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Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore

JOTARO URABE*, JUN TOGARI* and JAMES J. ELSER†
Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Biological Buildings, Aoba, Sendai 990-8578, Japan, †Department of Biology, Arizona State University, Tempe, Arizona 85287-1501, USA

Abstract

The partial pressure of carbon dioxide (pCO_2) in lake ecosystems varies over four orders of magnitude and is affected by local and global environmental perturbations associated with both natural and anthropogenic processes. Little is known, however, about how changes in pCO_2 extend into the function and structure of food webs in freshwater ecosystems. To fill this gap, we performed laboratory experiments using the ecologically important planktonic herbivore *Daphnia* and its algal prey under a natural range of pCO_2 with low light and phosphorus supplies. The experiment showed that increased pCO_2 stimulated algal growth but reduced algal P : C ratio. When feeding on algae grown under high pCO_2, herbivore growth decreased regardless of algal abundance. Thus, high CO_2-raised algae were poor food for *Daphnia*. Short-term experimental supplementation of PO_4 raised the P content of the high CO_2-raised algae and improved *Daphnia* growth, indicating that low *Daphnia* growth rates under high pCO_2 conditions were due to lowered P content in the algal food. These results suggest that, in freshwater ecosystems with low nutrient supplies, natural processes as well as anthropogenic perturbations resulting in increased pCO_2 enhance algal production but reduce energy and mass transfer efficiency to herbivores by decreasing algal nutritional quality.

Keywords: *Daphnia pulicaria*, food quality, green algae, lakes, pCO_2, nutrients

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Introduction

The aqueous concentration of CO_2 (expressed as a partial pressure, or pCO_2 in gaseous form) in freshwater ecosystems is regulated by complex chemical, geological and biological processes in addition to physical gas exchange at the air–water interface (Wetzel, 2001). In general, pCO_2 in thermally stratified lakes is higher in aphotic layers, where decomposition of organic matter generates CO_2 but its photosynthetic consumption is low. However, in most lakes, pCO_2 is supersaturated even at the surface and ranges over four orders of magnitude (1–20 000 μatom with an average of ~1000 μatom) (Cole *et al*., 1994). This supersaturation is caused mainly by higher internal CO_2 production due to decomposition of allochthonous organic matter relative to internal CO_2 consumption by photosynthesis (Cole & Caraco, 2001; Prairie *et al*., 2002) or by direct external inputs from streams and groundwater (Dillon & Molot, 1997; Jones & Mulholland, 1998).

Growing evidence shows that the two- to three-fold increase in atmospheric CO_2 expected during the next 100 years (Houghton & Intergovernmental Panel on Climate Change, 2001) may have large impacts on the structure and function of terrestrial ecosystems (Lincoln, 1993; Cotrufo *et al*., 1998; Jones *et al*., 1998; Agrell *et al*., 2000). In addition, it has recently been shown that the nutritional quality of foliage and leaf litter from plants grown under elevated CO_2 is lower when consumed by aquatic decomposers and insects (Tuchman *et al*., 2002). However, despite the greater variance of pCO_2 in freshwater ecosystems and the likelihood of its sensitivity to local and global anthropogenic perturbations, little information is available on the effects of changes in pCO_2 on the internal dynamics of food webs within freshwater habitats.

In aquatic ecosystems, algal nutrient content (relative to C or total biomass) is strongly affected by the balance of

Correspondence: Jotaro Urabe, Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Biological Buildings, Aoba, Sendai 990-8578, Japan,
tel. + 81 22 217 6681, fax + 81 22 217 6686,
e-mail: urabe@mail.cc.tohoku.ac.jp

light and nutrient supplies because photosynthesis and nutrient uptake rate are not perfectly coupled (Urabe & Sterner, 1996; Hessen *et al*., 2002). *Daphnia* is a planktonic herbivore that plays a crucial role in structuring community composition (Carpenter & Kitchell, 1993) and regulating material cycling in the pelagic zone of lakes (Elser & Urabe, 2001). Previous studies have shown that, when nutrient supply is low, increased algal growth due to increased light intensity lowers individual and population growth of *Daphnia* because algae develop lower nutrient content under high light conditions (Urabe & Sterner, 1996; Sterner *et al*., 1998; Urabe *et al*., 2002). Under elevated pCO_2 conditions and low nutrient supply, a similar stoichiometric effect on the algae–herbivore interface may operate if increases in pCO_2 disproportionately enhance algal carbon fixation rates.

