Freshwater Biology (2008) **53,** 1291–1302 doi:10.1111/j.1365-2427.2008.01962.x

Stoichiometric relationships in vernal pond plankton communities

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SUMMARY

1. The light-nutrient hypothesis (LNH) predicts that changes in light supply can alter the balance of nutrient and energy limitation in primary producers. We tested this prediction by examining temporal changes in vernal forest ponds, which are highly dynamic systems with respect to seasonal change in light and nutrient supply. In three vernal ponds that differ in productivity, we measured changes in light, total and seston nitrogen and phosphorus, and seston carbon and chlorophyll during the spring, before and after tree leaf-out. We also quantified changes in the population dynamics of the major zooplankton grazers in these systems.
2. In each pond, nutrient levels increased and light levels declined, creating a temporal shift in light-nutrient supply to the plankton. Results generally supported predictions of stoichiometric theory and the LNH, but there were notable exceptions.
3. Seston C : N : P ratios rapidly changed in response to dramatic increases in N and P supply rates. However, seston N : P was typically lower than values for total N : P in the water. Furthermore, as predicted, we observed a decline in seston C : P as the light : nutrient ratio declined, but seston C : N simultaneously increased. These results suggest an unexpected shift towards potential nitrogen limitation. Alternatively, this change in nutrient ratios may be driven by a seasonal change in phytoplankton composition or nutritional mode.
4. Seston carbon concentrations remained stable despite seasonal changes in grazing intensity associated with the phenology of large-bodied *Daphnia* grazers. However, chlorophyll concentrations declined dramatically as the season progressed, resulting in a simultaneous decline in the C : Chlorophyll ratio of seston. Both pond shading and increased grazing probably contributed to the decline in chlorophyll.

Keywords: carbon, *Daphnia*, light-nutrient hypothesis, nitrogen, phosphorus

Introduction

Ecological stoichiometry, the balance of nutrients and energy in ecological interactions, provides a valuable framework for investigating trophic interactions (Sterner & Elser, 2002). By tracking supply rates of elements such as carbon, nitrogen and phosphorus through the primary producers and into grazers, questions of energy versus nutrient limitation can be addressed at multiple trophic levels (Hassett *et al.*, 1997; Elser *et al.*, 2000; Hall *et al.*, 2006). The elemental composition of primary producers fluctuates in response to external changes in nutrient supply whereas that of grazers is more constant (Andersen & Hessen, 1991; Hessen & Faafeng, 2000). Hence, stoichiometric theory predicts thresholds of change where, depending upon the nutrient supply ratios, it is expected that grazers can become limited by nutrients (food quality) rather than energy (food

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quantity). Such transitions impact energy flow and nutrient recycling within ecosystems, can shift competitive relationships among species, and shape the evolution of organism physiology and life history (Andersen, 1997; Sterner & Elser, 2002).

The stoichiometric framework considers more than just the supply of elemental nutrients. Light is also important, as is the interaction between light and nutrients. In their light-nutrient hypothesis (LNH), Sterner *et al.* (1997) make several predictions regarding how nutrient use efficiency should vary across systems experiencing different light to nutrient ratios. The general concept is that as light becomes more limiting to primary producers, their elemental composition should become enriched in nutrients such as nitrogen and phosphorus relative to carbon, resulting in increased food quality for grazers and higher trophic transfer efficiency. Of course, low light conditions will also reduce overall primary productivity, decreasing food quantity for grazers. However, the evidence available from lakes suggests that limitation via food quality (including stoichiometry, phytoplankton assemblage, fatty acid composition, etc.) is often more important than is limitation by food quantity (Sterner & Schulz, 1998).

Several predictions of the LNH have been confirmed by focusing on comparisons among lakes that differ in mixing depth (mean light conditions for the phytoplankton), transparency and productivity either by using short-term manipulations such as mesocosm experiments or broad-scale comparative studies (e.g. Urabe & Sterner, 1996; Sterner *et al.*, 1997; Hessen, Færøvig & Andersen, 2002; Diehl, Berger & Wohrl, 2005; Schade *et al.*, 2005; Dickman, Vanni & Horgan, 2006). Hall *et al.* (2007) extended the LNH to include ponds that differ in light caused by tree shading, but still considered only spatial variation among communities. However, light limitation has long been viewed as central in explaining *temporal* variation in primary productivity in most temperate ecosystems, and is pivotal to our understanding of seasonal succession in the plankton (Sommer *et al.*, 1986). The LNH predicts that an annual reduction in light level may cause a shift in the balance of nutrient and light limitation in the phytoplankton. However, this prediction has received limited testing (Chrzanowski & Grover, 2001).

Vernal ponds are intriguing systems in which to address these stoichiometric concepts for a number of reasons. First, these systems tend to exhibit dramatic temporal changes in light and nutrient supply levels (Colburn, 2004). In forested ponds, tree leaves predictably open out over the course of a few weeks, decreasing light levels dramatically. In addition, vernal ponds form from nutrient-dilute snowmelt and rain, but should experience a large increase in nutrient supply due to decomposition from forest floor litter (Wilbur, 1997). Second, temporary ponds are often dominated by mixotrophic algae (Colburn, 2004). As the season progresses, this ability to change nutritional mode as light and nutrient supply ratios are altered, coupled with overall changes in species composition, may contribute to variance in the stoichometric ratios of the seston. Third, the influence of top-down factors on seston stoichiometry can fluctuate just as dramatically as the bottom-up factors of light and nutrients (DeMott & Tessier, 2002). Hall *et al.* (2007) showed evidence that grazers may play a key role in explaining field patterns of variation in seston stoichiometry. In particular, top-down effects of grazers increase turnover rate of the producers and recycling rates of elements, both of which are expected to influence the stoichiometry of producers. Large-bodied daphniid species, which are a hallmark of temporary ponds (Schneider & Frost, 1996; Colburn, 2004), hatch from diapausing eggs and can quickly attain high biomass. Just as quickly, these populations can disappear from the water entirely, as the result of producing diapausing rather than immediately hatching eggs and increased predation. This creates a highly dynamic system with respect to grazer-imposed mortality rates on the primary producers.

