. T., L. M. Ver, C. Sabine, M. Lane, and A. Lerman. 1993. C, N, P, S global biogeochemical cycles and modeling of global change. Pages 1–61 *in* R. Wollast, F. T. Mackenzie, and L. Chou, editors. Interactions of C, N, P and S, biogeochemical cycles and global change. Springer-Verlag, Berlin, Germany.
Martin, J. H. et al. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature **371**:123–129.
Martin, J. H., and S. E. Fitzwater. 1988. Iron deficiency limits phytoplankton growth in the northeast pacific subarctic. Nature **331**:341–343.
Meybeck, M. 1982. Carbon, nitrogen, and phosphorus transport by world rivers. American Journal of Science **282**: 401–450.
Osenberg, C. W., and G. G. Mittelbach. 1996. The relative importance of resource limitation and predator limitation in food chains. Pages 134–148 *in* G. A. Polis and K. O. Winemiller, editors. Food webs: integration of patterns and dynamics, Chapman & Hall, New York, New York, USA.
Osenberg, C. W., O. Sarnelle, and S. D. Cooper. 1997. Effect size in ecological experiments: the applicaton of biological models to meta-analysis. American Naturalist **150**:798–803.
Osenberg, C. W., O. Sarnelle, S. D. Cooper, and R. D. Holt. 1999. Resolving ecological questions through meta-analysis: goals, metrics, and models. Ecology **80**:1105–1117.
Oviatt, C., P. Doering, B. Nowicki, L. Reed, J. Cole, and J. Frithsen. 1995. An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. Marine Ecology Progress Series **116**:171–179.
Paerl, H. W., and D. Bowles. 1987. Dilution bioassays: their application to assessments of nutrient limitation in hypereutrophic waters. Hydrobiologia **146**:265–273.
Pedersen, M. F., and J. Borum. 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Marine Ecology Progress Series **142**:261–272.
Redfield, A. C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. Pages 176–192 *in* James Johnstone Memorial Volume. University Press, Liverpool, UK.
———. 1958. The biological control of chemical factors in the environment. American Scientist. **46**:205–221.
Redfield, A. C., B. H. Ketchum, and F. A. Richards. 1963. The influence of organisms on the composition of sea-water. Pages 26–77 *in* M. N. Hill, editor. The sea. Volume 2. John Wiley, New York, New York, USA.
Ritschard, R. L. 1992. Marine algae as a CO_2 sink. Water, Air, and Soil Pollution **64**:289–303.
Rosenberg, M. S., D. C. Adams, and J. Gurevitch. 1997. MetaWin. Statistical software for conducting meta-analysis: fixed effects models, mixed effects models, and resampling tests. Version 1.0. Sinauer Associates, Sunderland, Massachusetts, USA.
Rudek, J., H. W. Paerl, M. A. Mallin, and P. W. Bates. 1991. Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. Marine Ecology Progress Series **75**:133–142.
Ryther, J. H., and W. M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science **171**:1008–1013.
Schaffer, W. M. 1981. Ecological abstraction: the consequences of reduced dimensionality in ecological models. Ecological Monographs **51**:383–401.
Schindler, D. W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnology and Oceanography **23**:478–486.
Short, F. T., M. W. Davis, R. A. Gibson, and C. F. Zimmermann. 1985. Evidence for phosphorus limitation in carbonate sediments of the seagrass *Syringodium filiforme*. Estuarine, Coastal and Shelf Science **20**:419–430.
Smith, S. V. 1984. Phosphorus vs. nitrogen limitation in the marine environment. Limnology and Oceanography **29**: 1149–1160.
Taylor, D., S. Nixon, S. Granger, and B. Buckley. 1995. Nutrient limitation and the eutrophication of coastal lagoons. Marine Ecology Progress Series **127**:235–244.
Thomas, H. W., D. L. R. Seibert, and A. N. Dodson. 1974. Phytoplankton enrichment experiments and bioassays in natural coastal sea water and in sewage outfall receiving waters off southern California. Estuarine and Coastal Marine Science**2**:191–206.
Valiela, I. 1995. Marine ecological processes. Springer-Verlag, New York, New York, USA.
Vince, S., and I. Valiela. 1973. The effects of ammonium and phosphate enrichments on chlorophyll a, pigment ratio, and species composition of phytoplankton of Vineyard Sound. Marine Biology **19**:69–73.
Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry **13**:87–115.

APPENDIX

Marine nutrient-enrichment data from 16 different papers (published dates: 1971–1995) and used in this paper are available in digital form from ESA's Electronic Data Archive: *Ecological Archives* E080-009. The data are also available from the data depository at the National Center for Ecological Analysis and Synthesis (NCEAS), Santa Barbara, California, USA.