In most algae, the activity of ribulose-1, 5-bisphosphate carboxylase/oxygease (Rubisco) is less than half-saturated under pCO_2 levels at equilibrium with the atmosphere (Kirk, 1994; Badger *et al*., 1998), suggesting that CO_2 concentration is a potential factor limiting carbon fixation rate in aquatic ecosystems. Indeed, manipulation experiments have shown that increased dissolved CO_2 concentration can stimulate photosynthetic rates of natural and cultured freshwater algae (Hein, 1997; Saxby-Rouen *et al*., 1998; Qui & Gao, 2002). However, it has also been shown that a variety of algal species can use HCO_3^- via so-called carbon-concentration mechanisms (CCMs) that include active transport of CO_2 and HCO_3^- into the algal cell and active conversion of HCO_3^- to CO_2 by carbonic anhydrase. The relative importance of HCO_3^- utilization for carbon fixation is the subject of debate and probably differs among algal species (Torrell, 2000; Cassar *et al*., 2002). If algae can use HCO_3^- efficiently for carbon fixation via CCMs, elevation of pCO_2 would have less of an impact on carbon fixation rate and thus, potentially, on the growth rate of planktonic algae (Raven, 1997) because dissolved HCO_3^- concentrations are much higher than CO_2 concentrations in most lakes (pH = 7–9; Wetzel, 2001). However, because of their metabolic and energetic costs, CCMs seem to operate less efficiently under suboptimal conditions in terms of light and some nutrients (Raven, 1997; Beardall *et al*., 1998). This possibility implies that algal growth is more likely to be stimulated by increases in pCO_2 under low light conditions. In many lakes, light intensity in the water column is suboptimal for planktonic algae (Kirk, 1994; Sterner *et al*., 1997; Urabe *et al*., 1999). Therefore, it seems that there is a real potential for pCO_2 impacts on algal carbon fixation in nature. If this is the case, increases in pCO_2 may reduce the nutrient contents of algae relative to carbon, which in turn may affect herbivore growth rate.

The present study was designed to examine this possibility. Specifically, we manipulated pCO_2 under conditions of naturally low light and nutrient supplies and monitored the growth and nutrient contents of the green alga *Scenedesmus acutus* and individual growth rate of *Daphnia pulicaria* in semibatch cultures. Few studies, regardless of habitat, have tried to separate the effects of low nutrient content in autotrophs grown under high CO_2 from possible effects due to changes in plant secondary metabolites. In the present study therefore we also assessed the effects of lowered nutrient content in autotroph biomass on the herbivore growth by directly increasing the relative nutrient contents of the algae grown under elevated pCO_2.

Methods

To mimic the natural range of pCO_2 experienced by lake phytoplankton, experiments were conducted using incubation chambers with ambient (0.036% in air) or high CO_2 concentrations (0.15 and 0.35% CO_2 in air). Since pCO_2 in most lakes is supersaturated, we employed these high gas phase CO_2 concentrations to set pCO_2 in the experimental medium well within the natural range (Cole *et al*., 1994). Here we focused on phosphorus as the potentially limiting nutrient for algae because algal growth is frequently limited by P in lakes (Elser *et al*., 1990), and because algal P to C ratio (P : C) has been shown to regulate growth and dynamics of planktonic herbivores, especially *Daphnia* (Urabe *et al*., 1997; Elser & Urabe, 2001). The total P concentration in the flasks corresponded to that found in meso- to eutrophic lakes (Wetzel, 2001) while light levels were also set to match average levels for lake photic zones (Fee *et al*., 1992; Sterner *et al*., 1997).

Scenedesmus acutus and *Daphnia pulicaria* were obtained from stock cultures maintained for greater than two years under constant lab conditions. The semibatch cultures were performed at 21 °C in CO_2 incubation chambers (ShinMaywa VCB33M) with defined atmospheric CO_2 concentrations and initiated by inoculating the algae into flasks containing one litre of COMBO medium (Kilham *et al*., 1998). To avoid any direct effects of changes in pH on algal physiology, we included TES buffer in the growth medium at 200 mg L^{-1} to stabilize pH (Kilham *et al*., 1998), which was adjusted to 7.0. Each treatment was performed with 5–7 replicates. To ensure P-deficient conditions relative to N, inorganic phosphorus concentration was set at 1.5 μM with an N : P atomic ratio of 80. Constant light was provided by cool-white fluorescent bulbs with a target irradiance of 50 μmoles m^{-2} s^{-1} inside the flasks. A small aerator was also placed in each flask and the suspension was mixed by mild aeration for 1 min every 1 h.