In this study, we ask how temporal changes in light and nutrient supply coupled with seasonal changes in grazer abundance relate to seston stoichiometry in three temporary ponds located in southwestern MI, U.S.A. Specifically, we addressed the following questions (1) Are the patterns of temporal changes in seston stoichiometry similar across ponds that differ in trophic state (nutrient supply)? (2) Are these temporal changes related to the reduction in light supply caused by tree leaf-out? In addition, we discuss how seasonal changes in grazer abundance may influence, or be influenced by, seston stoichiometry. Our study was motivated by the premise that temporal shifts in elemental ratios, including light, may be particularly important in forested temporary

ponds, where canopy closure, temporal variation in supply rates and variable grazing pressure can create substantial seasonal changes.

Methods

We studied three natural, vernal forest ponds that differ in productivity as reflected by nutrient concentrations of nitrogen and phosphorus (Roughwood, Woodfrog and West Gull). The ponds, located in Kalamazoo County, MI, U.S.A., fill with snowmelt and rainwater in early spring (March–early April), at which time they achieve their greatest volume. This is also the time of peak sunlight to these ponds. Deciduous trees in and around the ponds form a forest canopy that shades the ponds after leaf-out, which is typically in May. Hence, each pond experiences a temporal gradient in light over the course of only 2 months. The ponds typically dry in June, but Woodfrog has a higher groundwater input than the other two ponds and can remain wet in some years (S. Hamilton, pers. comm).

To quantify temporal changes in light, nutrient supply rate, seston stoichiometry and grazer dynamics, we began sampling the ponds immediately after ice out on 29 March 2001 for Roughwood and Woodfrog Ponds, and on 6 April 2001 for West Gull Pond. We sampled weekly until 20 June, after which time shallow water precluded sampling. We measured light levels at the pond surface on each sampling date in April and May using a LI-COR Quantum meter and PAR sensor (LI-COR Biosciences, Lincoln, NE, U.S.A.). These values were expressed as a percent of full sun values by making simultaneous light measurements in open areas adjacent to each pond. We collected grab samples of water for nutrient analysis from the middle of the water column, making certain to avoid inclusion of sediment. Water samples were frozen for later analysis of total phosphorus (molybdate-ascorbic acid method; APHA, 1980; Prepas & Rigler, 1982) and total nitrogen (Bachmann & Canfield, 1996). We also measured the C, P and N content of the seston by passing the water through a 62 μm sieve and then filtering it onto pre-combusted, GF/F glass fibre filters (Whatman, Clifton, NJ, U.S.A.). Particulate nitrogen and carbon were measured using a Carlo-Erba C : H : N analyzer (Carlo-Erba, Milan, Italy) and particulate phosphorus was measured by the molybdate-ascorbic acid method. Samples for chlorophyll *a* (total, <62 μm, <15 μm and <3 μm) were also filtered onto GF/F glass-fibre filters, extracted in cold 95% ethanol and measured by use of narrow band fluorometry (Welschmeyer, 1994).

To sample the zooplankton assemblage, we collected three replicate zooplankton samples (12–21 L each) on each date by pooling 4–7 (depending on pond size) grab samples, taken with a 3-L pitcher. The grab samples were taken while walking a transect through the middle of each pond, taking care not to resample previously disturbed areas (Dudycha, 2004). Two of the samples were passed through a 170 μm sieve to concentrate the zooplankton and were individually preserved in >70% ethanol and processed under a dissecting microscope. All individuals were identified and counted to species (*Daphnia* and *Daphniopsis*), genus (all other cladocerans) or order (copepods). *Daphnia* adults and juveniles were counted separately, and for *Daphnia pulex* Leydig which was the most common species, a random sample of adults was measured for body length and fecundity. These body length measurements were used to estimate grazing rate, based on the equation given in Knoechel & Holtby (1986). The third zooplankton sample was used to estimate total grazer biomass. That sample was returned to the laboratory on ice and filtered live onto a pre-weighed glass fibre filter, dried overnight at 55 °C, and weighed using an analytical balance.

We measured pond surface dimensions and depth on each sampling date. Using these morphometric parameters, we estimated pond volume on each date, so that zooplankton abundances could be expressed in terms of total pond volume. Analyzing zooplankton values as number L^{-1} can confound temporal changes in population dynamics with a concentration of individuals as the pond dries (Hairston, 1988).

