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Moving on up: can results from simple aquatic mesocosm experiments be applied across broad spatial scales?

AMANDA C. SPIVAK, MICHAEL J. VANNI AND ELIZABETH M. METTE
Zoology Department, Miami University, Oxford, OH, U.S.A.

SUMMARY

1. Aquatic ecologists use mesocosm experiments to understand mechanisms driving ecological processes. Comparisons across experiments, and extrapolations to larger scales, are complicated by the use of mesocosms with varying dimensions. We conducted a mesocosm experiment over a volumetric scale spanning five orders of magnitude (from 4 L to whole ponds) to determine the generality of algal responses to nutrient enrichment. Recognising that mesocosm dimensions may affect algal growth, we also manipulated the ratio of mesocosm surface area to volume (SA : V) over two levels (high versus low). We used mesocosm tanks of similar size and construction to those commonly used in aquatic experiments to increase the generality of our results.

2. Volume was generally a stronger determinant of algal responses than mesocosm shape (i.e. SA : V). However, the effects of both volume and shape on algae were weak and explained a small portion of the variance in response variables. In addition, there was no consistent, directional relationship (positive or neutral) between mesocosm volume and algal abundance (estimated by chlorophyll concentration). Combined, our findings suggest that results from small-scale experiments, examining the direct response of algae to nutrient enrichment, can probably be 'moved on up' and applied to larger, more natural aquatic systems.

3. Algal response to nutrient enrichment (e.g. nutrient use efficiency and effect size) varied strongly with time. This underscores the importance of choosing an experimental timescale appropriate to the biological and/or ecological process of interest.

4. We compared our results to those from a recent meta-analysis of nutrient-limitation studies that included 359 freshwater pelagic experiments, spanning a wide range of volumetric and temporal scales. Similar findings between this experiment and the meta-analysis indicate that algal response to nutrient enrichment varies little across spatial scales. Therefore, it is probable that results from small-scale pelagic algal nutrient-limitation experiments are relevant to large-scale processes, such as eutrophication.

Keywords: chlorophyll, experimental design, mesocosm, nutrients, scaling

Introduction

A goal of ecology is to elucidate mechanisms driving patterns and processes observed in nature. Ideally, experiments designed to reveal these mechanisms are conducted at the proper scale. While experiments conducted at the ecosystem scale may be most realistic (Carpenter *et al*., 1995; Pace *et al*., 2004), such experiments suffer from limitations including low replication and reduced experimental control (Hurlbert, 1984; Drenner & Mazumder, 1999). Because of these limitations and the intractability of manipulating entire ecosystems, many researchers employ mesocosms that are designed to mimic natural

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Correspondence: Amanda C. Spivak, Miami University, Zoology Department, Pearson Hall Rm 212, Oxford, OH 45056, U.S.A.
E-mail: spivak.amanda@epa.gov
Present address: Amanda C. Spivak, US EPA, Gulf Ecology Division, 1 Sabine Island Dr., Gulf Breeze, FL 32561, U.S.A.

systems (Odum, 1984; Drenner & Mazumder, 1999). Experiments in mesocosms have contributed to our understanding of community ecology and ecosystem functioning (Fraser & Keddy, 1997; Jessup *et al.*, 2004; Spivak *et al.*, 2009b), informed theory (Pfisterer & Schmid, 2002; Cardinale *et al.*, 2006), and provided insight to global processes (Benton *et al.*, 2007; Duffy, 2009; O'Connor *et al.*, 2009). However, mesocosm experiments are criticised by some as being unrealistic simplifications with limited relevance to natural ecosystems (Carpenter, 1996; Schindler, 1998; Haag & Matschonat, 2001). While both whole-ecosystem and mesocosm manipulations have limitations, including generality and applicability of results to systems with different physical parameters (e.g. latitude, altitude, size and morphology), species compositions and/or environmental connectedness, there is a recent focus on extrapolating findings from mesocosm experiments to larger, natural ecosystems (Levin, 1992; Ives *et al.*, 1996; Kemp, Petersen & Gardner, 2001; Smith *et al.*, 2005; Bulling *et al.*, 2006).

The purpose of scaling results from mesocosm experiments to ecosystems is to address larger-scale ecological problems and/or environmental policy questions. For instance, eutrophication is a widespread problem in marine and freshwater ecosystems driven by an over-abundance of available nutrients, such as nitrogen and phosphorus. As such, a common experiment in aquatic ecology is to determine the nutrient(s) limiting algal growth using a standard bioassay approach (Gerhart & Likens, 1975; Francoeur, 2001). Because nutrient limitation of primary production is an important ecological constraint, it has been studied experimentally in a variety of ecosystems and at many scales (Downing, Osenberg & Sarnelle, 1999; Francoeur, 2001; Elser *et al.*, 2007). Although results of small-scale nutrient-limitation assays are often scaled to pond, lake and even oceanic systems, controversy still exists over whether the severity of algal nutrient limitation and/or the identity of the limiting nutrient in intact ecosystems can be predicted from mesocosm experiments (Lewis & Wurtsbaugh, 2008; Schindler *et al.*, 2008; Conley *et al.*, 2009).