To initiate the experiment (Experiment 1) examining effects of three levels of pCO_2 on algal P : C and *Daphnia*

growth, a small quantity of algal suspension was inoculated and 30% of the culture suspension was replaced with fresh growth medium every two 2 days. When algal abundance reached a saturation level after eight days, 8 neonates of *D. pulicaria* (born within a 24-h time span from the second clutch of a single female) were introduced to each flask where they remained for six days. Subsamples of neonates were collected to determine initial body mass. During the 6-day period, algae continued to be diluted every two days but animals were retained. At the end of experiments, *Daphnia* individuals in the flasks were pooled and their dry mass was measured with a Mettler model UMX2 microbalance. Individual growth rate of *Daphnia* was estimated assuming exponential change in body mass (Elser *et al*., 2001).

Concentrations of algal C and P, dissolved inorganic C (DIC), and pH in the flasks were examined using the culture suspensions collected for replacement. Known aliquots of the suspension were filtered onto pre-combusted glass fibre filters and analyzed for algal P content by spectrophotometric methods after oxidation by persulphate (APHA, 1992) and for algal C content by using a Perkin-Elmer model 2400. DIC and pH were measured using a Shimadzu TOC-500 and a Denver Instrument AP15 pH Meter. The value of pCO_2 in culture suspension was calculated based on measured DIC and pH values (DOE, 1994).

We also examined potential direct effects of increased algal food and pCO_2 on animal growth rate (Experiment 2). In this experiment, semi-batch algal cultures were established under 0.036 and 0.35% CO_2 in air as described above. When algal abundance reached a saturation level after eight days, algal density was reduced by 90% by adding 100 mL of one of these algal suspensions to 1-L flasks containing 900 mL of fresh growth medium without inorganic nitrogen and phosphorus (food abundance treatment). Each flask received 8 neonates and was placed in the incubation chamber with either control (0.036% in air) or high CO_2 concentrations (0.35% in air) in the dark ('location' treatment). Thus, food abundance and location treatments were cross-classified. The feeding suspensions were replaced every two days and *Daphnia* growth was monitored after six days. Algal C and P contents and pCO_2 were determined as above.

Finally, to determine if the impaired growth of *Daphnia* was indeed caused by nutrient deficiency in algal food, a P supplementation experiment was performed (Experiment 3) using algae grown under 0.35% CO_2 in air as above. Algal food was prepared as in Experiment 2 and 8 neonates were introduced to flasks containing growth medium with algae grown under 0.35% CO_2. In contrast to Experiment 2, however, we prepared feeding suspensions and transferred *Daphnia* to the flask containing fresh algae daily. On each day, we added 3 μM P as KH_2PO_4 to a half of the experimental flasks 6 h before it received the daily transfer of *Daphnia* (+P treatments). No nutrient enrichment was made in unenriched treatments. Six hours is sufficient to raise algal P content but not long enough to induce major changes in algal abundance, digestibility, or biochemical composition (Rothhaupt, 1995; Elser *et al*., 2001). Thus, *Daphnia* in the +P treatment fed on unenriched algae for 18 h each day and spent the rest of the day (6 h) consuming algae with a higher P : C ratio due to the PO_4 addition, while those in the controls fed on unenriched algae for whole days. In both treatments, *Daphnia* were rinsed with P-free water several times at the daily transfer to avoid carry over of P from the old feeding suspensions. These flasks were placed in the dark with the ambient CO_2 concentration and *Daphnia* growth was monitored for 6 days. Every other day, algae were collected from the culture suspensions for replacement and their C and P contents were analyzed.

Results

Increased atmospheric CO_2 in the incubation chamber increased pCO_2 in the experimental water (Fig. 1a). As algal biomass increased to a 'saturation' level during the incubation period, aqueous CO_2 in the 0.15 and 0.35% CO_2 treatments decreased to values seen in the control treatment (0.036% CO_2). Thus, at these biomass levels CO_2 uptake rates of algae exceeded the diffusion rate of CO_2 from the atmosphere. pH values at the algal saturation level were 7.0–7.3 and no significant difference was detected among treatments ($F_{2,15} = 0.67$, $P > 0.05$). Algal abundance reached the saturation level within eight days and was higher for higher CO_2 treatments (Fig. 1b), indicating that algal abundance in the control treatment was CO_2 limited. Final algal P : C ratio was 3.61×10^{-3} (by atoms) in the control treatments but was significantly lower in elevated CO_2 treatments (Fig. 1c). These data show that algal growth can be limited by CO_2 and that increased pCO_2 reduces P : C ratio of algal cells under low nutrient supply conditions.

