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Response of phytoplankton in an alpine lake to inputs of dissolved organic matter through nutrient enrichment and trophic forcing

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Abstract

Inputs of terrestrially derived dissolved organic matter (DOM) are increasing in alpine lakes due to multiple drivers such as climate change, tree line advancement, and insect epidemics. A 21 d microcosm experiment investigated three potential mechanisms by which increased inputs of terrestrial DOM subsidies might affect phytoplankton density, growth, and species assemblage: (1) directly, by providing nutrients enhancing growth of select phytoplankton species (nutrient stimulation hypothesis); (2) indirectly, through trophic forcing of zooplankton uniformly increasing the total biomass of all zooplankton that selectively graze on phytoplankton (trophic intensity hypothesis); and (3) indirectly, through trophic forcing of zooplankton by favoring zooplankton species that selectively graze on phytoplankton (trophic shift hypothesis). We manipulated DOM (terrestrial DOM additions vs. unmanipulated control), zooplankton (presence vs. absence), and incubation depth (epilimnion vs. hypolimnion) in a full 3×3 factorial design. Phytoplankton density and growth increased substantially and species assemblage shifted to near dominance by *Asterionella formosa* in the presence of DOM. Zooplankton biomass and growth increased with the addition of DOM, yet the species assemblage remained stable across treatments, and contributed to selective grazing effects on phytoplankton. Our data support the nutrient stimulation and trophic intensity hypotheses. While DOM effects have been classically attributed to stimulation by addition of fixed carbon, our experiments indicate that nutrient stimulation is also important. Additionally, the indirect DOM effect of trophic forcing can occur in the absence of selective effects of DOM on zooplankton.

Increasing concentrations of dissolved organic matter (DOM) have been documented in surface waters of many forested watersheds in the northern hemisphere (Monteith et al. 2007; Weyhenmeyer and Karlsson 2009; SanClements et al. 2012), with these changes attributed to changing sulfur and nitrogen emissions as well as increasing air temperatures and changing hydrology. These studies primarily focus on lakes in forested watersheds with generally moderate to high DOM concentrations. In contrast, high-elevation lakes are situated in sparsely vegetated watersheds and subsequently have very low (< 2 mg L^{-1} as dissolved organic carbon [DOC]) lakewater DOM. However, these watersheds are undergoing rapid vegetation changes due to multiple drivers, including climate change (Roush et al. 2007) and insect epidemics (Raffa et al. 2008), and these watershed changes affect lakewater chemistry. Tree line advance is occurring in many alpine regions as a result of temperature and precipitation changes (Roush et al. 2007; Harsch et al. 2009), with the establishment of trees potentially increasing the concentration of DOC in lakes (Vinebrooke and Leavitt 1998; Sommaruga et al. 1999). The mountain pine beetle (*Dendroctonus ponderosae*) is also causing rapid terrestrial change in high-elevation regions of western North America, inducing high rates of pine (*Pinus contorta*, *P. flexilis*, and *P. ponderosa*) mortality that are accompanied by substantial increases in litterfall to soils, potentially altering DOC concentrations in stream and lake ecosystems (Clow et al. 2011).

DOM is a fundamental regulator of aquatic ecosystems (Williamson et al. 1999), and when DOC concentrations are low (< 2 mg L^{-1}), even small changes in DOM exert strong effects on plankton. DOM can shade phytoplankton from harmful ultraviolet (UV) radiation, resulting in increased phytoplankton biomass in high-UV systems (Williamson et al. 2010). Yet, DOM can also increase attenuation of photosynthetically active radiation (PAR) in the water column, reducing phytoplankton biomass (Klug 2002). Terrestrial DOM subsidies have the potential to directly alter phytoplankton species assemblage by increasing density stratification, resulting in shallower mixing depths (Fee et al. 1996). Terrestrial DOM subsidies can promote periphyton (Frost et al. 2007) and epilithon biomass (Vinebrooke and Leavitt 1998), and species-specific phytoplankton density (Scott et al. 2009), likely through added nutrients enhancing growth of nutrient-limited species (Graneli et al. 1999; Klug 2002).

DOM can affect zooplankton biomass and species assemblage in highly transparent systems through a number of mechanisms. By reducing lake transparency and altering the timing and intensity of clear water phases (Williamson et al. 2008, 2009), DOM may alter zooplankton biomass and seasonal succession patterns. DOM additions can also shade zooplankton from harmful UV

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radiation, resulting in increased density and assemblage shifts towards less UV-tolerant species (Cooke et al. 2006*a,b*). DOM additions may also lower pH, selectively favoring pH-tolerant zooplankton species (Cooke et al. 2006*a*). In mesocosm experiments DOM additions selectively increased nauplii density (Vinebrooke and Leavitt 1998), egg production of *Daphnia catawba* and *Cyclops scutifer* (Cooke et al. 2006*a*), and survival and reproduction of *Leptodiaptomus ashlandi* (Cooke et al. 2006*b*). Lastly, DOM may alter food resources for zooplankton, indirectly affecting zooplankton biomass. By adding biolabile carbon that stimulates bacterial production via the microbial loop, DOM has indirectly increased the biomass of metazoan consumers (Berggren et al. 2010) and calanoid copepods (Faithfull et al. 2012; but *see* Faithfull et al. 2011). On the other hand, by adding nitrogen (N) and phosphorus (P) stimulating autotrophic production (Graneli et al. 1999; Klug 2002) and available food resources, DOM may also indirectly increase zooplankton biomass. Yet, if DOM additions increase attenuation of PAR to an extent that results in decreased primary productivity (Klug 2002), zooplankton biomass may also decrease due to reduced food resources. Ultimately, DOM additions have been shown to alter zooplankton species diversity (Shurin et al. 2010).

DOM-induced changes in the zooplankton may indirectly affect phytoplankton by a phenomenon we label trophic forcing. We define trophic forcing as forcing by any environmental factor that manifests its effects on one trophic level through its influence on a higher trophic level. For example, DOM has been shown to affect zooplankton biomass via enhanced bacterial production (Faithfull et al. 2012), and this DOM-induced change in zooplankton biomass can subsequently alter the zooplankton grazing intensity and selectivity on phytoplankton which in turn may alter phytoplankton species assemblages.

Here we investigate three mechanisms by which terrestrial DOM subsidies can affect phytoplankton species assemblages: direct stimulation of phytoplankton due to the nutrient inputs from DOM, and two indirect effects of DOM through trophic forcing by consumers. The first mechanism (*nutrient stimulation hypothesis*) may alter phytoplankton species assemblages in oligotrophic lakes by favoring species with moderate to high nutrient requirements because DOM subsidies may contain nutrients that could selectively stimulate algal production and biomass (Graneli et al. 1999; Klug 2002). The second and third mechanisms involve indirect DOM effects through trophic forcing: DOM may alter either the total biomass (*trophic intensity hypothesis*) or species composition (*trophic shift hypothesis*) of zooplankton through altered food resources, with consequent changes in phytoplankton species assemblage due to changes in selective grazing. The trophic intensity hypothesis argues that DOM may influence the phytoplankton species assemblage by uniformly increasing all species of selectively feeding zooplankton. If food resources are of poor quality (Danielsdottir et al. 2007), hard to digest (DeMott and Tessier 2002), have toxic defenses (Lurling 2003), or are difficult to ingest due to large size (e.g., *Asterionella formosa*; Kagami et al. 2005, 2011), then the zooplankton assemblage will be less likely to graze on these species. Thus, the trophic intensity hypothesis posits that selective grazing by the zooplankton assemblage as a whole will promote populations of less edible phytoplankton taxa, while reducing populations of preferred species that are more edible.

The trophic shift hypothesis is based on the fact that selective grazing varies among zooplankton taxa. For example, daphniids feed selectively on smaller particles and diaptomids on larger particles (Sanders et al. 1996; Kagami et al. 2002). Daphniids graze on small species of diatoms such as *Stephanodiscus* spp., whereas diaptomid copepods can graze on some larger colonial diatom species, such as *Fragilaria* spp. (Kagami et al. 2002), but not others, like *A. formosa* (Kagami et al. 2011). Thus, the trophic shift hypothesis posits that a zooplankton species assemblage that is altered by DOM will have altered grazing rates on phytoplankton that reflect the dominant species' grazing selectivity. The constrained range of zooplankton stoichiometry relative to phytoplankton may make these higher trophic levels less resilient and thus more sensitive to changes in DOM inputs.

Zooplankton consumers have been shown to respond differently to DOM at varying depths in the water column. This varying response of zooplankton may subsequently result in different trophic forcing effects on phytoplankton. Zooplankton rely less heavily on terrestrial DOM in the hypolimnion of oligotrophic clear water lakes (Matthews and Mazumder 2006; Francis et al. 2011) because autotrophic food sources in the hypolimnion are often of higher quality compared to allochthonous sources (Brett et al. 2009) and because DOM may not be as available at deeper depths due to lack of mixing in the hypolimnion during thermal stratification. Likewise, abiotic conditions vary by depth in the water column which may also influence DOM processing and uptake. To address potential differences in DOM effects on zooplankton with depth and the subsequent trophic forcing effects on phytoplankton, we test these three hypotheses with a series of in situ experiments that manipulate DOM and zooplankton in an alpine lake in the epilimnion and in the hypolimnion and examine the response of both zooplankton and phytoplankton species assemblages. Both the nutrient stimulation and trophic intensity hypotheses were supported by the data, whereas the trophic shift hypothesis was not. The results demonstrate that the direct effects of DOM on pelagic ecosystems may involve food web stimulation by nutrients in addition to the previously recognized stimulation of the microbial loop by fixed carbon (which we do not investigate here) and that indirect DOM effects through trophic forcing may occur even in the absence of selective effects on the consumer species assemblage.

Methods

Study site—The Beartooth Mountain Range is located in the Absaroka-Beartooth Wilderness Area of Montana and Wyoming, U.S.A., and is adjacent to Yellowstone National Park. The region has a multitude of permanent and temporary lakes that range in elevation from 1900 to

Table 1. Summary characteristics of Glacier Lake (source) and Emerald Lake (incubation). LA : WA indicates lake area to watershed area ratio. Epilimnetic (Epi) DOC, hypolimnetic (Hypo) DOC, chlorophyll *a* (Chl *a*), and depth (Z) of 1% surface irradiance of PAR are average values over the 21 d experiment (± standard error, $n = 3$ Glacier Lake; $n = 5$ Emerald Lake).