To assess the possibility of temporal changes in food availability to the grazers, we used the egg ratio method to calculate birth rates (day^{-1}) for *D. pulex* (Edmondson, 1960; Paloheimo, 1974): $b = ((\ln(E/N)+1))/D$. This equation is based on the number of eggs per individual (E/N) and the temperature-dependent development time D. Development times were calculated based on the equation given in Bottrell *et al.* (1976). The temperature we used in calculating egg development times was the mean of the minimum and maximum temperatures that

occurred between each sampling date, as recorded from a max–min thermometer left in each pond in a shaded location. Population growth rates (r, day^{-1}) were estimated from the logarithms of the water-column abundance (N) on two successive sampling dates (t_2 and t_1): $r = (\ln N_2 - \ln N_1)/(t_2\text{-}t_1)$ (e.g. Hall, 1964). Death rates (d, day^{-1}) were then estimated by taking the difference between birth and realized growth rates.

Seasonal changes in the three ponds were statistically modelled using a repeated measures analysis of variation (SAS, 2002, ver 9.1, Cary, NC, U.S.A.), following log transformation (except for light). For most variables, we were interested in changes that occurred in response to shading of the ponds by leaf-out, i.e. the contrast of the first 4 weeks of sampling (open) to the second 4 weeks of sampling (shaded). To compare the responses of the three ponds, for each pond, we treated samples collected in the first 4 weeks as replicates for the open time period and samples collected in the second 4 weeks as replicates for the shaded period.

In testing stoichiometric predictions, we treated our estimates of TN and TP as proxies of nutrient supply rates (Hall *et al.*, 2005, 2007). Similarly, we used our estimates of per cent of full sun as a relative measure of light resource to create ratios with TN or TP in testing the light : nutrient ratio hypothesis. Temporal variation in seston elemental composition (C : N : P) was related to these resource supply ratios, and to grazer biomass (log transformed), using ANCOVA, with pond treated as a categorical factor (SYSTAT, 2000 ver.10; San Jose, CA, U.S.A.). For each ANCOVA model, we first confirmed the homogeneity of slopes (all pond*factor interactions $P > 0.16$).

Results

The three ponds differed in size and trophic status, as measured by TP, chlorophyll and particulate carbon concentrations in early April (Table 1, Fig. 1). Roughwood is oligotrophic with chlorophyll of only 2 μg L^{-1}, Woodfrog is typical of mesotrophic conditions, with chlorophyll values of 4–6 μg L^{-1}, and West Gull is eutrophic with chlorophyll values exceeding 20 μg L^{-1}. Similarly, particulate carbon is low (~400 μg L^{-1}) in Roughwood, but nearly double that in Woodfrog, and nearly double that again in West Gull (Fig. 1b).

Light levels dramatically declined from April to May following leaf-out of the canopy (RM ANOVA – time $F_{1,8} = 20.39$, $P = 0.002$, Fig. 1a). By early May, the average amount of light reaching the surface of the three ponds was only 22% of that in open areas (Table 1). Of course, total hours of daylight increased from 12 h, 43 min in early April to 15 h, 5 min on 31 May. However, the additional hours of daylight represent <20% increase, as compared to the 84% reduction of light reaching the ponds surface due to leaf-out. We represented light levels as percentage of the open area to avoid confounding temporal decreases caused by leaf-out with temporal variance in cloud cover or angle of sun. Nevertheless, the amount of PAR we measured at the pond surface in May was considerably lower than April values.

The results of the cholorphyll size fractionation confirmed that the phytoplankton assemblage was composed primarily of small cells in all three ponds (Table 1). Hence, the <62 μm fraction, which contained >90% of the total chlorophyll, represented the bulk of the seston. Not surprisingly, the reduction in

Table 1 The three ponds differed in size and trophic status, as measured by total phosphorus, chlorophyll and particulate carbon concentrations in early April

Pond	Max volume (m^3)	April TP (μg L^{-1})	April light (%)	May light (%)	%Chl-*a* <3 μm	%Chl-*a* <15 μm	%Chl-*a* <62 μm	April C : Chl	May C : Chl
Roughwood	760	14.3	39	9	24	94	99	205	423
Woodfrog	230	59.4	69	37	26	72	91	61	782
West Gull	2160	212.8	54	20	24	89	94	168	838

Values for April and May light levels are the percentage of full sun values reaching the pond surface, estimated by making simultaneous light measurements in the pond and at open areas adjacent to each pond. The size structure (% of total Chl-*a* represented by the <3 μm, <15 μm, <62 μm fraction) of the phytoplankton was relatively constant through time. Hence, the average percentage of chlorophyll in each size class (<3 μm, <15 μm, <62 μm) are averages for the entire sampling period. April and May cabon : chlorophyll ratios (C : Chl) are for the <62 μm size fraction.