After algae reached saturation levels, juvenile *Daphnia* were introduced to experimental flasks to assess the nutritional suitability of the algal biomass produced. In the control treatment, growth rate of *Daphnia* individuals was as high as $0.3\,d^{-1}$. However, in the 0.15 and 0.35% CO_2 treatments, their growth rate decreased in spite of higher algal abundance (Fig. 1d). ANOVA with Tukeys' pairwise test showed that *Daphnia* growth rate in these high CO_2 treatments was significantly lower than in the control treatment ($F_{2,15} = 5.98$, $P < 0.05$), suggesting that the high CO_2-raised algae were poor quality food for *Daphnia*.

However, the low *Daphnia* growth rate in the high CO_2 treatments might be due to direct effects of increased pCO_2 on the animals. Alternatively, higher algal abundance

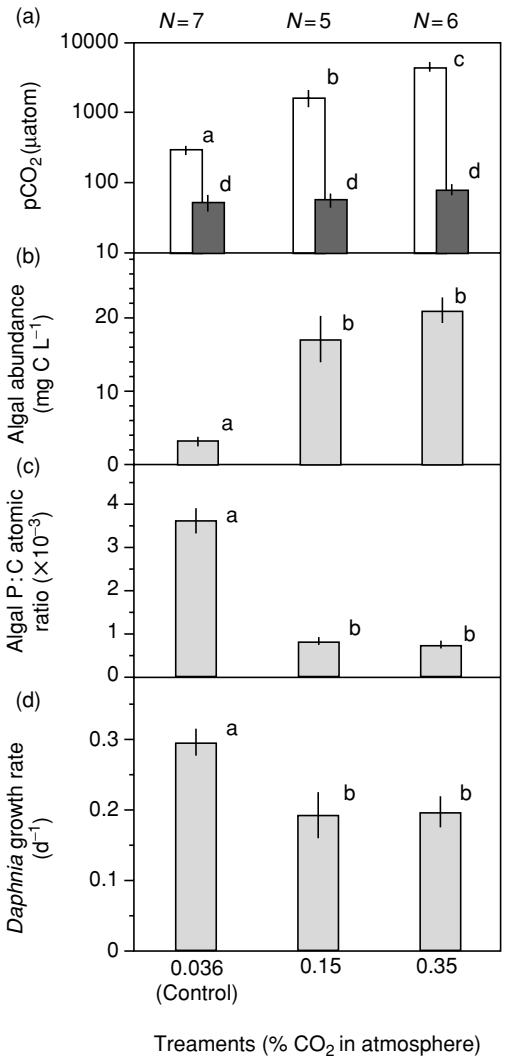

Fig. 1 Effects of atmospheric CO_2 concentration on (a) pCO_2 in growth medium (b) saturation level of algal abundance (c) algal P : C ratio when algae reached saturation abundance, and (d) *Daphnia* growth rate in Experiment 1. Means (±SE) are given. In panel (a), mean pCO_2 in the growth medium before (2 days after algal inoculation; open bars) and after attaining saturation levels of algal abundance (10–14 days; grey bars) are shown. For each variable, differences among treatments were evaluated by one-way ANOVA. Treatments that were significantly different ($P < 0.05$, Tukey's pairwise comparison) are denoted by different letters on the bars. Number of replicates is denoted by N.