Our goal in this study was to quantify how spatial and temporal scales mediate algal response to nutrient enrichment. We choose this response for two reasons. First, uncertainty exists over whether nutrient-limitation responses are scale independent. Secondly, the response of algae to nutrients is a well-studied, direct interaction with a clear mechanistic basis. This should make it relatively easy to interpret how experimental scale mediates algal responses and we anticipate that our results will be relevant to discussions about the appropriateness of applying findings from nutrient-limitation experiments to larger systems. To determine whether variation in mesocosm dimensions affects algal response to nutrient enrichment, we conducted an experiment with containers that differed in volume and the surface area : volume ratio (SA : V). Our experiment differs from prior mesocosm scaling work in that we varied the volumetric scale over five orders of magnitude and we used containers common to aquatic mesocosm experiments; this should increase the generality of our results. We tested two hypotheses. First, nutrient enrichment will increase algal biomass; this is consistent with previous experiments (Francoeur, 2001; Elser *et al.*, 2007). Second, algal response to nutrient enrichment will not vary with mesocosm volume but will be greater in treatments with a higher SA : V. We expected a positive relationship between algal response and SA : V because mesocosm surface area may control light availability to water column algae. If we find that mesocosm volume is an important determinant of algal response in this simple experiment, then we predict that container size will be important in more complex experiments, such as those examining direct and indirect food web interactions. However, if we find that algal response is independent of mesocosm volume, then our results can be viewed as a first step; the next step would be to design experiments that vary container size but embrace more complexity, such as multiple trophic levels and abiotic factors (e.g. top-down and bottom-up effects).

Methods

Experimental design

We designed an experiment testing the singular and interactive effects of mesocosm volume and shape and nutrient enrichment on algal responses. Mesocosm volume varied over five levels, and orders of magnitude, as experimental tanks were 4, 20, 1000, 5000, or 500 000 L. Mesocosm shape was manipulated by establishing two sets of 4, 20 and 1000 L tanks that differed in the surface area : volume ratio (SA : V); one set had a high SA : V and a second set had a low SA : V. The 5000 and 500 000 L mesocosms were only present at the low SA : V shape as a second, high SA : V set was unavailable at these sizes. Nutrient levels were raised in half of the mesocosms by a single addition of nitrogen and phosphorus as solutions of NH_4NO_3 and $NaH_2PO_4.H_2O$, respectively [equal to 750 μg N and 75 μg P per L of mesocosm water (Vanni *et al.*, 2006a,b)]. Because N and P were added in a 10 : 1 ratio by mass, which is lower than Redfield proportions (i.e. 7 : 1), P availability was expected to limit algal growth. There were 16 treatments with three replicates each for a total of 48 mesocosms. The experiment lasted 7 days in September 2008.

Mesocosms were filled simultaneously with well-mixed, unfiltered water from a pond at the Ecology Research Center (ERC) of Miami University, OH, U.S.A., to ensure that all replicates received similar nutrient and plankton compositions. After filling the mesocosms with pond water, we took samples for initial (Day 0) nutrient and chlorophyll *a* (chl *a*) concentrations. Dissolved NH_4NO_3 and $NaH_2PO_4.H_2O$ solutions were then applied to nutrient enrichment treatments. The largest mesocosms were 500 000 L experimental ponds (Pilati *et al.*, 2009), which are similar in size (~0.07 ha) to many natural ponds. The 5000 L mesocosms were the same as those used in prior food web studies (e.g. Dickman *et al.*, 2008). The 1000 L high SA : V mesocosms were polyethylene cattle tanks (e.g. Nowlin *et al.*, 2007), while the low SA : V mesocosms were constructed with plastic sheeting and suspended in the 500 000 L ponds. The 1000 L low SA : V mesocosms were 'bags' commonly used in lake food web experiments (e.g. Sommer *et al.*, 2003). We used plastic containers as the 20 and 4 L mesocosms. Mesocosm dimensions, SA : V categorisation, description and location are reported in Table 1. Mesocosms were assigned to a low or high SA : V category based on ratios within a volume level. The 20, 1000, 5000 and 500 000 L mesocosms were exposed to ambient weather conditions at the ERC. To minimise the effects of fluctuating temperatures on the smaller volume mesocosms, the 20 L containers were kept in a water bath while 4 L treatments were maintained indoors in two environmental chambers. The environmental chambers were set to 25 °C, and light levels were adjusted to 300 $\mu mol\ m^{-2}\ s^{-1}$. The experimental design was blocked to statistically account for the spatial distribution of the 500 000 L ponds across a three-tiered field (see photo in Pilati *et al.*, 2009).

This combination of different mesocosms and incubation conditions reflects the diversity of conditions under which nutrient-limitation experiments are conducted (Elser *et al.*, 2007). As such we anticipate that this experimental setup will increase the generality of our results. If we do not find strong volumetric or shape (i.e. SA : V) effects on algal responses, then it is probable that results from different nutrient-limitation experiments can be directly compared without correcting for mesocosm dimensions.

Temperature was continuously monitored in high and low SA : V treatments of the 4, 20, 1000 and the 5000 L tanks for the duration of experiment using Thermochron iButtons model DS1921G (Embedded Data Systems, Lawrenceburg, KY, U.S.A.). Because the 1000 L low SA : V mesocosms were suspended in the 500 000 L ponds, we assumed that water temperature was the same for both volumes.

Light levels in the outdoor mesocosms were monitored over several days prior to beginning the experiment. We determined that light varied with mesocosm size and that mesh screens placed over all of the outdoor treatments, except for the 500 000 L ponds, ensured that the tanks experienced similar

Table 1 Mesocosm volume, dimensions, SA : V category, description and location are reported. Mesocosm dimensions are in metres and in the form of diameter × depth or length × width × depth

Volume (L)	Dimensions	SA : V	Description	Location
500 000	46.05 × 15.56 × 3.00	Low	Experimental pond	ERC
5000	2.24 × 1.57	Low	Polyethylene tank	ERC
1000	0.95 × 1.47	Low	Plastic sheeting 'bag'	ERC
1000	1.68 × 1.52 × 0.64	High	Polyethylene cattle tank	ERC
20	0.30 × 0.37	Low	Plastic container	ERC
20	0.53 × 0.39 × 0.14	High	Plastic container	ERC
4	0.15 × 0.25	Low	Plastic container	Incubator
4	0.32 × 0.18 × 0.11	High	Plastic container	Incubator

light levels. Light levels in the indoor, environmental chambers were based on mid-afternoon light levels in the outdoor tanks. Light meters were suspended approximately 0.5 m below the waters surface in the centre of one 5000 L mesocosm and one 500 000 L pond on days 2–4. Because of equipment availability, we were unable to monitor light levels throughout the entire 7- day experiment.