Lake	Elevation (m)	Latitude (°N)	Longitude (°W)	LA : WA	Epi DOC (mg L^{-1})	Hypo DOC (mg L^{-1})	Chl *a* (μg L^{-1})	$Z_{1\%}$ PAR (m)
Glacier	2955	45°00′30″	109°32′05″	0.07	0.5±0.03	0.5±0.03	3.8±0.6	16.8±0.8
Emerald	2973	44°59′50″	109°31′30″	0.18	0.5±0.02	0.6±0.01	2.1±0.8	15.0±1.8

3900 m and have moderate silica (Si) and low N and P concentrations (Saros et al. 2003). Tree line in this area varies between 2700 and 3100 m.

From 06 to 27 August 2008, a 21 d experiment spanning both the critical plankton growing season and duration of lake thermal stratification was conducted in Emerald Lake using Glacier Lake organisms and water. Glacier and Emerald Lakes are located adjacent to each other and are both characterized by small, steep watersheds with sparse vegetation (Table 1). Previous field surveys conducted by our group have shown that although Glacier Lake contains more zooplankton and phytoplankton species than Emerald Lake, it is unsuitable for incubating a large multi-week experiment because of frequent high winds and wave action. Therefore, water and organisms were collected from Glacier Lake and deployed in watertight Bitran bags in Emerald Lake. This similar transplant approach was successful previously in the region (Williamson et al. 2010).

Microcosm experimental design—A full factorial design was implemented with three factor variables: (1) ± terrestrially derived DOM, (2) ± zooplankton, and (3) incubation in one of two strata, epilimnion (incubation depth 1.5 m) or hypolimnion (incubation depth 8.0 m); each combination was replicated three times. The microcosm design allowed for assessment of the mechanism(s) by which DOM affects producers and consumers in the epilimnion and hypolimnion and provided ample replication and statistical power to detect patterns. Note that both light and temperature vary between the two incubation strata. In our experiment, we combined light and temperature into a single "stratum" term for ecological realism, as we did not separate out their individual effects. On 04 August 2008, ~ 300 liters of Glacier Lake water was collected from the deep chlorophyll maximum (Z = 9 m; determined using a Turner Designs in vivo fluorometer [model number 10-AU]) to maximize phytoplankton biomass, and filtered through a 100 μm mesh net to remove zooplankton. The filtered water included the natural assemblage of phytoplankton. Filtered water was transported to Emerald Lake and used to fill 48 polyethylene (UV transparent) watertight Bitran S Series bags of volume 3.8 liters.

DOM amendments were made from the leachate of dead lodgepole pine (*Pinus contorta*) needles collected adjacent to Glacier and Emerald Lakes. Lodgepole pine is one of the dominant tree species in the watersheds of both lakes. Dead needles were collected in August 2007, just downhill from the experiment site. Needles were kept frozen until 3 weeks before the experiment, when 10 g of needles were weighed, minced, and mixed into 1 liter of Millipore water. DOM leached rapidly from needles and 48 h was sufficient to attain a high DOC concentrate. Leachate optical characteristics (dissolved absorption coefficients and spectral slopes) were comparable to the DOM in other nearby lakes and bog water (K. Rose unpubl.) and published values (McKnight et al. 1997), indicating that the leachate could serve as a reasonable proxy of natural allochthonous DOM. After 48 h, needles were removed and the resulting solution was 0.2 μm sterile filtered. Final DOC concentration of the DOM amendment solution was measured using a Shimadzu Total Organic Carbon (TOC) Analyzer (model number TOC-V_{CPH}) and dissolved absorbance was measured using a Shimadzu UV-Visible UV-1650_{PC} spectrophotometer. DOC was measured in standard sensitivity mode, subtracting Milli-Q deionized water (EMD Millipore Corporation) blanks (~ 0.2 mg C L^{-1}) from standards and samples, and calibrating to dilutions of a certified DOC standard (Aqua Solutions, 50 mg L^{-1} potassium biphthalate). Dissolved absorbance was corrected by subtracting Milli-Q water blanks and the average of absorbance at 775–800 nm. The DOM amendment added 1.1 mg C L^{-1} of DOC, 1.33 μg PO_4-P L^{-1}, 0.01 μg NH_4-N L^{-1}, and 0.12 μg NO_3-N L^{-1}.

Zooplankton were collected from an integrated vertical tow in the top 10 m in Glacier Lake, with a 243 μm mesh zooplankton net of 30 cm diameter. We added the natural assemblage of zooplankton from Glacier Lake to zooplankton treatment bags at approximately natural density (3.43 *Daphnia pulicaria* L^{-1} and 1.68 copepods L^{-1}).

Lake and microcosm sampling—To assess lake conditions between the source and incubation lake, abiotic and biotic samples were taken at the beginning (0 d), on day 7 (7 d), and at the end of the experiment (21 d). Temperature, pH, conductivity, and in vivo chlorophyll *a* profiles from the water column of both lakes were measured using a Hydrolab multisensor probe with a Turner Designs 10-AU field fluorometer attachment. PAR was collected using a Biospherical Instruments Cosine submersible radiometer (Biospherical Instruments). The depth where 1% of surface PAR remained in the water column ($Z_{1\%}$) was determined as in Rose et al. (2009).

Abiotic and biotic samples were taken from the microcosms at the beginning (0 d), on day 7 (7 d), and at the end of the experiment (21 d). Initial nutrient samples were taken as the microcosm bags were filled, whereas day 7 and day 21 samples were taken when the experimental bags were removed from the lake. Dissolved nutrient concentrations were measured by filtering the sample through prerinsed 0.4 μm polycarbonate filters and processed by standard methodology (American Public Health

Association 2000). Soluble reactive phosphorus (SRP) was determined using the ascorbic acid method, and nitrate plus nitrite was determined using the cadmium reduction method. Three replicate 50 mL initial phytoplankton samples were taken from the source water carboys used to fill each microcosm bag, and three replicate 50 mL day 7 and day 21 phytoplankton samples were taken from each treatment bag when they were removed from the lake and preserved in Lugol's solution. Phytoplankton were enumerated as in Saros et al. (2005*b*) with at least 1000 total individuals across all species counted in each sample. We restricted our phytoplankton analyses to the four dominant phytoplankton species (*Cyclotella stelligera*, *Asterionella formosa*, *Fragilaria crotonensis*, and *Fragilaria brevistriata*). These four species contributed between 85–100% of the phytoplankton biomass in initial, day 7, and day 21 treatments. Non-diatom taxa (e.g., *Dinobryon* spp. and *Rhodomonas* spp.) were enumerated but were excluded from subsequent analyses due to low densities across all treatments. Initial zooplankton samples were taken as the zooplankton were added to each microcosm bag. Day 7 and day 21 zooplankton samples were taken by filtering treatment bag water through 48 μm mesh, transferred to scintillation vials, and preserved in 95% ethanol. Zooplankton were identified to species level and enumerated in a Bogorov chamber using a Wild M54 dissecting microscope at 40–250× magnification. Temperature was measured every 30 min at the fixed incubation depth of 1.5 m in the epilimnion and 8 m in the hypolimnion for the entirety of the experiment using Thermochron I-Buttons attached to experimental racks holding the microcosm bags.

Zooplankton biomass estimates—Zooplankton lengths for *Bosmina longirostris*, *Arctodiaptomus arapahoensis*, *Acanthocyclops vernalis*, *Daphnia pulicaria*, and nauplii were measured for the first 50 individuals of each species per sample. Zooplankton biomass was estimated using literature values of length–weight regressions for individual species (Culver et al. 1985). The cyclopoid *A. vernalis* which was $< 11\%$ of the initial zooplankton biomass and $< 4\%$ of the day 7 and $< 2\%$ of the day 21 zooplankton biomass in all treatments, respectively, was excluded from analyses because of their low abundance as well as their more carnivorous feeding habits (Reid and Williamson 2010). The low abundance (often zero biomass) and population growth rates for nauplii and *B. longirostris* in the treatments on days 7 and 21 prevented statisical analysis. Thus, zooplankton were combined into two functional groups for analyses. The calanoid group represents the combined biomass of *A. arapahoensis* and nauplii, and the cladocerans group represents the combined biomass of *D. pulicaria* and *B. longirostris*.

Exponential phytoplankton and zooplankton growth rate and treatment effects—Phytoplankton and zooplankton exponential growth rate was calculated using the following equation adapted from Reid and Williamson (2010):

$$r = (\ln D_{ct} - \ln D_{\text{initial}}) \div \text{d} \quad (1)$$

where r is exponential growth rate, D_{ct} and D_{initial} are the densities of phytoplankton or zooplankton biomass in the control bags (phytoplankton control DOM−ZP−; zooplankton control DOM−ZP+) on either day 7 or on day 21 of the experiment, and the initial bags at the start of the experiment, respectively, and d is number of days. Thus, r represents the daily growth rate in phytoplankton density or zooplankton biomass on day 7 and on day 21, in the absence of DOM additions and zooplankton.

DOM and zooplankton treatment effects on phytoplankton densities, and the DOM treatment effect on zooplankton biomass in both strata were calculated by using the following equation:

$$r_{\text{treatment}} = (\ln D_{\text{treatment}} - \ln D_{\text{initial}}) \div \text{d} \quad (2)$$

where $r_{\text{treatment}}$ is the exponential growth rate in the various treatments, $D_{\text{treatment}}$ and D_{initial} are the densities of phytoplankton or zooplankton biomass in the treatment bags on day 7 or day 21 of the experiment and the initial bags at the start of the experiment, respectively, and d is number of days. Treatment effects were obtained by subtracting control growth rates from corresponding treatment growth rates. For example to determine the effect of DOM on phytoplankton densities, we calculated $r_{\text{DOM+ZP-}} - r_{\text{DOM-ZP-}}$, where $r_{\text{DOM+ZP-}}$ is the average daily exponential growth rate of phytoplankton on day 7 and day 21 in the presence of DOM but absence of zooplankton and $r_{\text{DOM-ZP-}}$ is the average daily exponential phytoplankton growth rate on day 7 and day 21 in the absence of both DOM and zooplankton. Standard error (SE) for treatment effects was calculated as the square root of the sum of the separate SE values from each average exponential growth estimate, squared.