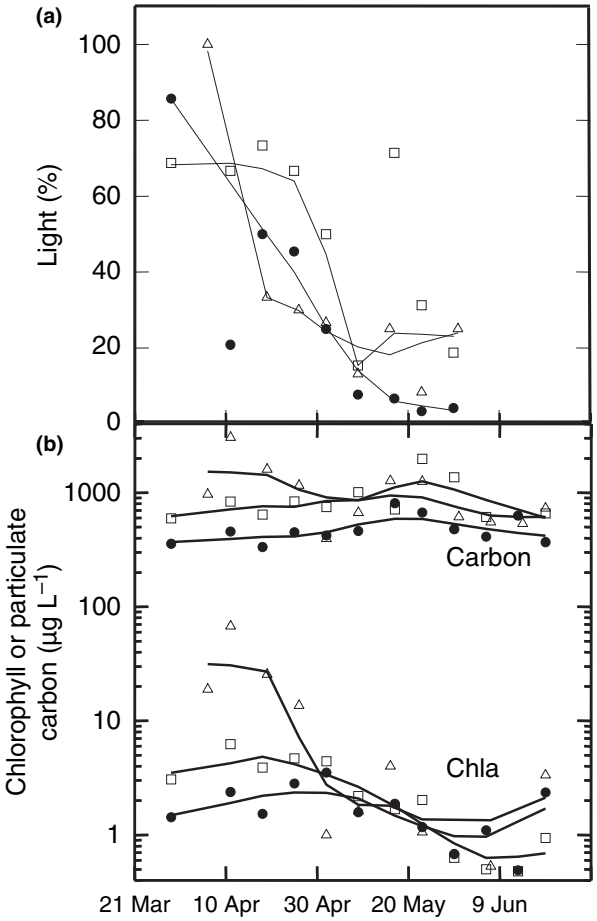

Fig. 1 (a) Light levels in each pond expressed as the percentage of ambient light reaching the pond surface. The lines represent a LOWESS smoother with a tension of 0.6. (b) Temporal changes in concentrations of particulate carbon and chlorophyll *a*. Both are the <62 μm fraction. The lines represent a LOWESS smoother with tension of 0.5. In both panels, triangles are for West Gull Pond, squares for Woodfrog and circles for Roughwood.

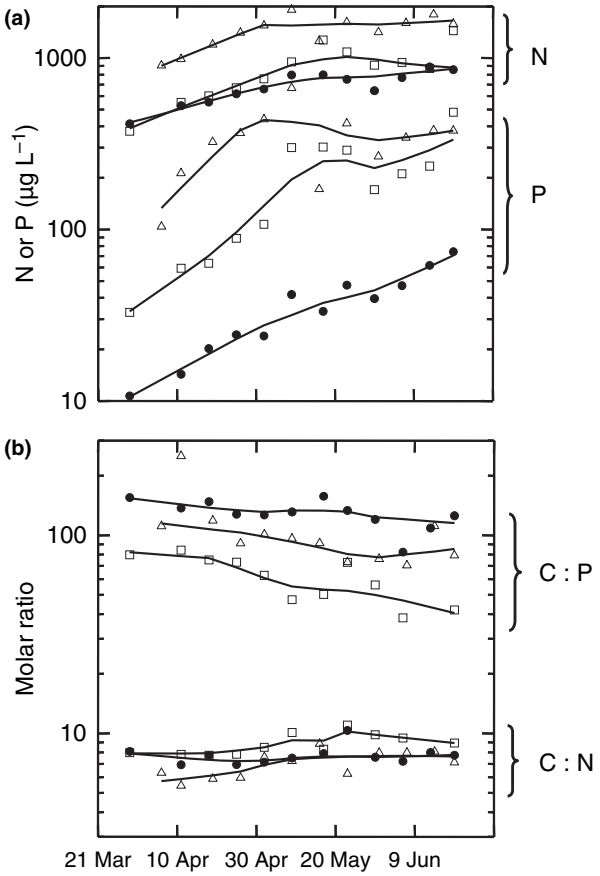

Fig. 2 (a) Concentrations of total nitrogen (TN) and total phosphorus (TP) through time. Although both TN and TP increase through time, the rate of increase in TP is much faster than that of TN. (b) Molar ratio of carbon : phosphorus and carbon : nitrogen of the seston (<62 μm). In both panels, triangles are West Gull, squares are Woodfrog and circles are Roughwood.

light levels was associated with decreases in chlorophyll concentrations (RM ANOVA – time, $F_{1,9} = 56.7$; $P < 0.0001$, Fig. 1b); furthermore, the large differences among ponds in April chlorophyll essentially disappeared in May as total chlorophyll dropped to <1 μg L^{-1} (RM ANOVA – pond*time interaction, $F_{2,9} = 9.5$, $P = 0.006$). Despite seasonal declines in chlorophyll, seston carbon stayed relatively constant throughout the sampling period (RM ANOVA – time, $F_{1,9} = 0.02$; $P = 0.88$), although the ponds differed in average seston carbon as reflected by their productivity (RM ANOVA – pond, $F_{1,9} = 8.1$, $P = 0.01$). Consequently, the C : Chlorophyll ratio underwent a drastic increase in all ponds (Table 1). When light levels were high in April, average C : Chlorophyll was 144, but in May this ratio averaged 681.

In all three ponds, the concentrations of TN and TP greatly increased from April to May (Fig. 2a, RM ANOVA – time, TN, $F_{1,9} = 58.8$, $P < 0.0001$; TP $F_{1,9} = 28.0$, $P = 0.0005$). The ponds differed in mean nutrient concentrations, (RM ANOVA – pond, TN, $F_{2,9} = 23.1$, $P = 0.0003$; TP, $F_{2,9} = 48.1$, $P < 0.001$) and they retained their rank differences through time (TN pond*time interaction $F_{2,9} = 2.38$, $P = 0.15$; TP time*-pond interaction $F_{2,9} = 2.36$, $P = 0.15$). TP displayed greater proportionate increases than did TN.

The three ponds were clearly distinguished in C : P of their seston (Fig. 2b, RM ANOVA – pond; $F_{2,9} = 20.1$, $P = 0.0005$). Furthermore, sestonic C : P decreased seasonally in all three ponds (RM ANOVA –

time; $F_{1,9}$ = 9.2, P = 0.014), with no significant pond* time interaction ($F_{2,9}$ = 1.8, P = 0.22). Hence, in both comparisons among ponds, or temporally within each pond, seston C : P reflected changes in supply rates of P (measured by TP), as predicted by stoichiometric theory. The C : N of the seston also differed among ponds (Fig. 2b; RM ANOVA – pond $F_{2,9}$ = 16.4, P = 0.001) and seasonally (RM ANOVA – time $F_{1,9}$ = 12.9, P = 0.006), but in the opposite direction. Woodfrog, which had the lowest seston C : P values, had the highest C : N ratios. Through time, pond C : N values increased instead of decreasing as with C : P.