Depth-integrated water samples were taken from every mesocosm for chl *a* and total phosphorus (TP) concentrations on days 0, 2, 3, 5 and 7. Because of the size of the 500 000 L ponds, two replicate samples were taken from different locations in each. Samples were filtered for chl *a* using Pall A/E glass fibre filters (1.0- μm nominal pore size; Ann Arbor, MI, U.S.A.) and frozen until analysis. Chl *a* was later extracted from the filter with acetone and stored in the dark at 4 °C for 2–24 h until analysis with a fluorometer (Turner Designs, Sunnyvale, CA, U.S.A.). Total P samples were acidified and stored at 4 °C until digestion with potassium persulfate and analysis using a Lachat QC 8000 FIA auto-analyzer (Lachat Instruments, Loveland, CO, U.S.A.).

Statistical methods

To determine whether abiotic conditions (i.e. temperature and light), chl *a*, TP and algal responses varied with experimental treatment and day, we used a repeated measures ANOVA (SAS v. 9.1 for Windows, Cary, NC, U.S.A.). Temperature data were natural log (ln) transformed and analysed with volume (d.f. = 3) and shape (d.f. = 1) as fixed factors. Differences in light levels in the 500 000 L ponds and 5000 L mesocosms were examined with volume (d.f. = 1) as a fixed factor. Light data were analysed from days 2, 3 and 4 as those were the only days during which irradiance was recorded in both mesocosms. Block effects were not analysed for either temperature or light because these factors were not monitored in more than one replicate per block.

To determine whether nutrient enrichment affected chl *a* and TP concentrations throughout experiment, we conducted a repeated measures ANOVA with block (d.f. = 2) and nutrients (d.f. = 1) as fixed factors. To determine whether mesocosm volume and shape moderated algal response to nutrient enrichment, we used a repeated measures ANOVA with experimental block (d.f. = 2), mesocosm volume (d.f. = 4) and mesocosm shape (d.f. = 1) as fixed factors. We used chlorophyll effect size (chl *a* in enriched : ambient nutrient treatments) and algal nutrient use efficiency (chl *a* : TP) as metrics of algal response and analysed these variables on days 2, 3, 5 and 7 since nutrient enrichment effects on chl *a* did not occur until day 2. To determine whether tank dimensions affected variability in algal biomass, we conducted a repeated measures ANOVA of the coefficient of variation (CV) in chl *a* with mesocosm volume (d.f. = 4) and shape (d.f. = 1) as fixed factors. When TP and algal responses were significantly affected by experimental day, the data were separated according to day and re-analysed with multivariate ANOVAS; these analyses tested the singular effect of experimental block and the singular and interactive effects of nutrients, mesocosm volume and mesocosm shape. In addition, to look for specific effects of mesocosm size, we performed regression analyses of chl *a* and effect size against log-transformed volume.

Data were transformed as necessary to maintain homogeneity of variance. From the 3-way ANOVAS, we calculated the magnitude of main and interactive effects (ω^2, per cent variance explained [Olejnik & Algina, 2003; Duffy, Richardson & France, 2005; Spivak *et al*., 2007, 2009a)]. *Post hoc* Student Newman–Keuls (SNK) analyses were performed to examine differences between volume and shape levels.

Results

Temperature and light

Light levels, averaged over daily 12-h periods, were slightly higher in the 5000 L mesocosms (229 μmol m^{-2} s^{-1} ± 15 SE) than in the 500 000 L ponds (183 μmol m^{-2} s^{-1} ± 9 SE). During the 3-day period when irradiance was simultaneously measured in both volumes, there was no statistical difference in light levels (data not shown). Light levels in the 5000 L mesocosms and 500 000 L ponds were lower than in the 4 L high SA : V (~293 μmol m^{-2} s^{-1}) and low SA : V (~264 μmol m^{-2} s^{-1}) mesocosms that were incubated indoors. A possible explanation for the disparity in light levels between the outdoor and indoor mesocosms was that light was held constant for 12-h periods in the environmental chambers while outdoor mesocosms experienced daily fluctuations in light levels. However, light generally exceeded 300 μmol m^{-2} s^{-1} in the outdoor mesocosms from 11:00 to 16:00 hours daily. Although light levels differed in the indoor versus outdoor mesocosms, this would only affect the comparison of algal responses (e.g. effect sizes) if there is an interaction between the effects of nutrients and light.

Temperature, averaged over 24-h, varied with mesocosm volume and shape (Table 2). The lowest average 24-h temperature (16.5 °C) was recorded in the 20 L high SA : V mesocosms; the highest (26.2 °C) was in the 4 L low SA : V mesocosm. Water temperature tended to be most variable in the 20 L high SA : V mesocosms and least variable in the 1000 L and 5000 L low SA : V mesocosms. Although water column temperature was influenced by mesocosm shape and volume, this would affect the comparison of effect sizes among mesocosm sizes only if there was an interaction between the effects of nutrients and temperature.

Total phosphorus (TP)

After Day 0, TP concentrations were consistently higher in the enriched versus ambient nutrient treatments (Fig. 1a–e; Table 3). In nutrient-enriched mesocosms, TP peaked on day 2 and steadily declined until day 7 (101.4 μg L^{-1} ± 3.9 and 56.4 μg L^{-1} ± 3.4, respectively). Thus, in the nutrient-enriched treatments, we achieved our target concentration of 75 μg P L^{-1} above ambient TP (26.3 ± 1.2 μg P L^{-1}). The decline in TP concentrations over the 7- day experiment resulted in a nutrient by day interaction effect and was probably due to algal sedimentation (repeated measures ANOVA $P < 0.001$). Total P concentrations were 2.4 (day 7) – 3.9 (day 2) times higher in enriched versus ambient nutrient treatments. Volume influenced TP concentrations on Day 2, but this explained a small per cent of the variance in the data (i.e. $\omega^2 < 5\%$; Table 3).