Statistical analyses—Four-way analysis of variance (ANOVA) was used to determine the effects of DOM additions, presence or absence of zooplankton, phytoplankton species, and incubation stratum on phytoplankton density and exponential growth on day 7 and day 21 of the experiment. Due to significant interactions with phytoplankton species on day 21, three-way ANOVA was then implemented to assess the effects of DOM additions, presence or absence of zooplankton, and incubation stratum on each phytoplankton species on day 21. Three-way ANOVA was used to assess the effects of DOM additions, incubation stratum, and zooplankton functional group on zooplankton biomass and exponential growth on day 7 and day 21 of the experiment. Proportional data were natural log transformed to achieve normality of residuals. All data were analyzed using Systat version 11.0.

Results

Temperature and transparency (PAR irradiance as calculated from epilimnetic diffuse attenuation coefficients) differed on day 1 and day 21 between the surface stratum (Z = 1.5 m) and the deeper stratum (Z = 8.0 m). Day 1 average temperature in the epilimnion was 7.7°C and the hypolimnion was 4.7°C. Day 21 average temperatures in the

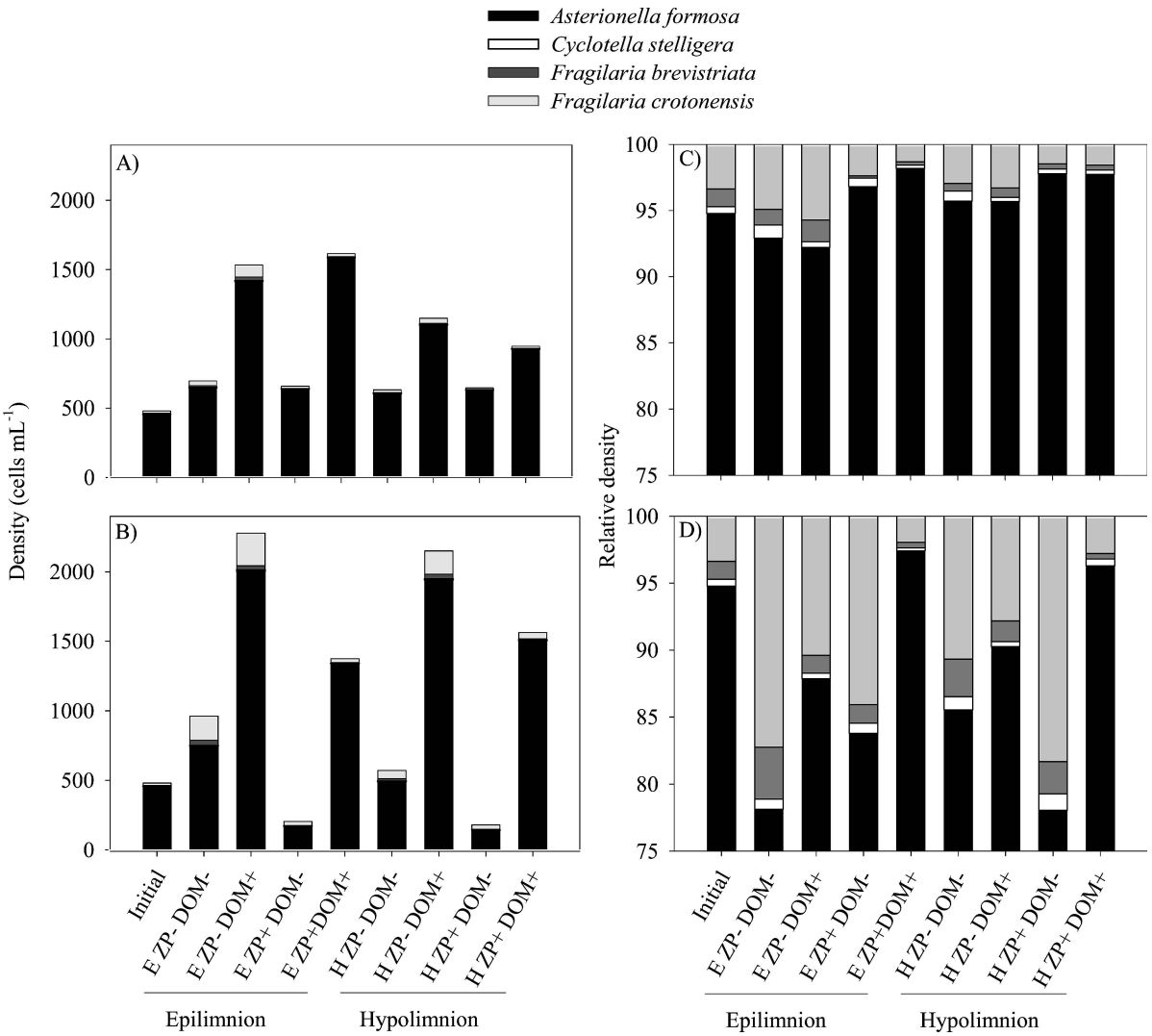

Fig. 1. (A, B) Absolute density (cells L^{-1}) and (C, D) relative density of the four dominant phytoplankton species in initial controls and in all treatments in the presence and absence of dissolved organic matter (DOM) additions and zooplankton (ZP) in the epilimnetic (E) and hypolimnetic (H) incubations on (A, C) day 7 and on (B, D) day 21 of the experiment. Note that the y-axis starts at 75% for panels C and D.

epilimnion and hypolimnion were 10.2°C and 6.0°C, respectively. I-Button temperature data recorded at 30 min intervals throughout the experiment revealed that temperature ranged from 6.0–12.0°C in the epilimnion, and from 4.0–8.0°C in the hypolimnion over the 21 d. PAR values from day 1 in the epilimnion and hypolimnion were 55.8% and 4.4% of surface, respectively, whereas on day 21 PAR values were 63.6% and 9.0% of surface, respectively. Overall, the depth of 1% of surface PAR transparency increased by 3.5 m from day 1 (11.8 m) to day 21 (15.3 m).

Diatoms comprised > 87% of the phytoplankton density on day 7 and *A. formosa* comprised > 92% of the relative phytoplankton density in all treatments (Fig. 1A,C). *A. arapahoensis* and *D. pulicaria* dominated the zooplankton assemblage on day 7 as well, contributing to 96% of the overall zooplankton biomass (Fig. 2A). *A. arapahoensis* comprised > 70% of the relative abundance, whereas *D. pulicaria* comprised up to 30% of the zooplankton relative abundance on day 7 (Fig. 2C). Phytoplankton density and growth were significantly affected by DOM additions (Tables 2, 3; Fig. 1A; DOM $\text{effect}_{\text{density}}$ $F_{1,61} = 30.971$, $p < 0.001$; DOM $\text{effect}_{\text{growth}}$ $F_{1,61} = 30.971$, $p < 0.001$) and these responses varied by species (DOM × Species [SPP] $\text{effect}_{\text{density}}$ $F_{3,61} = 5.553$, $p = 0.002$; DOM × SPP $\text{effect}_{\text{growth}}$ $F_{3,61} = 5.553$, $p = 0.002$). Phytoplankton densities and growth were lower in the hypolimnion on day 7 (Tables 2, 3; Fig. 1A; Stratum (STRATUM) $\text{effect}_{\text{density}}$ $F_{1,61} = 22.30$, $p < 0.001$; STRATUM $\text{effect}_{\text{growth}}$ $F_{1,61} = 22.30$, $p < 0.001$), likely due to colder temperatures slowing population growth, yet by day 21 this stratum difference is absent (Tables 2, 3; Figs. 1B, 3; STRATUM $\text{effect}_{\text{density}}$ $F_{1,63} = 0.3$, $p = 0.610$; STRATUM $\text{effect}_{\text{growth}}$ $F_{1,63} = 0.3$, $p = 0.610$). Lastly, due to cold temperatures and slower generation times of zooplankton, the 7 d time period is likely not long enough to see the full effect of zooplankton grazing on phytoplankton, as zooplankton