We tested for a relationship between C : P ratio of seston and the light : TP ratio in the ponds (Fig. 3a). As a joint consequence of both decreased light from tree leaf shading, and increased TP in the water, the light : TP ratio decreased by more than an order of magnitude in each pond during April and May. The ponds showed the predicted decrease in seston C : P with decreased light : TP ratio (ANCOVA $F_{1,20}$ = 6.2, P = 0.02, Fig. 3a). Surprisingly, seston C : N also exhibited a marked change in response to decreased light : TP ratio ($F_{1,20}$ = 4.6, P = 0.04, Fig. 3b), but in the opposite direction of seston C : P. The qualitative pattern of decreasing seston C : P and increasing seston C : N is similar for both light : TP or light : TN as the surrogate for light : nutrient ratio. However, since there was much less variance over time in the light : TN as compared to the light : TP ratio, significance values were 0.068 C : P and 0.098 for C : N for the models considering the effect of light : TN.

We also tested for a relationship between the N : P ratio of the seston and the TN : TP ratio ($F_{1,31}$ = 7.89, P = 0.001; Fig. 4). West Gull exhibited little change in TN : TP, but for the two other ponds (Roughwood and Woodfrog) a strong seasonal decrease in TN : TP produced the expected positive relationship with seston N : P. However, seston N : P is consistently lower than TN : TP (below a 1 : 1 line) in these ponds. In West Gull, which was the richest in TP (lowest in TN : TP), the seston N : P was initially higher than expected but quickly decreased to match TN : TP within a few weeks.

Zooplankton abundance fluctuated in all three ponds (Fig. 5). *Daphniopsis ephemeralis* Schwartz and Herbert, *Daphnia pulex*, *Ceriodaphnia* sp., *Scapholeberis* sp., *Simocephalus* sp., cyclopoids, ostracods and mosquito larvae were found in all ponds and *Daphnia laevis* Birge was found in small numbers in West Gull pond. Most taxa were rare; hence we only consider the dynamics of the three most abundant taxa. *Daphniopsis ephemeralis* peaked first in all three ponds, and this was also the first species to return to dormancy and disappear from the water column by early May. This species largely completed its life cycle prior to pond shading. In contrast, *D. pulex* and cyclopoid copepods dominated the water column after the reduction in light caused by tree leaf-out. Although both the *D. pulex* population and the cyclopoids declined sharply during May, a second

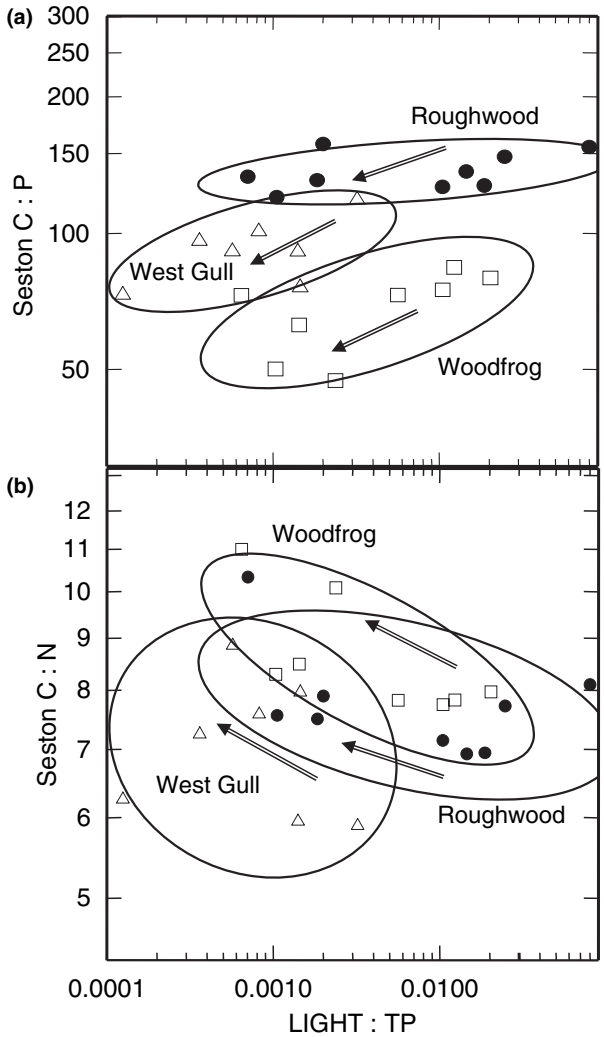

Fig. 3 Seston (<62 μm) carbon : phosphorus ratio (a) and nitrogen : phosphorus ratio (b) as a function of the light : TP ratio. For each pond, the arrow represents the change in values over time, from early April to mid May. The ellipses indicate bivariate SE.