Chlorophyll a (chl a)

Nutrient enrichment consistently increased chl *a* concentrations after day 0; the magnitude of the nutrient effect varied with time and resulted in a nutrient by day interaction effect (repeated measures ANOVA $P < 0.001$; Fig. 1f–j). Volume and shape affected chl *a* concentrations on day 0, suggesting that, despite mixing, algae were not uniformly delivered to all of the mesocosms (Table 3); however, differences among volume and shape treatments were relatively small (Fig. 1f–j). Since there was no main

Table 2 (a) Water temperature varied according to mesocosm volume and shape (SA : V) and day. Temperature data were natural log transformed, to maintain homogeneity of variance, and analysed with repeated measures ANOVA. Significant effects ($P < 0.05$) are in bold. (b) Means and standard error of mesocosm temperature according to volume and shape

a

Day		Volume		Shape		Day × Volume		Day × Shape		Volume × Shape		D × V × S	
MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
0.51	**<0.001**	10.05	**<0.001**	7.69	**<0.001**	0.09	**<0.001**	0.09	**<0.001**	0.07	0.389	0.08	**<0.001**

b

	4 L High SA : V		4 L Low SA : V		20 L High SA : V		20 L Low SA : V		1000 L High SA : V		1000 L Low SA : V		5000 L Low SA : V	
Day	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	24.75	0.82	24.25	0.88	18.85	0.50	20.87	0.32	22.20	0.27	23.17	0.07	21.05	0.06
1	21.80	0.28	22.73	0.25	16.97	0.42	19.16	0.24	20.82	0.21	22.80	0.07	20.42	0.06
2	20.71	0.16	26.16	0.08	16.51	0.35	18.04	0.19	19.47	0.16	21.81	0.06	19.74	0.05
3	20.60	0.15	24.43	0.12	18.61	0.16	19.13	0.08	19.86	0.07	21.49	0.04	20.00	0.00
4	20.56	0.15	23.47	0.11	18.33	0.33	19.28	0.19	19.99	0.19	21.27	0.08	19.46	0.04
5	20.76	0.18	23.83	0.14	17.40	0.35	19.06	0.17	19.95	0.16	21.44	0.06	19.20	0.04
6	20.93	0.20	23.01	0.12	17.36	0.19	18.69	0.12	19.79	0.09	21.28	0.05	19.22	0.03
7	20.75	0.16	22.85	0.20	17.25	0.48	18.54	0.34	19.50	0.27	20.84	0.10	20.02	0.21