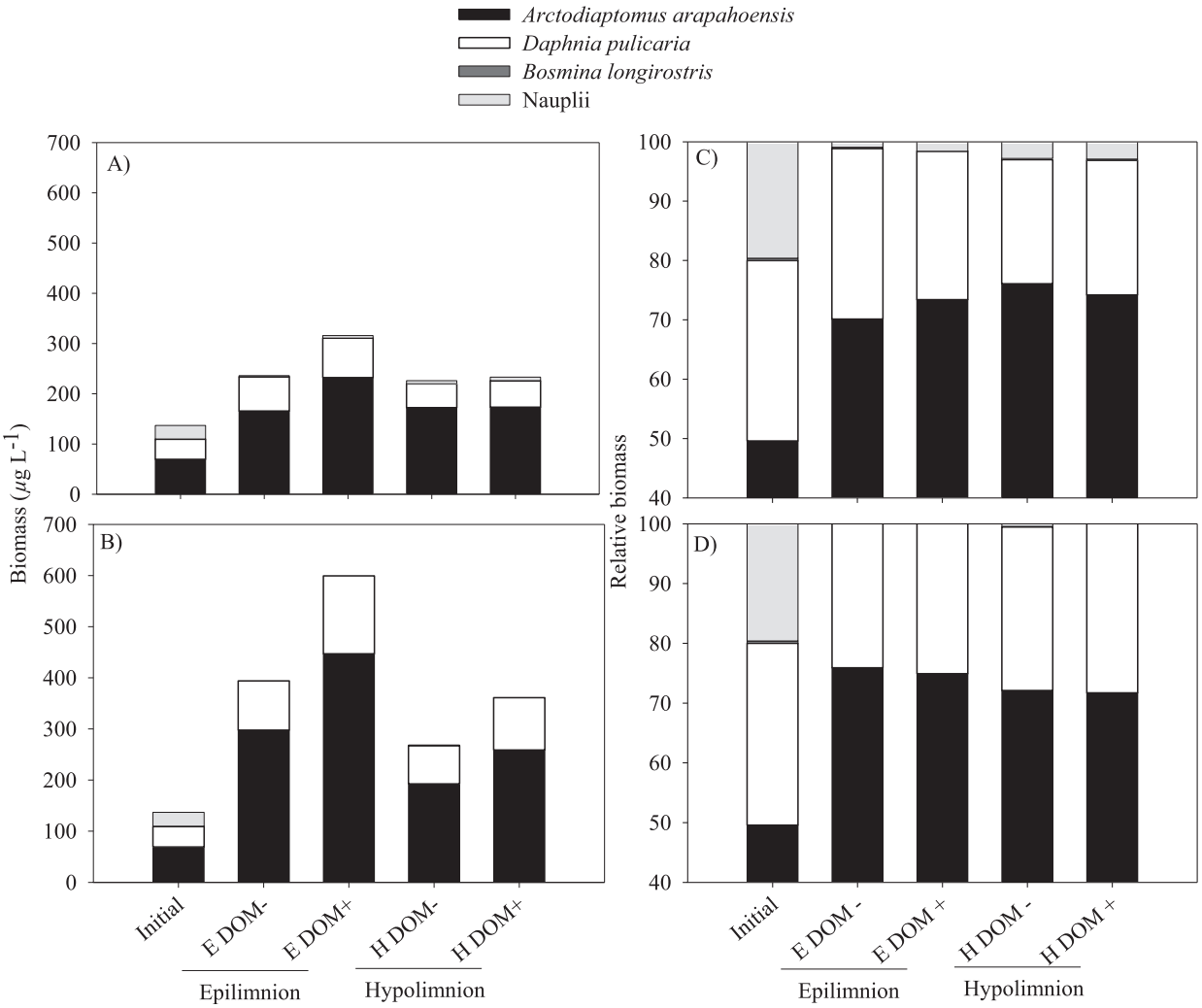

Fig. 2. (A, B) Absolute biomass (μg L^{-1}) and (C, D) relative biomass of four zooplankton species in the initial and in the (A, C) day 7 and (B, D) day 21 treatments in the presence and absence of dissolved organic matter (DOM) in the epilimnion (E) and hypolimnion (H). The cyclopoid *Acanthocyclops vernalis* which was < 11% of the initial zooplankton biomass and < 4% of the day 7 and < 2% of the day 21 zooplankton biomass in all treatments, respectively, was excluded from analyses because of their low abundance as well as their more carnivorous feeding habits (Reid and Williamson 2010). Note that the y-axis starts at 40% for panels C and D.

biomass responded to DOM amendments on day 7 (Table 4; Fig. 2A; DOM $\text{effect}_{\text{biomass}}$ $F_{1,16} = 7.145$, $p = 0.017$), but growth did not respond significantly to DOM (Tables 4, 5; DOM $\text{effect}_{\text{growth}}$ $F_{1,16} = 3.633$, $p = 0.075$). As a result, and to ensure capturing the most complete suite of DOM effects on phytoplankton, further analyses were restricted to day 21.

Diatoms comprised > 85% of the phytoplankton density in all treatments on day 21 and *A. formosa* was the most abundant phytoplankton species, comprising > 77% of the relative phytoplankton density in all treatments, and at both incubation depths (Fig. 1B,D). *A. arapahoensis* and *D. pulicaria* dominated the zooplankton biomass on day 21, making up > 97% of the total zooplankton biomass in all treatments. *A. arapahoensis* contributed about 70–75% of the zooplankton biomass and *D. pulicaria* made up 25–30% (Fig. 2B,D).

The very low phosphorus and chlorophyll concentrations in the initial incubation waters indicate severe phosphorus limitation in the treatments with no DOM additions. SRP concentrations were below quantification (limit of quantification = 1 μg L^{-1}), nitrate concentrations were 86 μg NO_3-N L^{-1}, and chlorophyll *a* concentrations were 4 μg L^{-1}. The addition of DOM increased SRP concentrations to 1.3 μg PO_4-P L^{-1} but did not add any measurable nitrate (< 1 μg NO_3-N L^{-1}). By the end of the 3 week incubation period in the DOM addition treatments, the SRP was drawn back down to 1 μg PO_4-P L^{-1} in the epilimnion and to unquantifiable levels in the hypolimnion. The DOM addition led to a severe drawdown (dropping to below quantification limits of 1 μg NO_3-N L^{-1}) of nitrate in the epilimnion and hypolimnion compared to the DOM treatments, suggesting that the addition of organic and inorganic forms of P with the DOM facilitated this nitrate

Table 2. Exponential growth rate (± standard error) of the four dominant phytoplankton species calculated on day 7 and on day 21 in the presence or absence of dissolved organic material (DOM) and zooplankton (ZP) additions in the epilimnetic (E) and hypolimnetic (H) incubations.

Treatment	*Cyclotella stelligera*	*Asterionella formosa*	*Fragilaria crotonensis*	*Fragilaria brevistriata*
Day 7				
E ZP− DOM−	0.16±0.01	0.05±0.01	0.1±0.02	0.03±0.03
E ZP− DOM+	0.15±0.03	0.16±0.00	0.24±0.00	0.19±0.02
E ZP+ DOM−	0.08±0.02	0.05±0.01	−0.01±0.04	−0.26±0.05
E ZP+ DOM+	0.07±0.04	0.18±0.01	0.03±0.04	0.01±0.00
H ZP− DOM−	0.09±0.05	0.04±0.01	0.01±0.04	−0.11±0.07
H ZP− DOM+	0.04±0.04	0.12±0.02	0.12±0.02	0.08±0.00
H ZP+ DOM−	0.00±0.02	0.05±0.01	−0.11±0.08	−0.07±0.02
H ZP+ DOM+	0.02±0.07	0.10±0.00	−0.07±0.09	−0.12±0.07
Day 21				
E ZP− DOM−	0.05±0.01	0.02±0.00	0.11±0.01	0.08±0.01
E ZP− DOM+	0.06±0.01	0.07±0.00	0.13±0.00	0.07±0.01
E ZP+ DOM−	−0.02±0.01	−0.05±0.01	0.02±0.01	−0.02±0.01
E ZP+ DOM+	0.01±0.00	0.05±0.00	0.02±0.01	−0.02±0.03
H ZP− DOM−	0.04±0.01	0.00±0.01	0.06±0.00	0.04±0.00
H ZP− DOM+	0.06±0.02	0.07±0.00	0.11±0.00	0.08±0.01
H ZP+ DOM−	0.00±0.01	−0.06±0.00	0.03±0.01	−0.03±0.02
H ZP+ DOM+	0.06±0.02	0.06±0.00	0.05±0.01	0.00±0.01

uptake. In the absence of DOM addition, SRP remained unquantifiable and nitrate declined to 71–73 μg NO_3-N L^{-1}.

Initial phytoplankton densities and zooplankton biomass were dominated by *A. formosa* and *A. arapahoensis*, respectively, at the beginning of the experiment and on day 21 (Figs. 1B,D, 2B,D). At the end of the experiment and in the absence of zooplankton, the addition of DOM had strong effects on phytoplankton density and growth at both incubation depths (Tables 2, 3; Figs. 1B, 3). DOM additions stimulated an increase in phytoplankton densities (DOM $\text{effect}_{\text{density}}$ $F_{1,63} = 98.4$, $p < 0.001$), and growth (DOM $\text{effect}_{\text{growth}}$ $F_{1,63} = 98.4$, $p < 0.001$) and the magnitude of the increase differed as a function of phytoplankton species (DOM × SPP $\text{effect}_{\text{density}}$ $F_{3,63} = 18.3$, $p < 0.001$; DOM × SPP $\text{effect}_{\text{growth}}$ $F_{3,63} = 18.3$, $p < 0.001$). In the presence of DOM, densities of *C. stelligera*, *A. formosa*, and *F. crotonensis* increased (Table 6; Fig. 1B,D; DOM $\text{effect}_{\text{density}}$ $F_{1,16} = 8.84$–438, $p < 0.009$), whereas *F. brevistriata* did not differ (Table 6; Fig. 1B,D; DOM $\text{effect}_{\text{density}}$ $F_{1,16} = 1.449$, $p = 0.247$). In the presence of DOM, *A. formosa* density increased by 170% and 297% and exponential growth rates increased by > 200% and 1400% relative to control treatments in the epilimnion and hypolimnion, respectively (Figs. 1B, 3). *F. crotonensis* density increased by 36% and 180% in the presence of DOM in the epilimnion and hypolimnion and increased by 15% and 47%, respectively, for *C. stelligera* compared to controls (Fig. 1B). Exponential

Table 3. Results of a four-way ANOVA on the effects of dissolved organic material (DOM), zooplankton (ZP), depth stratum (STRATUM), and phytoplankton species (SPP) on phytoplankton density and exponential growth rate on day 7 and day 21 of the experiment. Note that the phytoplankton density and phytoplankton exponential growth rate four-way ANOVA results were identical on day 7, and identical on day 21; therefore, only one *F*-ratio and *p*-value is listed for each day. Bold indicates significance at $\alpha < 0.05$.