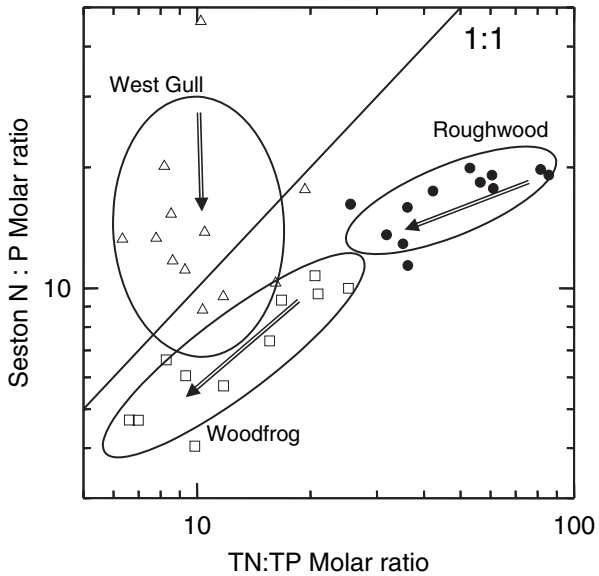

Fig. 4 Seston (<62 μm) nitrogen : phosphorus ratio as a function of supply rates (Total N : P). For each pond, the arrow represents the change in values over time, from early April to mid May. The ellipses indicate bivariate SE.

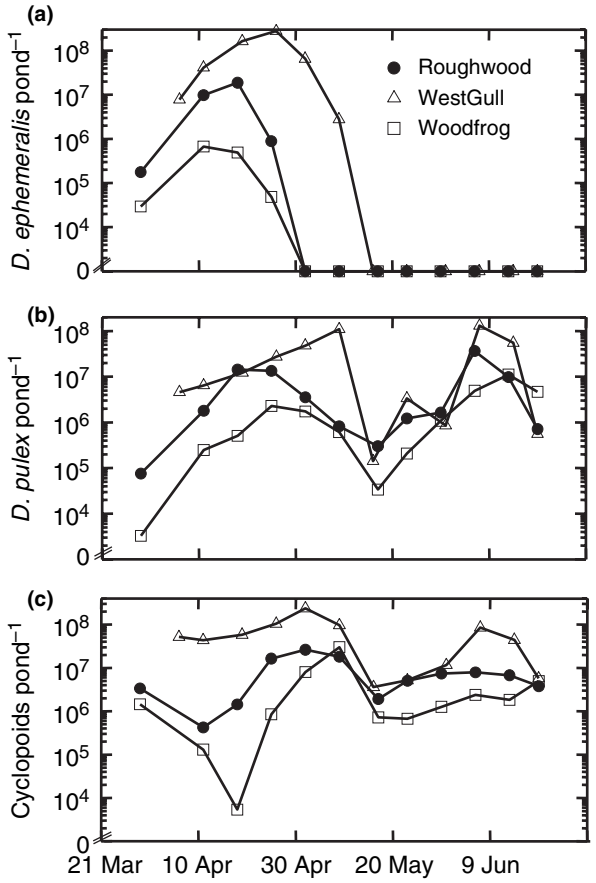

Fig. 5 Seasonal dynamics of the three most abundant zooplankton taxa. Densities are expressed as number pond^{-1} to avoid confounding temporal changes in population dynamics with a concentration of individuals as the pond dries.

cohort of each was observed in all three ponds, suggesting the availability of suitable food resources well after the canopy closed.

Estimated grazing rates suggest that at peak *Daphnia* densities in late April, grazers should be imposing a strong mortality on the seston. Peak estimates of clearance rates were 0.37 of the pond water d^{-1} in Woodfrog, 0.90 d^{-1} in Roughwood and 2.9 d^{-1} in West Gull. However, the population abundance of the *Daphnia* underwent dramatic changes over short periods of time. Hence, estimates of grazing rates were highly variable throughout the season. The realized impact of this grazing on seston abundance is apparently small; only in West Gull, which had extremely high grazing rates, was there any evidence of a reduction in seston carbon during April (Fig. 1b). Moreover, the seasonal variability in grazing did not appear to influence seston stoichiometry; we found no relationship between our estimate of grazer biomass and the N : P or C : P ratio of the seston (ANCOVA C : N, $F_{1,31} = 1.1$, $P = 0.28$; C : P, $F_{1,31} = 1.32$, $P = 0.26$).

Although seston stoichiometry did not seem to be determined by grazer abundance, we were also interested in whether changes in seston stoichiometry resulted in food-limitation for the grazers. Because *D. pulex* was the dominant grazer throughout the period of transition from light to shade, we examined its population dynamics in more detail. Such an analysis can suggest times at which the grazers may be food-limited. In late March and early April, the population growth rate of *D. pulex* was greater than expected from birth rates. Consequently, negative death rates were calculated (Fig. 6), suggesting hatching from dormant eggs. During April, the populations grew quickly as a result of extremely high birth rates, despite a drastic increase in death rates. In May, the switch to diapausing egg production (data not shown) reduced birth rates. This reduction, combined with increasing death rates, resulted in sharp declines in population abundances. In mid-May, we observed birth rates of zero because adult *D. pulex* were not detected in the water column. The continued presence of water in the ponds allowed a second cohort of *D. pulex* to invade the water column from the egg