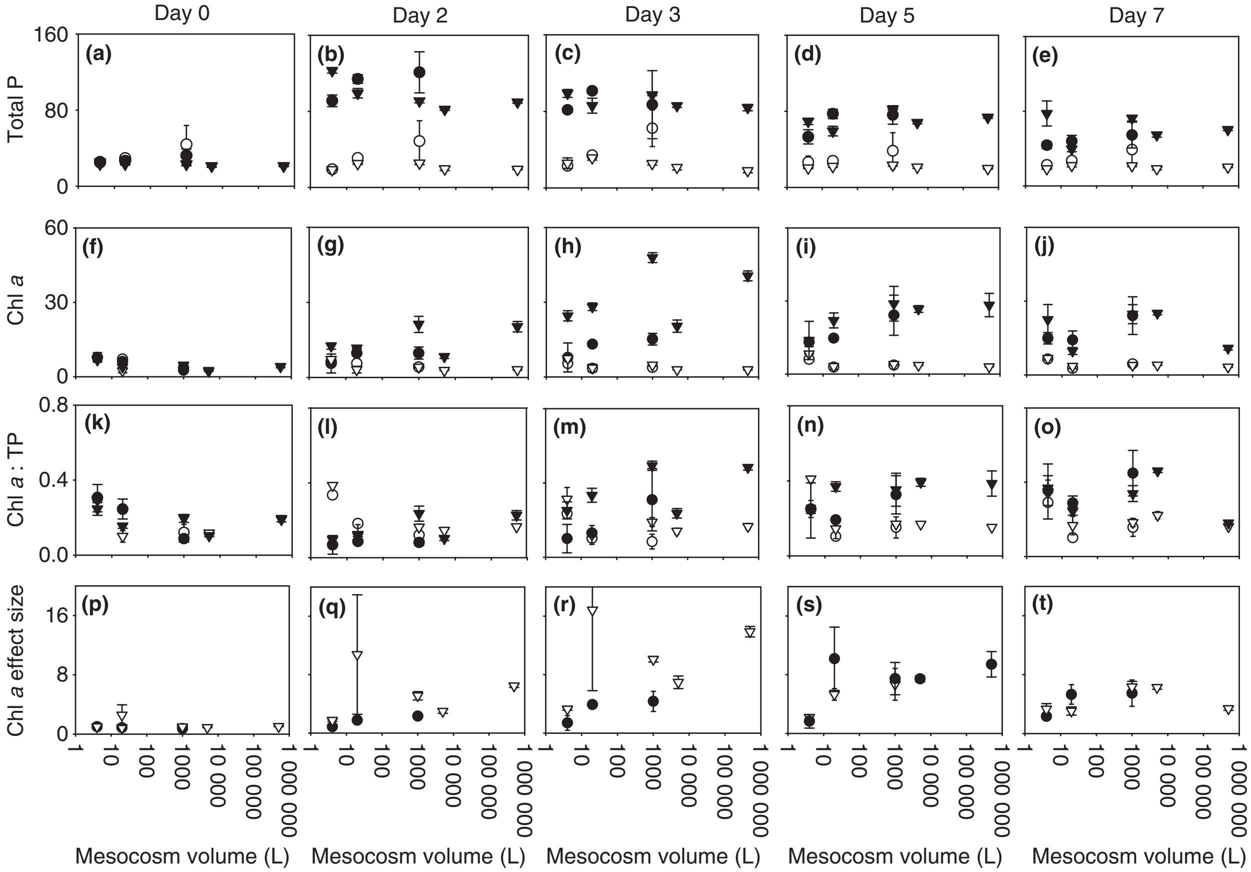

Fig. 1 TP concentrations (μg L^{-1}; a–e), chlorophyll *a* concentrations (chl *a*; μg L^{-1}; f–j), algal nutrient use efficiency (chl *a* : TP; k–o) and chlorophyll *a* effect size (chl *a* in enriched : ambient nutrient treatments; p–t) varied with mesocosm shape and volume throughout the 7- day experiment. Circles represent high SA:V treatments, triangles represent low SA:V treatments, filled symbols represent treatments with nutrient additions and empty symbols represent ambient nutrient treatments. For panels p–t, filled symbols represent high SA:V treatments while empty symbols are low SA:V treatments. Statistical results are reported in Table 3; error bars are standard error.

effect of volume on days 2–5, initial differences in algal concentrations probably did not influence chl *a* for the remainder of the experiment. Nutrient enrichment effects varied with mesocosm volume on days 2–7. While this suggests that volume mediated nutrient effects, the magnitude of the interactive effects varied across days. There were no significant correlations between chl *a* and volume on days 2–7 (data not shown). However, in nutrient-enriched treatments only, there were positive relationships between chl *a* and volume on days 2–3 ($P < 0.05$, $r^2 = 0.20–0.24$), but not on day 5 or 7. Nutrient enrichment effects were generally stronger in low SA : V treatments on days 2–3, resulting in a nutrient by shape interaction effect (Table 3). Since shape effects disappeared after day 3, it is probable that the importance of this factor in determining chl *a* concentrations changes over time. The CV of chl *a* was unaffected by mesocosm dimensions and did not vary with day (repeated measures ANOVA $P > 0.05$; data not shown).

Chlorophyll a: total P

The efficiency with which algae used phosphorus to build biomass varied throughout the 7- day experiment, resulting in a significant day effect (repeated measures ANOVA $P < 0.001$; Fig. 1k–o). Nutrient enrichment consistently increased chl *a* : TP after day 2 (Table 3); this effect was strongest at the end of the experiment as indicated by ω^2. The positive effect of nutrient enrichment on chl *a* : TP varied with mesocosm volume, resulting in an interaction effect after day 0 (Table 3). However, there was no clear directionality in the chl *a* : TP response to nutrient

Table 3 Results from multivariate ANOVAs testing the singular effect of experimental block and the singular and interactive effects of nutrient enrichment, mesocosm volume and mesocosm shape on concentrations of total P (TP) and chlorophyll *a* (chl *a*), the ratio of chl *a*: TP, and chl *a* effect size for days 0–7. Type III ANOVA results are reported as the design was unbalanced. The estimated magnitudes of effect (ω^2) of the manipulated variables on the response variables were calculated. Student Newman–Keuls analyses for shape and volume effects are listed; treatments with different means are noted with different superscripts. Data were transformed as necessary to maintain homogeniety of variance

	Block			Nutrients			Volume			Shape			Interaction	Model		
Day	MS	*P*	ω^2	MS	*P*	ω^2	MS	*P*	ω^2	MS	*P*	ω^2	(MS, *P*)	ω^2	SNK of Volume	SNK of Shape
log TP (μg L^{-1}) (N = 48)																
0	0.01	0.262	0.02	0.00	0.720	0.00	0.01	0.485	0.00	0.05	**0.029**	0.08		0.92	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{b}$
2	0.00	0.787	0.00	4.14	**<0.001**	0.78	0.03	**0.013**	0.02	0.03	0.068	0.00	N × V (0.02) 0.044 V × S (0.04) 0.022	0.18	$4^{bc}20^{ab}1000^{a}5000^{c}500000^{c}$	$H^{a}L^{b}$
3	0.01	0.661	0.00	2.62	**<0.001**	0.62	0.03	0.332	0.00	0.01	0.611	0.00		0.35	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
5	0.02	0.157	0.01	2.30	**<0.001**	0.73	0.01	0.310	0.00	0.02	0.204	0.00		0.27	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
7	0.01	0.437	0.01	1.47	**<0.001**	0.59	0.02	0.218	0.01	0.01	0.810	0.00	N × S (0.11) 0.003	0.34	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
ln Chl *a* (μg L^{-1}) (N = 48)																
0	0.28	**0.017**	0.04	0.03	0.511	0.00	1.13	**<0.001**	0.38	0.29	**0.038**	0.02	N × S (0.32) 0.028 V × S (1.19) <0.001	0.32	$4^{a}20^{b}1000^{b}5000^{c}500000^{b}$	$H^{a}L^{b}$
2	0.51	0.111	0.02	8.05	**<0.001**	0.27	0.34	0.214	0.02	0.52	0.134	0.01	N × S (2.61) 0.002 N × V (0.88) 0.010 V × S (1.00) 0.018	0.47	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
3	0.37	0.189	0.01	25.93	**<0.001**	0.48	0.42	0.123	0.02	3.89	**<0.001**	0.07	N × S (2.92) <0.001 N × V (1.09) 0.003	0.31	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{b}L^{a}$
5	0.39	0.090	0.01	28.81	**<0.001**	0.69	0.19	0.308	0.00	0.11	0.407	0.00	N × V (1.19) <0.001	0.21	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
7	0.02	0.774	0.00	21.28	**<0.001**	0.68	0.89	**<0.001**	0.10	0.01	0.726	0.00	N × V (0.28) 0.035	0.20	$4^{a}20^{b}1000^{a}5000^{a}500000^{b}$	$H^{a}L^{a}$
ln Chl *a* : TP (N = 48)																
0	0.12	0.380	0.00	0.01	0.817	0.00	1.25	**<0.001**	0.35	0.00	0.975	0.00	V × S (1.67) <0.001	0.45	$4^{a}20^{bc}1000^{bc}5000^{c}500000^{b}$	$H^{a}L$
2	0.45	0.211	0.01	3.42	**0.001**	0.12	0.28	0.408	0.00	1.25	**0.041**	0.04	N × V (1.55) 0.002 N × S (1.72) 0.018	0.54	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{b}L^{a}$
3	0.32	0.426	0.00	1.84	**0.032**	0.05	0.35	0.440	0.00	4.65	**0.001**	0.14	N × V (1.49) 0.009	0.63	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{b}L^{a}$
5	0.67	**0.044**	0.06	3.51	**<0.001**	0.19	0.22	0.360	0.01	0.40	0.158	0.01	N × V (1.06) 0.002	0.52	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
7	0.12	0.382	0.00	3.31	**<0.001**	0.26	0.79	**0.001**	0.22	0.03	0.628	0.00	N × V (0.47) 0.010 N × S (0.53) 0.041	0.42	$4^{a}20^{b}1000^{ab}5000^{a}500000^{b}$	$H^{a}L^{a}$
ln Chl *a* effect size (N = 24)																
0	0.01	0.929	0.00				0.21	0.278	0.06	0.65	0.054	0.13		0.84	$4^{a}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
2	0.29	0.558	0.00				1.75	**0.032**	0.23	5.22	**0.005**	0.21		0.62	$4^{b}20^{ab}1000^{ab}5000^{ab}500000^{a}$	$H^{b}L^{a}$
3	0.39	0.416	0.00				2.18	**0.009**	0.28	5.83	**0.002**	0.22		0.53	$4^{b}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{b}L^{a}$
5	0.21	0.517	0.00				2.38	**0.002**	0.53	0.00	0.924	0.00		0.49	$4^{b}20^{a}1000^{a}5000^{a}500000^{a}$	$H^{a}L^{a}$
7	0.28	0.153	0.05				0.56	**0.017**	0.32	0.00	0.874	0.00		0.57	$4^{b}20^{ab}1000^{a}5000^{a}500000^{ab}$	$H^{a}L^{a}$