Source	Day 7		Day 21	
	$F_{1,61}$	p	$F_{1,63}$	p
DOM	**30.97**	**0.000**	**98.4**	**0.000**
ZP	**47.51**	**0.000**	**274.9**	**0.000**
STRATUM	**22.30**	**0.000**	0.3	0.610
SPP	**676.6**	**0.000**	**32.9**	**0.000**
DOM×ZP	1.027	0.315	**4.2**	**0.044**
DOM×STRATUM	3.704	0.059	**9.3**	**0.003**
DOM×SPP	**5.553**	**0.002**	**18.3**	**0.000**
ZP×STRATUM	2.555	0.115	**17.4**	**0.000**
ZP×SPP	**7.713**	**0.000**	**9.1**	**0.000**
STRATUM×SPP	1.327	0.274	1.5	0.233
DOM×ZP×STRATUM	1.710	0.196	0.2	0.629
DOM×ZP×SPP	0.949	0.423	**5.4**	**0.002**
DOM×STRATUM×SPP	1.378	0.258	0.2	0.893
ZP×STRATUM×SPP	1.784	0.160	1.4	0.249
DOM×ZP×STRATUM×SPP	2.754	0.050	0.7	0.541

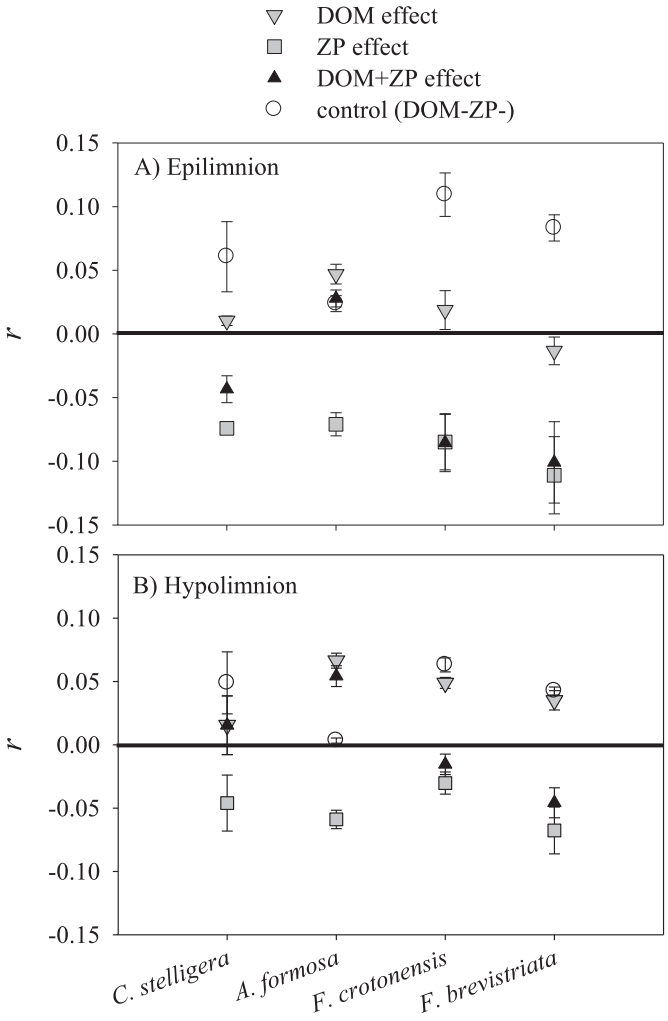

Fig. 3. Exponential population growth rates (± standard error) of the four dominant phytoplankton species in control treatments with no added dissolved organic matter (DOM) or zooplankton (ZP; DOM−ZP−), and the incremental effect of DOM, zooplankton, and DOM+ zooplankton treatments (± standard error) on these growth rates in the (A) epilimnetic and (B) hypolimnetic incubations at the end of the 21 d experimental period. The incremental effects are expressed as the daily exponential rate of growth or decline relative to phytoplankton growth in control treatments.

growth rates for *F. crotonensis* and *C. stelligera* in the DOM+ treatments increased a more modest 17% and 27% in the epilimnion and 33% and 77% in the hypolimnion relative to controls (Fig. 3). *F. brevistriata* density decreased by 23% and exponential growth rates decreased by 12% in the epilimnion, yet density increased 115% and exponential growth increased by 78% in the hypolimnion relative to controls (Figs. 1B, 3). The magnitude of the DOM effect was also greater in the hypolimnion (Tables 2, 3; Figs. 1B, 3; DOM × STRATUM $\text{effect}_{\text{density}}$ $F_{1,63} = 9.3$, $p = 0.003$; DOM × STRATUM $\text{effect}_{\text{growth}}$ $F_{1,63} = 9.3$, $p = 0.003$).

DOM additions also stimulated substantial increases in the zooplankton biomass (DOM $\text{effect}_{\text{biomass}}$ $F_{1,16} = 15.255$, $p = 0.001$) and growth (DOM $\text{effect}_{\text{growth}}$ $F_{1,16} = 8.539$, $p = 0.01$) across all treatments on day 21 (Tables 4, 5; Figs. 2B,D, 4). Calanoid and cladoceran functional groups did not significantly differ in their biomass or growth responses to DOM additions (Tables 4, 5; Figs. 2B, 4; DOM × GRP $\text{effect}_{\text{biomass}}$ $F_{1,16} = 3.033$, $p = 0.101$; DOM × GRP $\text{effect}_{\text{growth}}$ $F_{1,16} = 0.083$, $p = 0.777$). Thus, DOM had a positive, nonselective effect on zooplankton grazer functional groups. Zooplankton biomass and growth, in both the presence and absence of DOM, was significantly greater in the epilimnion (Tables 4, 5; Figs. 2B, 4; STRATUM $\text{effect}_{\text{biomass}}$ $F_{1,16} = 22.692$, $p < 0.001$; STRATUM $\text{effect}_{\text{growth}}$ $F_{1,16} = 7.926$, $p = 0.012$). Zooplankton grazing, on the other hand, was consistently stronger in the epilimnion than in the hypolimnion (Tables 2, 3; Figs. 1B, 3; ZP × STRATUM $\text{effect}_{\text{density}}$ $F_{1,63} = 17.4$, $p < 0.001$; ZP × STRATUM $\text{effect}_{\text{growth}}$ $F_{1,63} = 17.4$, $p < 0.001$). Zooplankton grazing was selective depending on phytoplankton species (Tables 2, 3; Figs. 1B, 3; ZP × SPP $\text{effect}_{\text{density}}$ $F_{3,63} = 9.1$, $p < 0.001$; ZP × SPP $\text{effect}_{\text{growth}}$ $F_{3,63} = 9.1$, $p < 0.001$) and varied in the presence and absence of DOM (Tables 2, 3; Figs. 1B, 3; DOM × ZP × SPP $\text{effect}_{\text{density}}$ $F_{3,63} = 5.4$, $p = 0.002$; DOM × ZP × SPP $\text{effect}_{\text{growth}}$ $F_{3,63} = 5.4$, $p = 0.002$).

Discussion

The results of our study indicate that increased terrestrial-based DOM inputs may have important effects on phytoplankton and zooplankton density, growth, and species assemblages in alpine lakes. Experimental addition of DOM resulted in selectively altered phytoplankton species assemblage on day 21. In the presence of DOM, *A. formosa* density and exponential growth rates increased by 1.7–2.8-fold and 2.0–14.0-fold, respectively, relative to controls. *F. crotonensis* and *C. stelligera* had modest increases in both strata, whereas *F. brevistriata* had minor decreases in the epilimnion and modest increases in the hypolimnion. These selective increases in species-specific phytoplankton density and growth rates in response to DOM additions support hypothesized mechanism 1 that DOM will alter phytoplankton species assemblages by differentially stimulating the density and growth of select phytoplankton species.

Biomass and exponential growth rates of calanoid and cladoceran functional groups increased significantly in the DOM+ treatments, but the response did not differ significantly by group. Despite the uniform increase in zooplankton functional group biomass and growth in the presence of DOM, the subsequent effects of zooplankton grazing on phytoplankton was selective. Grazing varied significantly on phytoplankton species (ZP × SPP effect), and the selective grazing varied in the presence and absence of DOM (DOM × ZP × SPP effect; Fig. 3). The net grazing effect of zooplankton in the absence of DOM was negative on all four phytoplankton species, but in the presence of DOM was positive on *A. formosa*. These selective grazing results likely also contributed to the observed species-specific shifts in the phytoplankton assemblage. These data support mechanism 2 that DOM

Table 4. Results of a three-way ANOVA testing for the effects of dissolved organic material (DOM), depth stratum (STRATUM), and zooplankton functional group (GRP) on zooplankton biomass and zooplankton exponential growth rate on day 7 and day 21 of the experiment. Bold indicates significance at $\alpha < 0.05$. Note that the low abundance (often zero biomass) and population growth rates for nauplii and *Bosmina longirostris* in the treatments on days 7 and 21 prevented statisical analysis. Thus zooplankton were combined into two functional groups. The calanoid group represents the combined biomass of *Arctodiaptomus arapahoensis* and nauplii, and the cladoceran group represents the combined biomass of *Daphnia pulicaria* and *B. longirostris*.

	Biomass		Exponential growth rate	
Source	$F_{1,16}$	p	$F_{1,16}$	p
Day 7				
DOM	**7.145**	**0.017**	3.633	0.075
STRATUM	**8.166**	**0.011**	**10.71**	**0.005**
GRP	**253.1**	**0.000**	**13.252**	**0.002**
DOM×STRATUM	**5.076**	**0.039**	1.406	0.253
DOM×GRP	2.917	0.107	0.139	0.714
STRATUM×GRP	0.000	0.996	3.457	0.081
DOM×STRATUM×GRP	3.844	0.068	0.899	0.357
Day 21				
DOM	**15.26**	**0.001**	**8.539**	**0.010**
STRATUM	**22.69**	**0.000**	**7.926**	**0.012**
GRP	**101.7**	**0.000**	2.024	0.174
DOM×STRATUM	2.196	0.158	0.283	0.602
DOM×GRP	3.033	0.101	0.083	0.777
STRATUM×GRP	**8.532**	**0.010**	0.497	0.491
DOM×STRATUM×GRP	0.485	0.496	0.026	0.874

uniformly increases the biomass of zooplankton, and through the subsequent intensified selective grazing of the zooplankton assemblage, can stimulate shifts in the phytoplankton species assemblage. There is no support for mechanism 3 in which DOM alters phytoplankton species assemblage by shifting the zooplankton grazer species assemblage.