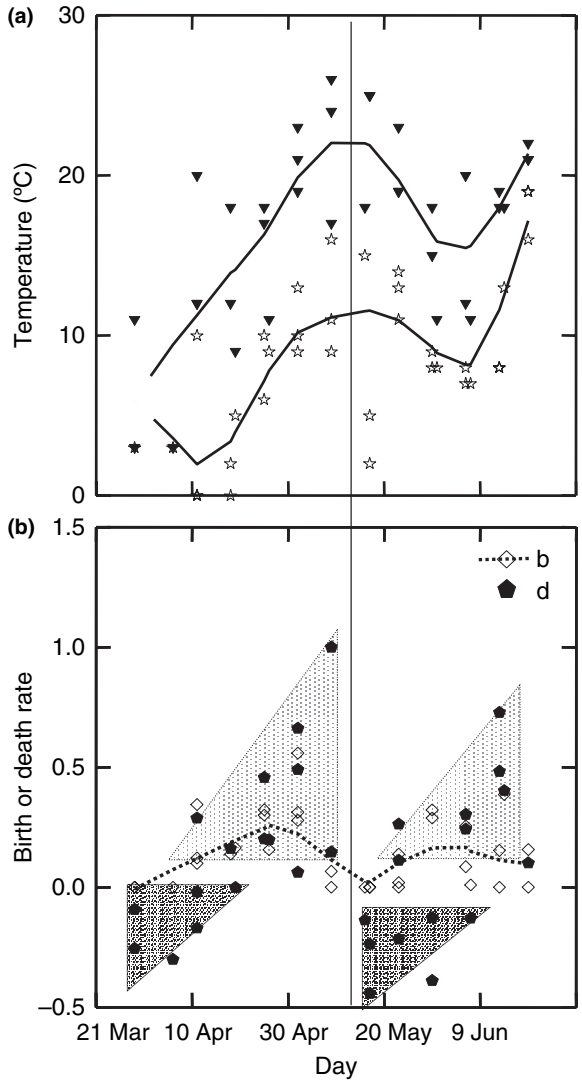

Fig. 6 (a) Maximum (triangle) and minimum (star) temperatures of all three ponds, as recorded by a max–min thermometer located in a shady location in each pond. Note the midseason decline in temperature prior to the onset of the second cohort (highlighted by the vertical line). The lines represent a LOWESS smoother with tension of 0.5. (b) Birth and death rates for the three populations of *Daphnia pulex*. The dotted line indicates the average birth rate of the three populations, and is a LOWESS smoother with tension of 0.5. Grey triangles highlight the two periods of negative death rate whereas the stippled triangles highlight the rapid increase in death rates for both cohorts.

bank in late May. As with the first cohort, negative death rates were commonly estimated in late May until an adult population was well established in the water column. Birth rates increased rapidly again until stabilizing in early June as some females began producing diapausing eggs again. As with the first cohort, death rates increased dramatically once the population became reestablished.

Discussion

Our results illustrate how forested ponds undergo rapid temporal change in the balance of light and nutrient supply and that this results in a shift in the elemental composition of seston. Despite large differences in the initial conditions with respect to trophic status, the three ponds exhibited nearly identical patterns of seasonal change in seston C : N : P and in grazer population dynamics. These parallel changes in ponds of varying size and trophic state suggest that the temporal responses to shading that we observed are robust.

It was not unexpected that we would record rapid temporal changes in physical, chemical and ecological conditions in these vernal ponds. However, temporal changes in ephemeral aquatic habitats have received far less study than have similar seasonal dynamics in permanent lakes and reservoirs. Several studies point to the importance of leaf-out and decomposition in changing light and nutrients supply to forest ponds (Wiggins, Mackay & Smith, 1980; Colburn, 2004), but quantitative data such as we present are rare. Similarly, prior studies of plankton population dynamics and succession in temporary ponds corroborate our observations of synchronized hatching from dormant stages driving seasonal phenologies (Hairston & Olds, 1984; Taylor & Mahoney, 1990).

To a large extent, changes in seston stoichiometry were as predicted by the LNH (Sterner *et al.*, 1997). The C : P ratio of the seston declined rapidly in all three ponds in response to decreased light and increased phosphorus supply (measured as TP) as the season progressed. However, we also observed changes in seston C : N : P that were inconsistent with simple stoichiometric predictions. Seston N : P was typically less than expected based on nutrient supply in the water column, and despite a large temporal increase in nitrogen supply and little or no change in seston carbon, the C : N ratio of the seston increased. Prior studies have also reported that the N : P ratio of natural phytoplankton assemblages does not always match that expected from supply rates of N and P (Hall *et al.*, 2005). There are at least three potential explanations for these patterns.

The most common explanation for a lack of consistency between seston stoichiometry and changing supply rates has been physiological constraints of the algae, especially a greater plasticity to store P compared to N. It is also likely that much of the increase in TN produced by decomposition was in the form of dissolved organic nitrogen. The ability of phytoplankton to take up this form of nitrogen may be limited. Further, Ågren (2004) showed that N : C is expected to increase linearly, while P : C should increase quadratically as a function of growth rate of algae. This also will give rise to different patterns of N : P depending on the nutrient supply rate. Hence, physiological constraints may contribute to why total and seston N : P ratios did not match; the N : P of the seston was almost always less than what was predicted based on the supply rate ratio. Still, the seston N : P did decline seasonally with the decline in supply rate of TN : TP, confirming an important prediction of ecological stoichiometry.

However, it was unexpected that TN : TP would decline seasonally. Interestingly, it occurred only in the two less productive ponds, and was driven by a disproportionate increase in TP compared to TN. It is likely that at the whole pond basin scale, nitrogen may not accumulate as readily as phosphorus due to differential uptake by forest trees and shrubs. In addition, the ponds were observed to become quite low in oxygen in May suggesting that microbial denitrification might contribute to loss of nitrogen.