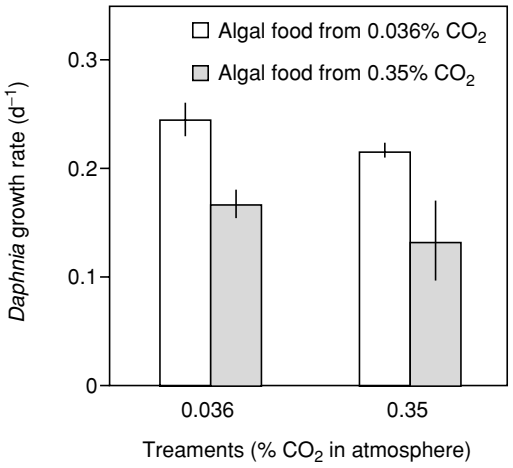

Fig. 2 Growth rates of *Daphnia* individuals fed algae grown in 0.036% CO_2 (open bars) and 0.35% CO_2 treatments (grey bars) but living in the flasks placed under 0.036% CO_2 and 0.35% CO_2 conditions (Experiment 2). Each treatment was made in duplicate. Means (±SE) are given. Algal abundance and P : C ratio are shown in Table 1.

achieved in the high CO_2 treatments itself might reduce *Daphnia* growth rate because excessive food levels can interfere with feeding activities (Porter *et al*., 1982). Results of Experiment 2 indicate that these factors did not play a role in causing reduced growth of *Daphnia* observed in Experiment 1. Algal abundance in feeding suspensions from the high CO_2 chamber was three-times higher than from the control chambers (Table 1) but comparable to food abundance in the control treatment (0.036% CO_2) in Experiment 1 (Fig. 1). As in the first experiment, *Daphnia* grew more slowly when fed low P : C algae grown under high CO_2 than when fed high P : C algae grown under control conditions (Fig. 2). Two-way ANOVA showed that the effect of algal food source on *Daphnia* growth rate was significant ($F_{1,4} = 14.6$, $P < 0.05$), but the direct effect of CO_2 and the food source x direct CO_2 interaction were not ($P > 0.05$). Thus, the low growth of *Daphnia* was due to the poor quality of the high CO_2-raised algae, rather than direct effects of pCO_2 on the animals or due to filtering interference by high algal densities.

Finally, to determine if the impaired growth of *Daphnia* feeding on algae grown under high pCO_2 was indeed caused by a nutrient deficiency in the algal food, a P supplementation experiment was performed using algae grown under 0.35% CO_2 in air (Experiment 3). Within 6 h, P enrichment did not induce significant changes in algal abundance and successfully increased relative P contents of the algae (Table 1). Thus, *Daphnia* in the +P treatment fed on enriched algae for 6 h each day and spent the rest of day (18 h) consuming algae with lower P : C ratio. Growth rate of these individuals was significantly higher than that of *Daphnia* fed unenriched algae for the whole day ($t = 3.69$, $P < 0.05$; Fig. 3). Note that the growth rate of *Daphnia* in +P treatment was as high as $0.3\,d^{-1}$, comparable to that of *Daphnia* fed a similar amount of algae grown under ambient CO_2 concentration (0.036% CO_2; Fig. 1d). Indeed, there was no significant difference between these growth rates ($t = 0.57$, $P > 0.05$). Thus, P enrichment restored the nutritional quality of the high CO_2-raised algae.

Table 1 Algal abundance and P : C ratio (mean ± SE) in feeding suspensions of experiments 2 and 3

Experiment	Treatment	Algal source	Number of replications	Algal abundance (mg C L^{-1})	P : C ratio ($\times 10^{-3}$)
Exp. 2	High CO_2	0.036% CO_2 culture	2	0.604 ± 0.048	3.64 ± 0.18
		0.35% CO_2 culture	2	2.303 ± 0.005	0.81 ± 0.05
	Control	0.036% CO_2 culture	2	0.719 ± 0.058	3.62 ± 0.99
		0.35% CO_2 culture	2	2.479 ± 0.147	0.80 ± 0.04
Exp. 3	P-enrichment	0.35% CO_2 culture	3	1.303 ± 0.024	3.64 ± 0.34
	Control	0.35% CO_2 culture	3	1.099 ± 0.045	0.80 ± 0.08

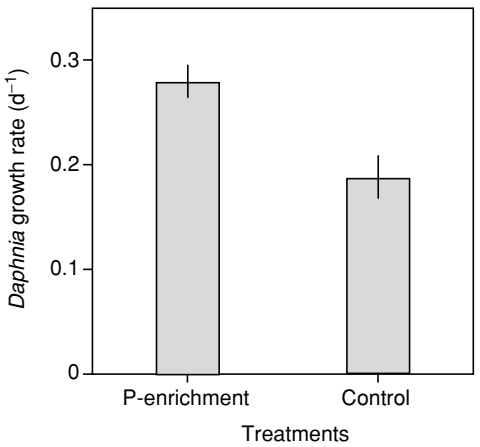

Fig. 3 Effects of feeding on P-enriched algae for 6 h per day on growth rate of *Daphnia* individuals fed algae grown under 0.35% CO_2 in air (Experiment 3). Means (±SE) are given. Food abundance and P : C ratio are shown in Table 1.