Whereas previous studies have found that DOM additions selectively altered phytoplankton communities (Vinebrooke and Leavitt 1998; Scott et al. 2009) and enhanced zooplankton density, survival, and reproduction (Vinebrooke and Leavitt 1998; Cooke et al. 2006*b*) in alpine lakes, the novel contribution of our experiment is that it enables an evaluation of the relative importance of three potential mechanisms by which DOM additions alter phytoplankton density, growth, and species assemblage. Two non–mutually exclusive mechanisms were identified as being responsible for the markedly different responses of the phytoplankton species to DOM: selective nutrient stimulation of phytoplankton species (*nutrient stimulation hypothesis*), and a uniform increase in zooplankton grazing (*trophic intensity hypothesis*). The relative importance of these two processes varied greatly with phytoplankton species, depth stratum, and in the presence or absence of DOM. In the epilimnion the highest grazing rates were on the two *Fragilaria* species, whereas in the hypolimnion grazing rates were highest on *A. formosa* and *F. brevistriata*. But, in the presence of DOM, the net zooplankton grazing effect reversed for *A. formosa*, most likely due to the zooplankton consumers being overwhelmed by the increased densities of this large colonial species. The negative effects of zooplankton grazing on algal growth rates were consistently stronger in the epilimnion than in the hypolimnion for all phytoplankton species, most likely due to greater zooplankton biomass in the epilimnion treatments on day 21. Because phytoplankton densities do not differ significantly between the epilimnion and hypolimnion across all treatments on day 21, stress on phytoplankton due to higher light conditions and greater exposure to UV radiation in the epilimnion is less likely to have contributed to this pattern. While it might be expected that bacterial abundance would be greater in the hypolimnion relative to phytoplankton abundance due to colder

Table 5. Exponential growth rate (± standard error) of calanoid and cladoceran zooplankton functional groups on day 7 and on day 21 in the presence or absence of dissolved organic material (DOM) additions in the epilimnetic (E) and hypolimnetic (H) incubations. Note that the low abundance (often zero biomass) and population growth rates for nauplii and *Bosmina longirostris* in the treatments on days 7 and 21 prevented statisical analysis. Thus zooplankton were combined into two functional groups. The calanoid group represents the combined biomass of *Arctodiaptomus arapahoensis* and nauplii, and the cladoceran group represents the combined biomass of *Daphnia pulicaria* and *B. longirostris*.

Treatment	Calanoid	Cladoceran
Day 7		
E DOM−	0.08±0.01	0.07±0.01
E DOM+	0.13±0.01	0.09±0.01
H DOM−	0.09±0.01	0.02±0.02
H DOM+	0.09±0.01	0.03±0.03
Day 21		
E DOM−	0.05±0.01	0.04±0.02
E DOM+	0.07±0.00	0.06±0.01
H DOM−	0.03±0.01	0.03±0.01
H DOM+	0.05±0.00	0.04±0.01

Table 6. Results from three-way ANOVA on the four dominant diatom species densities on day 21. Bold indicates significance at $\alpha < 0.05$.

Source	*C. stelligera*		*A. formosa*		*F. crotonensis*		*F. brevistriata*	
	$F_{1,16}$	p	$F_{1,16}$	p	$F_{1,16}$	p	$F_{1,15}$	p
DOM	**8.839**	**0.009**	**437.8**	**0.000**	**12.05**	**0.003**	1.449	0.247
ZP	**15.45**	**0.001**	**65.36**	**0.000**	**144.3**	**0.000**	**66.14**	**0.000**
STRATUM	0.929	0.349	0.536	0.475	1.666	0.215	0.209	0.654
DOM×ZP	0.576	0.459	0.510	0.485	**5.091**	**0.038**	0.031	0.862
DOM×STRATUM	2.080	0.169	2.375	0.143	3.686	0.073	2.203	0.158
ZP×STRATUM	**4.813**	**0.043**	3.267	0.09	**16.05**	**0.001**	1.247	0.282
DOM×ZP×STRATUM	0.903	0.356	0.000	0.987	0.394	0.539	0.640	0.436

and darker conditions that can influence both the processing of DOM and suppress phytoplankton, we saw no evidence of this in the phytoplankton response. Rather, the positive effects of DOM ecosystem subsidies on algal density and growth rates were generally stronger in the hypolimnion than in the epilimnion, with the strongest effects on *A. formosa*. *A. formosa* and *F. crotonensis* grow well in low temperatures and low light conditions (Interlandi et al. 1999), and increases in these species are attributed to nutrient enrichment (Ramstack et al. 2003). Therefore, in the hypolimnion, three favorable conditions likely contributed to the enhancement of *A. formosa*: light conditions were 92.8% and 85.8% lower than the epilimnion on days 1 and 21, respectively, temperature averaged 2–4°C lower than the epilimnion, and the DOM amendment added 1.3 μg PO_4-P L^{-1}. Overall, these effects led to pronounced selective changes in phytoplankton species assemblage characterized by *A. formosa* dominance in the presence of DOM, and in the combined presence of DOM and zooplankton. It is important to note that due to the experimental design, water with the same original suite of organisms obtained from the same location in the source lake and exposed to the identical DOM addition were placed in the epilimnion and hypolimnion strata. Because of design, we are likely assessing the effects of abiotic factors such as light and temperature on DOM responses between strata, rather than differences in DOM availability for organisms in the water column. While the negative effects of zooplankton grazing were generally stronger than the DOM enhancement effects in our experiment (Fig. 3, exceptions were *A. formosa* and *F. crotonensis* in the hypolimnion), these were fixed effects determined by the experimental design. Although the magnitude of the manipulations was designed to make the forcing by these variables realistic, the relative importance of future changes in DOM and zooplankton grazing will ultimately depend on the magnitude of changes in these variables in response to environmental change. It is important to note that because our experiment was incubated at two depths in the water column and we did not specifically intend to manipulate light with the DOM amendments, our observed results would likely be different if light had been manipulated. However, the nutrient stimulation results and differences in response among strata still hold, as light conditions should have minimal effects on these parameters.

Several lines of evidence indicate that P was the key nutrient underlying the enhancement observed in response to DOM additions in our experiments. The initial lake water added to all treatment bags had high seston N : P of 24, suggesting P-limited conditions. At the end of the experiment the DOM− treatments still had significant concentrations of nitrate and undetectable SRP concentrations in the epilimnion and hypolimnion. P contained in the DOM+ treatments likely facilitated the substantial nitrate drawn down to unquantifiable levels at both depths. Therefore, it is likely that the additional P contained in the DOM amendments helped alleviate the P limitation and stimulated phytoplankton growth in the DOM+ treatments. The fact that the two dominant species in our experiment, *A. formosa* and *F. crotonensis*, are opportunistic taxa that are frequently used as indicators of nutrient enrichment (Ramstack et al. 2003) further supports the idea that nutrient additions with DOM were involved with the strong response of phytoplankton density and growth to DOM. While *A. formosa* and *F. crotonensis* previously responded to N additions in low-nitrate lakes in this region (Saros et al. 2005*b*), the experimental lake tested here was already high in nitrate likely due to runoff from glacial remnants above the lake (Saros et al. 2010), suggesting that in this case, the P addition with DOM was likely the nutrient that stimulated growth. On the other hand, *C. stelligera* is found in a variety of nutrient conditions, and is generally less abundant when taxa of more productive systems are dominant (Clerk et al. 2000), which may help explain the modest response of *C. stelligera* to the DOM addition.

The similar increase in biomass and exponential growth rates of both cladoceran and calanoid functional groups in the DOM+ treatments provide support for enhanced trophic forcing by zooplankton due to uniform increases in all zooplankton taxa. This supports the trophic intensity hypothesis. The increased intensity of the selective grazing combined with a strong enhancement in the presence of DOM contributed to the shift in the phytoplankton assemblage to almost complete dominance by *A. formosa,* a large colony-forming diatom species that is difficult to ingest by both daphniids (Kagami et al. 2005) and diaptomids (Kagami et al. 2011). Likewise, *F. brevistriata* and *C. stelligera* are smaller in size and thus more subject to grazing, and *F. crotonensis*, *F. brevistriata*, and *C. stelligera* were all less responsive than *A. formosa* to DOM additions. This may be the cause of the significant decreases in the relative abundance of these three phytoplankton species in the DOM+ZP+ treatments.

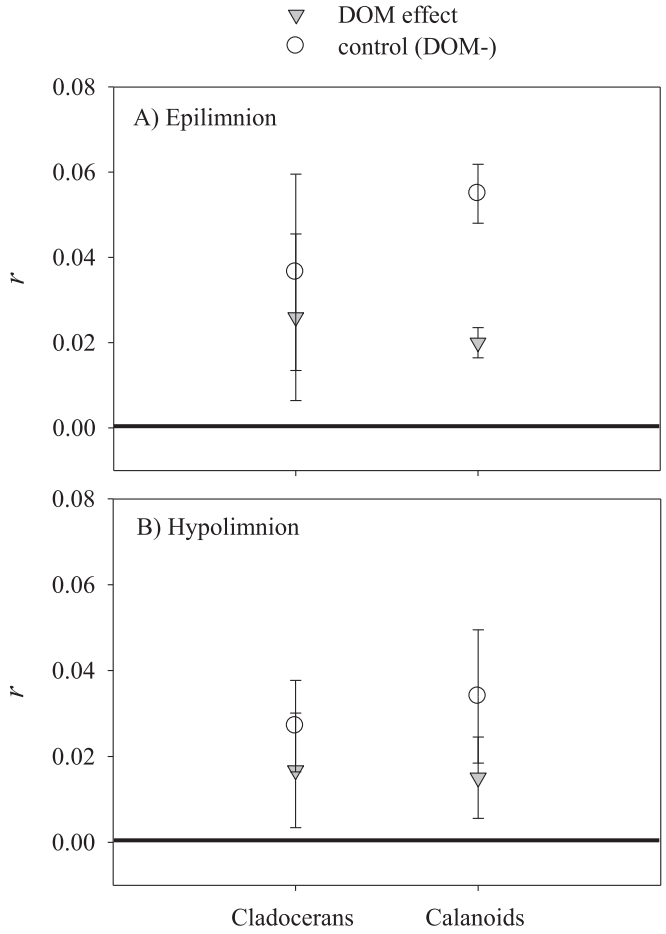

Fig. 4. Exponential population growth rates (± standard error) of the two dominant zooplankton groups in control treatments with no added dissolved organic material (DOM−), and the incremental effect of DOM (± standard error) on these growth rates in the (A) epilimnetic and (B) hypolimnetic incubations at the end of the 21 d experimental period. The incremental effects are expressed as the daily exponential rate of growth or decline relative to zooplankton growth rate in control treatments. Note, the low abundance (often zero biomass) and population growth rates for nauplii and *Bosmina longirostris* in the treatments on days 7 and 21 prevented statisical analysis. Thus zooplankton were combined into two functional groups. The calanoid group represents the combined biomass of *Arctodiaptomus arapahoensis* and nauplii, and the cladoceran group represents the combined biomass of *Daphnia pulicaria* and *B. longirostris*.