Interactive effects are listed followed by the mean squared in parentheses and the *P* value for significance; N refers to Nutrients, S to Shape and V to Volume. *P* values <0.05 are in bold.

enrichment and volume. Chl *a*: TP was higher in low versus high SA : V tanks on days 2 and 3 (Table 3); this resulted in a significant shape effect (repeated measures ANOVA $P = 0.031$). Combined, these data indicate that algal nutrient use was more strongly influenced by nutrient levels than by mesocosm dimensions.

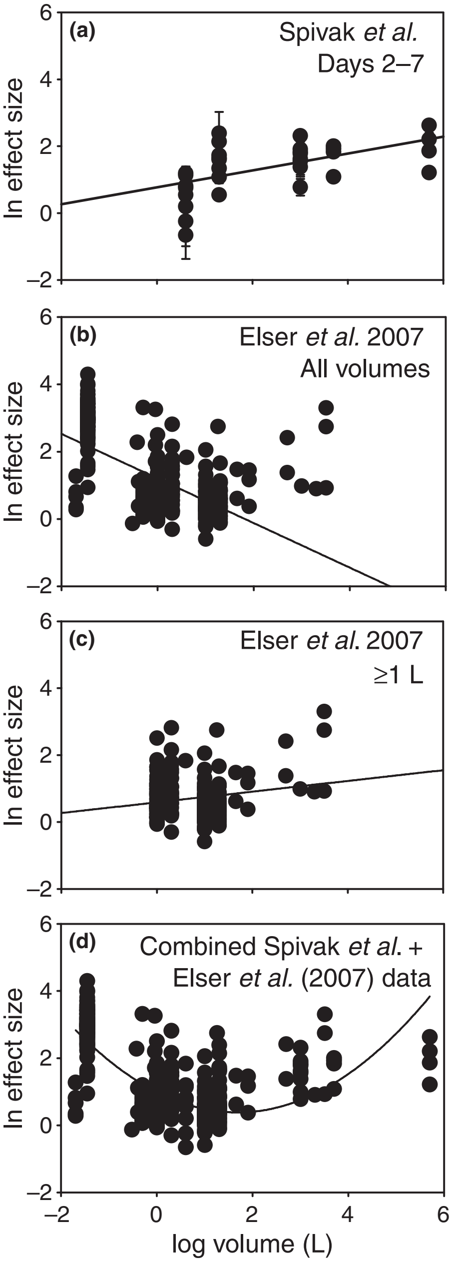

Fig. 2 Chlorophyll effect size versus volume from this experiment (a) ($y = 0.25x + 0.77$, $r^2 = 0.23$, $P < 0.001$) and the studies analysed by Elser *et al.* (2007; b, c). (b) Regression of all freshwater pelagic studies surveyed by Elser *et al.* (2007) versus volume ($y = -0.66x + 1.20$, $r^2 = 0.37$, $P < 0.001$). (c) Regression only includes studies from Elser *et al.* (2007) that were conducted in mesocosms ≥1 L ($y = 0.16x + 0.59$, $r^2 = 0.04$, $P = 0.003$). (d) Chlorophyll effect size from this study and those included in Elser *et al.* (2007; linear $y = -0.15x + 1.34$, $r^2 = 0.05$; polynomial $y = 0.17x^2 - 0.62x + 1.09$, $r^2 = 0.41$).