We observed not just a decline in N : P ratio of seston and nutrient supply, but an increase in the C : N ratio of seston. Others have also reported an increase in C : N ratio of seston with a decrease in light : nutrient ratio. Frenette, Vincent & Legendre (1998) observed that small-celled phytoplankton increased C : N after a typhoon decreased water transparency and Schiesari (2006) found that the C : N composition of algae sampled from open canopy ponds was lower than that of nearby closed canopy ponds. We know of no obvious physiological mechanism for why C : N should increase under lower irradiance, but these results from other diverse systems suggest the phenomenon is broader than just our study.

A second, general factor to consider in understanding relationships between nutrient supply and seston elemental composition in these ponds is nutritional mode and species turnover. Autotrophic phytoplankton are expected to have different N and P requirements than are heterotrophic or mixotrophic organisms (Katechakis *et al.*, 2005). Hence, seasonal changes in stoichiometric ratios would be expected based on seasonal changes in community structure. We also observed dramatic changes in C : Chlorophyll ratio when leaf-out shaded the pond, with the initial values in early April being indicative of an autotrophic assemblage (Sterner & Schulz, 1998). A dramatic shift to very high ratios occurred after canopy closure. If this were a high light environment, increasing C : Chlorophyll ratio would be considered evidence of nutrient limitation, but clearly that is not the case in these ponds. Visual observations of the phytoplankton in our study ponds and in forest ponds in general (Colburn, 2004), reveal a dominance of cryptomonads, chrysophytes, dinoflagellates and euglenoids; many of these taxa are believed to be mixotrophic and can shift from autotrophic to heterotrophic nutrition with changes in light and nutrient supply. We suggest that changes in nutritional mode or perhaps species compositional shift to greater heterotrophy in the phytoplankton assemblage, contributed to the unexpected increase in C : N of the seston despite increased supply of *N* from decomposition (Jansson *et al.*, 1996; Rothhaupt, 1996).

A third factor considers the grazers and top-down influences on plankton composition. Hall *et al.* (2007) suggested that these top-down factors could influence producer stoichiometry through a variety of mechanisms including reducing standing stocks, recycling of nutrients and forcing an increased turnover rate. The transition from open to closed canopy was also associated with dramatic increases in daphniid abundance; grazing rates in at least two of the ponds would be expected to impose very high mortalities on phytoplankton. Because the ponds had similar sized animals and similar temperatures, among-pond differences in our estimates of grazing rates were driven by grazer abundance. However, we found no relationship between changes in grazer biomass and seston stoichiometry. The fact that seston carbon remained relatively stable suggests that turnover rate of phytoplankton was very rapid. The increased C : Chlorophyll may reflect more than a change in nutrition; it is possible that the phytoplankton shifted (via species replacement or phenotypic plasticity) to forms that invest more in defensive structures to minimize grazing loss (Agrawal, 1998). This seems

unlikely in these ponds given the evidence of high productivity of the grazers and prior studies documenting high food quality (Tessier & Woodruff, 2002).

A related question asks how the observed seasonal changes in seston stoichiometry influence the dynamics of the grazers. The C : P ratio of the seston, which is often used as an indicator of food quality was well above the thresholds at which P-content is thought to become limiting for *Daphnia* (Sterner & Elser, 2002; DeMott & Pape, 2005). The apparent availability of P, coupled with the fact that birth rates of the grazers remain high for most of the season, suggests that neither food quantity nor quality are limiting. Hence, zooplankton dynamics are largely governed by hatching and production of diapausing eggs. *Daphniopsis ephemeralis* had a single cohort that was driven by a hatch of females, which essentially produced males and then diapausing eggs. *Daphnia pulex* had a more extensive occupation of the water column, but even this was composed of two episodes of diapausing egg hatch and production. Although birth rate declined just when leaf-out and shading took effect in late April, the decline in birth rates can be attributed primarily to the populations switching to diapausing egg production, rather than food limitation (Cáceres & Tessier, 2004). Our estimates of birth and death rates for this species were similar in all three ponds and indicate rich food conditions (high birth rates) and high extrinsic mortality. This has been reported previously by Dudycha (2004) and reflects high level of predation on the zooplankton. Hence, vernal ponds appear to be highly productive environments with strong top-down (mortality) influences that create rapid turnover in prey assemblages.

The light : nutrient hypothesis (Sterner *et al.*, 1997) provides a valuable framework for assessing the transfer of nutrients and energy among trophic levels. To date, most empirical and theoretical attention has focused on permanent lakes (but see Hall *et al.*, 2007). Ephemeral ponds, however, experience much more rapid temporal changes in light levels, nutrient supply rates and grazer dynamics; changes that are central to many of the predictions of ecological stoichiometry. Hence, the temporal dynamics of these forested ponds provide an excellent opportunity to examine ecological stoichiometry at the scale of whole food webs, and at the interface of terrestrial and aquatic ecosystems.

Acknowledgments

We thank P. Woodruff, S. Mulvany and A. Jaurigue for assistance in the laboratory and field. S.R. Hall and C.T. Milling provided helpful discussion on earlier drafts of the manuscript. Support for this project was provided by NSF grants DEB-9816047, DEB-9816191, DEB- 0235118 and DEB-0237438. Any opinions and conclusions expressed in this paper are those of the authors and do not reflect the views of the National Science Foundation. This is contribution number 1452 of the Kellogg Biological Station.

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(Manuscript accepted 5 January 2008)