Even though our experiment does not provide support for the trophic shift hypothesis, it is still important to consider additional mechanisms by which DOM may selectively alter zooplankton species and subsequently lead to changes in phytoplankton species assemblage. DOM selectively increased *Daphnia catawba* compared to copepod biomass in a microcosm experiment in Lake Giles, Pennsylvania, due to the amendment dropping pH and favoring acid-tolerant species (Cooke et al. 2006*a*). This negative effect of low pH was not observed in our experiment due to the relatively high pH of lakes in the Beartooth-Absaroka region (6.7–7.6; Saros et al. 2005*b*).

DOM may also selectively alter zooplankton species composition by stimulating the microbial loop, providing additional C for zooplankton through bacterial consumption (Berggren et al. 2010; Faithfull et al. 2012; but *see* Faithfull et al. 2011). For example, *Daphnia* are better able to ingest and utilize bacteria than diaptomids (Sanders et al. 1996), yet copepods have been found to selectively graze on higher quality food sources (i.e., ciliates) that ultimately derived their energy from consuming bacteria (Faithfull et al. 2012). In our study, the strong enhancement of phytoplankton density and growth rates by DOM additions in the epilimnion and hypolimnion combined with the similar responses of both zooplankton functional groups suggest that stimulation of autotrophic biomass likely contributed to the observed increases in zooplankton biomass. The lack of any assessment of the response of the microbial loop in our experiments prevents us from making any definitive statements about the relative contribution of enhanced autotrophic vs. heterotrophic biomass to the observed DOM stimulation of zooplankton consumers. However, if the effect of DOM additions had resulted in enhanced bacterial biomass and subsequently provided an additional food source for zooplankton, we would have expected much larger increases in zooplankton biomass and growth relative to that of phytoplankton. Moreover, we would not have expected the large density and growth responses from autotrophic diatoms, such as *A. formosa* and *F. crotonensis* which contributed to $> 95\%$ of the phytoplankton density in all treatments. The mean loss of DOC in treatments from day 1 to day 21 (0.14 mg L^{-1} for both strata) was similar to the increase in C from zooplankton biomass from day 1 to day 21 (mean increase = 0.15 mg L^{-1} for both strata). Using a 10% trophic transfer for the transfers from terrestrial DOM to microbes, and from microbes to zooplankton, the estimated C available from DOM to zooplankton would be $\sim 1\%$, or 0.0014 mg C L^{-1}, a similar estimate to the measured $< 2\%$ contribution of C from terrestrial DOC via the microbial pathway to zooplankton observed in small midwestern U.S. lakes (Cole et al. 2006). The estimated 1% of C available from the microbial pathway in the present study can only explain a minimal portion of the 0.15 mg C L^{-1} increase in the zooplankton, further suggesting DOM stimulation of phytoplankton density and growth likely contributed to a greater degree. Our results contrast studies indicating terrestrial carbon subsidies significantly increase zooplankton biomass via the microbial loop (Berggren et al. 2010; Faithfull et al. 2012), and support findings that DOM stimulates autotrophic production in nutrient-rich systems (Carpenter et al. 2005). In addition, our results support findings indicating zooplankton are comprised to greater degree by autotrophic-, rather than heterotrophic-derived C in a lab study (Brett et al. 2009) and in oligotrophic lakes with deep chlorophyll maxima (Francis et al. 2011).

Other factors that we did not look at in our experiment but that may lead to selective changes in the zooplankton assemblage and subsequent effects on phytoplankton (i.e., trophic shift hypothesis) include differing competitive abilities (Milbrink et al. 2003), preferences for different sized food particles (Sanders et al. 1996; Kagami et al.

2002), and increased UV tolerance from the DOM addition (Cooke et al. 2006*a*,*b*).

The microcosm experimental design of the present study provides benefits to addressing specific mechanisms, yet also presents limitations. The microcosms constrained phytoplankton and zooplankton in individual strata, thereby allowing us to directly test the three potential mechanisms by which DOM affects phytoplankton growth and species assemblages in the epilimnion and hypolimnion. In addition, microcosms allowed for ample treatment replication and statistical power to detect patterns. In contrast, microcosm experiments may not replicate whole lake conditions, limiting ecological realism. Microcosm bags are small, and may inhibit species growth and reproduction due to limited resources during long experimental periods. In the present study, phytoplankton densities and zooplankton biomass increased from day 1 to day 7, and from day 7 to day 21, indicating that phytoplankton and zooplankton were likely not affected greatly by bag size. Microcosms also inhibit vertical migration of zooplankton. Despite zooplankton in the epilimnion treatments being exposed to higher light intensities (including damaging UV radiation), zooplankton biomass was greater in the epilimnion, indicating low sensitivity to ambient UV in this lake. Zooplankton grazing and excretion has been shown to promote algal and bacterial growth in small bags (Roman and Rublee 1980). Phytoplankton density and growth was significantly suppressed by zooplankton in the absence of DOM additions, indicating that zooplankton were effective grazers on phytoplankton even in the event that algal growth was positively enhanced by presence of zooplankton grazers. Mesocosm bags also can prevent sinking of plankton species that are unable to regulate their position in the water column through buoyancy. However, diatoms can regulate their buoyancy and can maintain their position in the deep chlorophyll maximum (DCM) where nutrient concentrations are the highest in the water column (Saros et al. 2005*a*). Additionally, the diatoms in our experiment are found throughout the water column (J. Saros unpubl.), including the hypolimnion, likely due to buoyancy regulation. Lastly, this mesocosm experiment was conducted in only one lake. Therefore, caution must be used when extrapolating the results of this study to the whole lake and to other lake ecosystems.

Phytoplankton used in the study were obtained from the DCM ($Z = 9$ m) of the adjacent source lake, Glacier Lake, to ensure that phytoplankton density would be sufficient to support zooplankton grazers for the 21 d experiment. The species assemblage collected for the present study was confirmed to contain the same species that were present at various depths in Glacier Lake the previous August (J. Saros unpubl.). Collecting the phytoplankton from 9 m and then incubating half of the experimental bags at 1.5 m exposed the phytoplankton assemblage in the epilimnion treatments to warmer temperatures and higher light intensity than the collection depth conditions. However, the temperature difference between the DCM in Glacier Lake and the surface stratum incubation depth of 1.5 m in Emerald Lake on day 21 was $< 2^{\circ}$C, and the depth of the 1% surface irradiance of PAR in Glacier Lake was 6.7 m deeper than the DCM, indicating exposure to ample light prior to collection. Moreover, these species are found distributed at lower densities throughout the whole water column of both the source and incubation lakes during the growing season (J. Saros unpubl.) with the highest densities at the DCM likely for nutrient acquisition rather than to avoid harmful UV radiation (Saros et al. 2005*a*).

Our alpine source and incubation lakes are ice-free for short time periods due to cold temperatures and high winter snowfall. We deployed the experiment immediately following ice-off and we ran the experiment for 21 d using the spring assemblage of phytoplankton typically subjected to the maximum DOM pulse following snowmelt. The 21 d duration captured both the critical growing season for the phytoplankton and zooplankton and the more complete suite of DOM and grazer effects on phytoplankton, while encompassing the time period in which the lake was thermally stratified into two distinct strata. Soon after removal of the experiment, cold temperatures and low light resulted in whole water column mixing and the degradation of thermal stratification. These dynamic changes to the lake would likely affect phytoplankton and zooplankton, and decrease the ability to detect the mechanisms by which DOM affects phytoplankton density, growth, and species assemblage.

As a fundamental regulator of aquatic systems (Williamson et al. 1999) terrestrially derived organic matter can contribute significantly to the C content of zooplankton. In clear water and humic lakes, upwards of 40–50% of carbon in zooplankton can be derived from terrestrial organic matter (Pace et al. 2004; Cole et al. 2011). In contrast, autochthonous material may be the primary carbon source for zooplankton in oligotrophic lakes with strong deep chlorophyll maxima (Francis et al. 2011) as well as in nutrient-manipulated humic lakes (Pace et al. 2007). These alternative sources of carbon for pelagic food webs may be due in part to the fact that DOM contains nutrients that stimulate autochthonous primary production. This stimulation of the autotrophic food web by nutrients may be at least as important as stimulation of the heterotrophic microbial loop by the fixed organic carbon in alpine lakes. Here we show that DOM also results in enhanced trophic forcing that alters the producer species assemblage. These substantial changes in ecosystem structure and function are likely to influence alpine systems as climate change is increasing the elevation of tree line (Harsch et al. 2009) and hence the inputs of terrestrially derived DOM resource subsidies (Vinebrooke and Leavitt 1998; Sommaruga et al. 1999).

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References

American Public Health Association. 2000. Standard methods for the examination of water and wastewater, 20th ed. APHA.