Discussion

Our study clearly showed that, as in terrestrial plants (Lincoln, 1993; Cotrufo *et al*., 1998; Gill *et al*., 2002; Tuchman *et al*., 2002), increased pCO_2 stimulated algal carbon fixation and reduced nutrient content relative to carbon. The result contrasts to previous studies that failed to detect CO_2 effects on algal nutrient content (Burkhardt *et al*., 1999; Gervais & Riebesell, 2001). In contrast to our study, these studies have involved experiments under nutrient replete (Burkhardt *et al*., 1999) or high light (Gervais & Riebesell, 2001) conditions. Under nutrient replete conditions, changes in pCO_2 are expected to have minor effects on algal P : C ratio since algae are easily able to take up nutrients at rates proportional to carbon fixation (Sterner & Elser, 2002). Indeed, experiments by Burkhardt *et al*. (1999) showed that P : C ratio of measured algal species was relatively high and varied only slightly (10–16 $\times 10^{-3}$ in atomic ratio) over a range of CO_2 concentrations. Gervais & Riebesell (2001) showed that, when cultured at a low nutrient supply under a high light (150 μmoles $m^{-2} s^{-1}$), P : C ratio of *Skeletnema costatum* reached to a low level ($< 1.5 \times 10^{-3}$ in atomic ratio) regardless of CO_2 concentrations. We obtained a similar result for *Scenedesmum acutus* when cultured at high light (150 μmoles $m^{-2} s^{-1}$) with low nutrient supplies (Urabe and Togari, unpubl). Gervais & Riebesell (2001) further showed that $\delta^{13}C$ of *S. costatum* was higher in cultures at lower CO_2 supplies, indicating that, at low CO_2 supply, use of HCO_3^- for carbon fixation via CCMs was operating under high light intensities. In many lakes, however, light intensity in the water column is suboptimal for planktonic algae (Kirk, 1994; Sterner *et al*., 1997; Urabe *et al*., 1999) and nutrients, especially phosphorus, are in short supply relative to demands of the algae (Elser *et al*., 1990). Thus, the experimental conditions in our study were more realistic compared with those in the previous studies. Our data suggest that, in natural lakes where both nutrient and light are in limited supply, the operation of CCMs is less efficient and thus changes in pCO_2 can substantially affect growth rate and nutrient to carbon ratio of phytoplankton.

Other factors, such as trace metal supplies, must also be considered. Even under high light intensities, algal nutrient contents relative to C may be affected by CO_2 supply if supplies of zinc are insufficient because carbonic anhydrase is a zinc metalloenzyme. In our study, zinc was abundant in the present experimental medium (Kilham *et al*., 1998) and also appears to be present at relatively high concentrations in the waters of most lakes (Wetzel, 2001). However, concentration of this element is very low relative to demands of planktonic algae in deep oceans (Morel *et al*., 1994). Morel *et al*. (1994) showed that algal growth rate is highly limited by pCO_2 levels but not by total inorganic carbon concentration under very low concentrations of inorganic zinc. Thus, changes in pCO_2 supply may affect nutrient : carbon ratios of algae when zinc is deficient, as can be the case in the open ocean.

Our data also clearly showed that laboratory growth rates of the ecologically significant herbivore *Daphnia* decreased when they fed on algae raised under high pCO_2. These algae were apparently poor food for

Daphnia. Other than edibility and relative nutrient content, a number of aspects have been proposed as key determinants of algal food quality. For example, under P-limited conditions, Chlorophyte algae such as *Scenedesmus* can produce thickened cell walls that inhibit digestion by herbivorous zooplankton (van Donk *et al*., 1997). P limitation may also decrease the synthesis rate of some organic substances essential to herbivore growth, such as polyunsaturated fatty acids (Sterner & Schulz, 1998). Although we did not examine these factors, the present results accord well with quantitative predictions of stoichiometric theory that *Daphnia* growth is limited by P when feeding on algae with P : C ratio below 3.33×10^{-3} (Urabe *et al*., 1997; Sterner & Schulz, 1998). Algae grown under high pCO_2 showed P : C ratio much lower than this threshold elemental ratio and *Daphnia* feeding on these algae grew more slowly. Furthermore, experimental supplementation of P content in the high CO_2-raised algae returned growth rate of *Daphnia* to a level comparable to that of *Daphnia* fed a similar amount of algae grown under ambient CO_2 concentration. These results indicate that *Daphnia* growth was indeed P-limited when feeding on algae grown under high pCO_2 in our experiments.