Chlorophyll a effect size

Chl *a* effect size, calculated by normalising nutrient-enriched treatments to ambient nutrient treatments according to block, varied with experimental day (repeated measures ANOVA $P < 0.001$; Fig. 1p–t) and was influenced by mesocosm dimensions (repeated measures ANOVA volume $P = 0.001$, shape $P = 0.012$). Volume effects were strongest on days 2–7 (Table 3) and chl *a* effect size was generally lowest in the 4 L mesocosms (Table 3, SNK results). Chl *a* effect size, pooled across all treatments and days, correlated positively with log-transformed volume (Fig. 2a). This was driven by positive relationships between effect size and volume on days 2–5 ($P < 0.05$, $r^2 = 0.28$–0.34); there was no correlation between the variables on day 7 ($P = 0.14$, $r^2 = 0.09$). The influence of mesocosm shape on chl *a* effect size varied with day as chl *a* effect size was higher in low versus high SA : V mesocosms on days 2 and 3 (Table 3); this resulted in a significant day by shape interaction effect (repeated measures ANOVA $P < 0.001$). Combined, our data indicate that mesocosm volume was a stronger determinant of chl *a* effect size than mesocosm shape. Mesocosm volume and shape explained similar proportions of variance in the data on days 2 and 3, as estimated by ω^2 (Table 3). However, mesocosm volume strongly influenced chl *a* effect size throughout the experiment. This suggests that the relative importance of mesocosm dimensions (i.e. shape versus size) may vary over time.

Discussion

Over the 7- day experiment, nutrient enrichment and mesocosm dimensions influenced algal abundance, nutrient use efficiency and effect size. Nutrient enrichment increased algal biomass, supporting our first hypothesis. Mesocosm volume and shape effects on algae were idiosyncratic and generally weak. For instance, algal abundance (chl *a*), nutrient use efficiency (chl *a* : TP) and effect size showed positive or neutral relationships with increasing mesocosm volume depending on the experimental day. The importance of shape effects, as estimated by ω^2, tended to decline towards the end of the experiment (Table 3). The absence of consistent, directional relationships between mesocosm dimensions and algal responses, combined with the low correlation between chlorophyll effect size and volume ($r^2 = 0.28$–0.34), indicate that mesocosm volume and shape may not strongly modify phytoplankton response to nutrient enrichment and provides partial support for our second hypothesis. Further, differences in chlorophyll effect size were relatively small among volume treatments that are most commonly used in field experiments (i.e. 20–500 000 L). This suggests that phytoplankton responses to nutrient enrichment are not strongly influenced by scale and that results from field mesocosm experiments, testing the direct effects of stressors on phytoplankton responses, are probably ecologically meaningful to larger, natural ecosystems. Finally, because our data indicate that volume effects were inconsistent and generally weak, we are reasonably confident that, if mesocosm size does matter in experiments with greater complexity, it is not because of scale effects on the direct phytoplankton response to nutrient enrichment.

Idiosyncratic effects of mesocosm dimensions on algal response to nutrient enrichment

Algae were nutrient limited, rapidly taking up phosphorus and building biomass (Fig. 1). Nutrient enrichment effects on algae were modified by mesocosm shape (i.e. SA : V) early in the experiment, resulting in stronger chl *a* effect sizes in low SA : V mesocosms (Fig. 1). This was somewhat surprising as we hypothesised that algal responses would be higher in treatments with a greater SA : V and, potentially, higher light availability. The absence of singular effects of SA : V at the end of the experiment suggests mesocosm shape effects on algal responses were limited and temporary.

Mesocosm volume singularly and consistently influenced chl *a* effect size throughout the experiment while effects on algal biomass and nutrient use efficiency were more idiosyncratic (Table 3). This indicates that volume did not uniformly influence all types of algal responses and supports previous predictions (Gerhart & Likens, 1975). Interestingly, chl *a* effect size was generally smallest in the 4 L treatments and larger and more uniform across the higher volume treatments (i.e. 20–500 000 L; Fig. 1). This suggests that mesocosms used in field studies, which are typically larger than 20 L, may not artificially influence algal responses to direct manipulations. Overall, we did not find consistent relationships between response variables (chl *a*, chl *a*: TP, chl *a* effect size) and mesocosm volume, which supports our second hypothesis but is in contrast to prior assertions that environmentally meaningful results are obtained only by manipulating entire ecosystems (Carpenter, 1996, 1999; Schindler, 1998). Further, mesocosm dimensions had no effect on variability in algal biomass (data not shown). This contradicts predicted relationships between variability and experimental scale (Petersen, Cornwell & Kemp, 1999; Kemp *et al.*, 2001) and suggests that spatial patchiness in chl *a* concentrations did not vary with mesocosm dimensions (Weins, 2001).

One of the strongest patterns we observed was that mesocosm shape and volume effects on algal responses varied with day (Table 3; Fig. 1). For example, algal biomass (nutrient enriched treatments only) and chl *a* effect size were positively correlated with volume at various times in the experiment. This indicates that dimensional effects were temporary and underscores the importance of sampling times and experimental duration; arbitrary selection of sampling time points may lead to conclusions that do not reflect ecologically meaningful processes. Therefore, caution should be used when scaling mesocosm results to larger systems because, depending on experimental duration, chl *a* effect size can vary with mesocosm volume.

Comparison with a recent meta-analysis

Nutrient-limitation experiments are widely used to identify the factor(s) limiting primary production and to predict how productivity will change under different scenarios of nutrient availability (Downing *et al.*, 1999; Francoeur, 2001; Elser *et al.*, 2007). A recent meta-analysis by Elser *et al.* (2007) of 1060 nutrient enrichment experiments from freshwater, marine and terrestrial habitats concluded that N and P additions increase production across all three environments. We compared our results to Elser *et al.*'s (2007) data, which are publicly available through the National Center for Ecological Analysis and Synthesis (http://knb.ecoinformatics.org/knb/meta cat?action=read&qformat=nceas&docid=nceas.347), to examine the uniformity of freshwater algal responses to nutrient enrichment over broad spatial and temporal scales. Of the 1060 experiments, we included 359 freshwater pelagic experiments that lasted an average of ~7 days (1–71 day range) in mesocosms spanning 0.02–3200 L. The experiments received additions of N or P or both; the average N : P ratio of additions was 24 (2–147 range).