A recent compilation of stoichiometric data for autotrophs and invertebrate herbivores in terrestrial and aquatic food webs indicates that food quality in terms of elemental composition (e.g. P : C ratio) is suboptimal for herbivores in many ecosystems (Elser *et al*., 2000). This imbalance in P : C ratio between plants and herbivores has previously been attributed to high light intensities coupled to low nutrient supplies in various ecosystems (Urabe & Sterner, 1996; Sterner *et al*., 1998; Hessen *et al*., 2002; Urabe *et al*., 2002). Considering the fact that pCO_2 is supersaturated but highly variable in lakes, our data suggest that high CO_2 availability in combination with low nutrient supply may also generate poor quality algal food for planktonic herbivores. This would be especially likely for oligotrophic lakes where available inorganic P concentrations are even lower than in our experimental conditions.

Effects of elevated pCO_2 on algal elemental ratio may also affect sequestration of carbon in lake sediments. Empirical analysis shows that, with decreasing nutrient contents relative to carbon of plant tissue, the proportion of primary production that is consumed by herbivore decreases while the fraction entering into detrital food chains increases and the proportion becoming refractory detritus also increases (Cebrian, 1999). Thus, stoichiometric effects of increased pCO_2 in algae would act, on the one hand, to impinge on herbivore production and, on the other hand, to promote the 'biological pump' that transports carbon to the lake bottom. It is important to note that lake sediments may represent a nontrivial, but largely unappreciated, sink of organic C at the global scale (Dean & Gorham, 1998).

These possibilities imply that the effects of increased atmospheric CO_2 on terrestrial ecosystems now widely documented (Cotrufo *et al*., 1998; Jones *et al*., 1998; Gill *et al*., 2002) can extend into aquatic food webs. In many lakes, pCO_2 changes seasonally but is supersaturated in most seasons (Cole *et al*., 1994). Thus, a two- to three-fold increase in atmospheric CO_2 may have a minor impact on physical gas transfer processes from the atmosphere to lake waters. However, given the fact that the CO_2 status of lake waters is largely determined by the relative dominance of allochthonous organic matter inputs from the watershed (Cole *et al*., 1994), we would expect that the impacts on aquatic food webs of increasing atmospheric CO_2 will occur indirectly. Increased atmospheric CO_2 generally increases carbon fixation by terrestrial plants (DeLucia *et al*., 1999; Hendrey *et al*., 1999; Gill *et al*., 2002) and a substantial fraction of terrestrially fixed carbon is exported into freshwaters (Cole & Caraco, 2001; Pacala *et al*., 2001; Richey *et al*., 2002) as allochthonous organic matter or as dissolved inorganic C after being respired to CO_2 in soils. Thus, by increasing watershed export of C, increased atmospheric CO_2 may indirectly amplify the current state of CO_2 supersaturation in lakes and thus impair production of lake herbivores. Other anthropogenic impacts may also affect pCO_2 in lakes. For example, lake acidification by acid rain or altered land use affects dissolved CO_2 concentration in freshwaters by changing the equilibrium state of the inorganic carbon complex (Rebsdorf *et al*., 1991). It has frequently been observed that the abundance of large planktonic grazers such as *Daphnia* declines following lake acidification. While previous studies have attributed this to direct effects of decreased pH on *Daphnia* physiology (Brett, 1989; Havens *et al*., 1993), our data imply that stoichiometric effects of increased pCO_2 on algal food quality may have also played an unappreciated role in the decline of large planktonic grazers in acidified lakes.

In sum, our results suggests that any anthropogenic impact resulting in increased aqueous pCO_2 will negatively affect the structure and mass transfer efficiency of food webs in nutrient-limited freshwater ecosystems by lowering algal food quality for herbivores. In the present study, we used single algal and herbivore species. Adaptation to changes in light, pCO_2, nutrients and food quality may probably differ among species. Field tests with multiple species are required to make better prediction on effects of elevated pCO_2 on aquatic food webs.

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