In general, algal responses to nutrient enrichment were similar across our experiment and the freshwater pelagic studies analysed by Elser *et al.* (2007). The average ln effect size (defined as algae response in treatments that received N and P additions normalised to control treatments) was 1.37 (±0.09 SE) in our experiment and 1.25 (±0.06 SE) in Elser *et al.* (2007). Despite this, the correlation between ln effect size and log volume was positive in our experiment but negative in the studies surveyed by (Elser *et al.*, 2007; Fig. 2a,b). The negative correlation in the study of Elser *et al.* (2007) was driven by mesocosms with a volume <1 L; when 137 small volume (0.02–0.9 L) treatments were removed from the dataset, to equalise the range of volumes in this study and Elser *et al.* (2007), the relationship between log volume and ln chl *a* effect size was positive in the study of Elser *et al.* (2007) (Fig. 2c). Exclusion of mesocosms <1 L also had the effect of reducing the amount of variance in chl *a* effect size explained by volume. In addition, ln chl *a* effect size and the N : P ratio of additions was positively correlated in <1 L mesocosms ($r^2 = 0.20$; data not shown) but were unrelated in mesocosms ≥1 L ($r^2 < 0.01$; data not shown).

When the results from our experiment were pooled with the 359 freshwater experiments in Elser *et al.* (2007), the variance in the data was better explained by a polynomial model ($r^2 = 0.41$) than a linear model ($r^2 = 0.05$) and effect size was lowest in mesocosms of intermediate volume (Fig. 2d). There are two possible explanations for this result. First, algal response to nutrient enrichment was greater at volumes <1 L meaning that extrapolations of algal responses from small volume mesocosm experiments may overestimate ecosystem productivity. Second, intermediate-sized mesocosms may underestimate production (Fig. 2d). However, it is unclear why intermediately sized mesocosms would produce smaller algal responses than low- and high-volume mesocosms. Overall, the data do not allow us to distinguish between these different hypotheses. Combined, our results and the data from Elser *et al.* (2007) suggest that algal responses to nutrient enrichment may differ at low and intermediate mesocosm volumes but are similar in larger volume mesocosms (>20 L), which are typically used in field experiments.

The effect of scale on simple aquatic manipulations

Our results suggest that algal responses to simple, direct manipulations are generally similar across larger volume mesocosms. This is in contrast to multiple studies that analysed the effects of mesocosm dimensions, including volume, depth, radius and wall area, on ecological properties, processes and variability (Chen, Petersen & Kemp, 1997; Petersen *et al.*, 2003; Petersen & Englund, 2005). Results from these studies and others indicate that mesocosm dimensions can affect abiotic resources, including light availability, gas exchange and surface area, which, in turn, influence biological processes. In response to these findings, effort has been devoted to developing scaling rules that translate experimental results to entire ecosystems (Petersen & Hastings, 2001; Schneider, 2001 and references therein; Petersen *et al.*, 2003). In our experiment, mesocosm volume correlated positively to algal biomass (nutrient treatments only) and effect size. However, these correlations explained a small portion of the variance and were temporary, indicating that volume was a poor predictor of algal response to nutrient enrichment. In addition, variation in chlorophyll concentration was unaffected by and unrelated to mesocosm dimensions. Similar findings between this experiment and the freshwater pelagic studies surveyed by Elser *et al.* (2007) further reinforce that algal responses to direct manipulations in large volume mesocosms can probably be 'moved on up' to more natural systems without correcting for mesocosm dimensions.

The legitimacy of extending mesocosm findings to natural habitats is relevant to management decisions, including eutrophication controls in freshwater and coastal systems. For instance, the current paradigm, which asserts that reducing phosphorus inputs will reduce eutrophication, is based on long-term manipulations of entire lake ecosystems (Schindler *et al.*, 2008; Schindler & Hecky, 2009). Challenging this convention is the view that management approaches that simultaneously target phosphorus, nitrogen and, possibly, other nutrients, minerals and/or metals will more effectively control eutrophication; this recommendation is based largely on mesocosm studies (Lewis & Wurtsbaugh, 2008; Sterner, 2008; Conley *et al.*, 2009). While some argue that mesocosm systems are too small to adequately mimic natural ecosystems, we found relatively similar phytoplankton growth responses across mesocosms spanning several orders of magnitude. Further, the largest mesocosms in this experiment, the 500 000 L experimental ponds, were bigger than many productive and ecologically important ecosystems, including rock pools, tree holes and some natural ponds (Srivastava, 2005; Vanschoenwinkel *et al.*, 2007). Thus, our results provide support for using mesocosm systems in determining factors limiting aquatic primary producer growth and extrapolating those findings to larger aquatic systems (Drenner & Mazumder, 1999; Sterner, 2008).

Proponents of controlling eutrophication by reducing phosphorus contend that results of nutrient-limitation experiments from mesocosm systems have limited relevance to natural ecosystems because they do not account for long-term changes in community dynamics and biogeochemical processes (Carpenter *et al.*, 1995; Schindler, 1998; Schindler *et al.*, 2008). For instance, certain trophic and competitive interactions are sensitive to spatial scale (Bertolo, Lacroix & Lescher-Moutoue, 1999; Bergstrom & Englund, 2002; Ostman & Ives, 2003). In addition, algal growth is probably affected by benthic–pelagic coupling in shallow systems and erosional processes controlling mineral availability at longer timescales (Schindler, 1998). As such, our conclusions apply mainly to simple manipulations of direct interactions in pelagic systems at timescales relevant to phytoplankton growth. Responses involving indirect food web interactions and processes that occur over longer scales (e.g. numerical responses of consumers) may be more sensitive to variation in spatial scale; this hypothesis needs to be explicitly tested. Thus, experimental tests of the effects of mesocosm size on various ecosystem responses will continue to produce important insight into drivers of complex ecological and biogeochemical processes.

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