Berggren, M., and others. 2010. Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. Ecol. Lett. **13:** 870–880, doi:10.1111/j.1461-0248.2010.01483.x

Brett, M. T., M. J. Kainz, S. J. Talpale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proc. Natl. Acad. Sci. USA **106:** 21197–21201, doi:10.1073/pnas.0904129106

Carpenter, S. R., and others. 2005. Ecosystem subsidies: Terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. Ecology **86:** 2737–2750, doi:10.1890/04-1282

Clerk, S., R. Hall, R. Quinlan, and J. P. Smol. 2000. Quantitative inferences of past hypolimnetic anoxia and nutrient levels from a Canadian Precambrian Shield lake. J. Paleolimnol. **23:** 319–336, doi:10.1023/A:1008147127606

Clow, D. W., C. Rhoades, J. Briggs, M. Caldwell, and W. M. Lewis. 2011. Responses of soil and water chemistry to mountain pine beetle induced tree mortality in Grand County, Colorado, USA. Appl. Geochem. **26:** S174–S178, doi:10.1016/j.apgeochem.2011.03.096

Cole, J. J., S. R. Carpenter, J. Kitchell, M. L. Pace, C. T. Solomon, and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proc. Natl. Acad. Sci. USA **108:** 1975–1980, doi:10.1073/pnas.1012807108

———, ———, M. L. Pace, M. C. Van de Bogert, J. L. Kitchell, and J. R. Hodgson. 2006. Differential support of lake food webs by three types of terrestrial organic carbon. Ecol. Lett. **9:** 558–568, doi:10.1111/j.1461-0248.2006.00898.x

Cooke, S. L., C. E. Williamson, B. R. Hargreaves, and D. P. Morris. 2006*a*. Beneficial and detrimental interactive effects of dissolved organic matter and ultraviolet radiation on zooplankton in a transparent lake. Hydrobiologia **568:** 15–28. , doi:10.1007/s10750-005-0006-y

———, ———, and J. E. Saros. 2006*b*. How do temperature, dissolved organic matter and nutrients influence the response of *Leptodiaptomus ashlandi* to UV radiation in a subalpine lake? Freshw. Biol. **51:** 1827–1837, doi:10.1111/j.1365-2427.2006.01618.x

Culver, D. A., M. M. Boucherle, D. J. Bean, and J. W. Fletcher. 1985. Biomass of freshwater crustacean zooplankton from length-weight regressions. Can. J. Fish. Aquat. Sci. **42:** 1380–1390, doi:10.1139/f85-173

Danielsdottir, M. G., M. T. Brett, and G. B. Arhonditsis. 2007. Phytoplankton food quality control of planktonic food web processes. Hydrobiologia **589:** 29–41, doi:10.1007/s10750-007-0714-6

DeMott, W. R., and A. J. Tessier. 2002. Stoichiometric constraints vs. algal defenses: Testing mechanisms of zooplankton food limitation. Ecology **83:** 3426–3433, doi:10.1890/0012-9658(2002)083[3426:SCVADT]2.0.CO;2

Faithfull, C., M. Huss, T. Vrede, J. Karlsson, and A.-K. Bergstrom. 2012. Transfer of bacterial production based on labile carbon to higher trophic levels in an oligotrophic pelagic system. Can. J. Fish. Aquat. Sci. **69:** 85–93, doi:10.1139/f2011-142

———, ———, ———, and A.-K. Bergström. 2011. Bottom-up carbon subsidies and top-down predation pressure interact to affect aquatic food web structure. Oikos **120:** 311–320, doi:10.1111/j.1600-0706.2010.18683.x

Fee, E. J., R. E. Hecky, S. E. M. Kasian, and D. R. Cruikshank. 1996. Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. Limnol. Oceanogr. **41:** 912–920, doi:10.4319/lo.1996.41.5.0912

Francis, T. B., D. E. Schindler, G. W. Holtgrieve, E. R. Larson, M. D. Scheuerell, B. X. Semmens, and E. J. Ward. 2011. Habitat structure determines resource use by zooplankton in temperate lakes. Ecol. Lett. **14:** 364–372, doi:10.1111/j.1461-0248.2011.01597.x

Frost, P. C., C. T. Cherrier, J. H. Larson, S. Bridgham, and G. A. Lamberti. 2007. Effects of dissolved organic matter and ultraviolet radiation on the accrual, stoichiometry and algal taxonomy of stream periphyton. Freshw. Biol. **52:** 319–330, doi:10.1111/j.1365-2427.2006.01696.x

Graneli, E., P. Carlsson, and C. Legrand. 1999. The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. Aquat. Ecol. **33:** 17–27, doi:10.1023/A:1009925515059

Harsch, M. A., P. E. Hulme, M. S. McGlone, and R. P. Duncan. 2009. Are treelines advancing? A global meta-analysis of treeline response to climate warming. Ecol. Lett. **12:** 1040–1049, doi:10.1111/j.1461-0248.2009.01355.x

Interlandi, S. J., S. S. Kilham, and E. C. Theriot. 1999. Responses of phytoplankton to varied resource availability in large lakes of the greater Yellowstone ecosystem. Limnol. Oceanogr. **44:** 668–682, doi:10.4319/lo.1999.44.3.0668

Kagami, M., N. R. Helmsing, and E. van Donk. 2011. Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. Hydrobiologia **659:** 49–54, doi:10.1007/s10750-010-0274-z

———, B. W. Ibelings, A. de Bruin, and E. Van Donk. 2005. Vulnerability of *Asterionella formosa* to *Daphnia* grazing: The impact of a fungal parasite. Verh. Int. Ver. Limnol. **29:** 350–354.

———, T. Yoshida, T. B. Gurung, and J. Urabe. 2002. Direct and indirect effects of zooplankton on algal composition in in situ grazing experiments. Oecologia **133:** 356–363, doi:10.1007/s00442-002-1035-0

Klug, J. L. 2002. Positive and negative effects of allochthonous dissolved organic matter and inorganic nutrients on phytoplankton growth. Can. J. Fish. Aquat. Sci. **59:** 85–95, doi:10.1139/f01-194

Lurling, M. 2003. Effects of microcystin-free and microcystin containing strains of the cyanobacterium *Microcystis aeruginosa* on growth of the grazer *Daphnia magna*. Environ. Toxicol. **18:** 202–211, doi:10.1002/tox.10115

Matthews, B., and A. Mazumder. 2006. Habitat specialization and the exploitation of allochthonous carbon by zooplankton. Ecology **87:** 2800–2812, doi:10.1890/0012-9658(2006)87[2800:HSATEO]2.0.CO;2

McKnight, D. M., R. Harnish, R. L. Wershaw, J. S. Baron and S. Schiff. 1997. Chemical characteristics of particulate, colloidal and dissolved organic material in Loch Vale watershed, Rocky Mountain National Park. Biogeochemistry **36:** 99–124, doi:10.1023/A:1005783812730

Milbrink, G., M. L. Kruse, and J. Bengtsson. 2003. Competitive ability and life history strategies in four species of *Daphnia*: *D. obtusa*, *D. magna*, *D. pulex* and *D. longispina*. Arch. Hydrobiol. **157:** 433–453, doi:10.1127/0003-9136/2003/0157-0433

Monteith, D. T., and others. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature **450:** 537–541, doi:10.1038/nature06316

Pace, M. L., and others. 2004. Whole lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427:** 240–243, doi:10.1038/nature02227

———, and others. 2007. Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? Limnol. Oceanogr. **52:** 2177–2189, doi:10.4319/lo.2007.52.5.2177

Raffa, K. F., B. H. Aukema, B. J. Bentz, A. L. Carroll, J. A. Hicke, M. G. Turner, and W. H. Romme. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: The dynamics of bark beetle eruptions. BioScience **58:** 501–517, doi:10.1641/B580607

Ramstack, J. M., S. C. Fritz, D. R. Engstrom, and S. A. Heiskary. 2003. The application of a diatom-based transfer function to evaluate regional water-quality trends in Minnesota since 1970. J. Paleolimnol. **29:** 79–94, doi:10.1023/A:1022869205291

Reid, J. W., and C. E. Williamson. 2010. Copepoda, p. 915–944. *In* J. H. Thorp and A. P. Covich [eds.], Ecology and classification of North American freshwater invertebrates. Academic Press.

Roman, M. R., and P. A. Rublee. 1980. Containment effects in copepod grazing experiments: A plea to end the black box approach. Limnol. Oceanogr. **25:** 982–990, doi:10.4319/lo.1980.25.6.0982

Rose, K. C., C. E. Williamson, J. E. Saros, R. Sommaruga, and J. M. Fischer. 2009. Differences in UV transparency and thermal structure between alpine and subalpine lakes: Implications for organisms. Photochem. Photobiol. Sci. **8:** 1244–1256, doi:10.1039/b905616e

Roush, W., J. S. Munroe, and D. B. Fagre. 2007. Development of a spatial analysis method using ground-based repeat photography to detect changes in the alpine treeline ecotone, Glacier National Park, Montana, U.S.A. Arct. Antarct. Alp. Res. **39:** 297–308, doi:10.1657/1523-0430(2007)39[297:DOASAM]2.0.CO;2

SanClements, M. D., G. P. Oelsner, D. M. McKnight, J. L. Stoddard, and S. J. Nelson. 2012. New insights into the source of decadal increases of dissolved organic matter (DOM) in acid-sensitive lakes of the Northeastern U.S. Environ. Sci. Technol. **46:** 3212–3219, doi:10.1021/es204321x

Sanders, R. W., C. E. Williamson, P. L. Stutzman, R. E. Moeller, C. E. Goulden, and R. Aoki-Goldsmith. 1996. Reproductive success of "herbivorous" zooplankton fed algal and nonalgal food resources. Limnol. Oceanogr. **41:** 1295–1305, doi:10.4319/lo.1996.41.6.1295

Saros, J. E., S. J. Interlandi, S. Doyle, T. Michel, and C. E. Williamson. 2005*a*. Are the deep chlorophyll maxima in alpine lakes primarily induced by nutrient availability, not UV avoidance? Arct. Antarct. Alp. Res. **37:** 557–563, doi:10.1657/1523-0430(2005)037[0557:ATDCMI]2.0.CO;2

———, ———, A. P. Wolfe, and D. R. Engstrom. 2003. Recent changes in the diatom community structure of lakes in the Beartooth Mountain Range, U.S.A. Arct. Antarct. Alp. Res. **35:** 18–23, doi:10.1657/1523-0430(2003)035[0018:RCITDC]2.0.CO;2

———, T. J. Michel, S. J. Interlandi, and A. P. Wolfe. 2005*b*. Resource requirements of *Asterionella formosa* and *Fragilaria crotonensis* in oligotrophic alpine lakes: Implications for recent phytoplankton community reorganizations. Can. J. Fish. Aquat. Sci. **62:** 1681–1689, doi:10.1139/f05-077

———, and others. 2010. Melting alpine glaciers enrich high-elevation lakes with reactive nitrogen. Environ. Sci. Technol. **44:** 4891–4896, doi:10.1021/es100147j

Scott, C. E., J. E. Saros, C. E. Williamson, S. C. Peters, and D. L. Mitchell. 2009. Effects of nutrients and dissolved organic matter on the response of phytoplankton to ultraviolet radiation: Experimental comparison in spring versus summer. Hydrobiologia **619:** 155–166, doi:10.1007/s10750-008-9608-5

Shurin, J. B., and others. 2010. Environmental stability and lake zooplankton diversity—contrasting effects of chemical and thermal variability. Ecol. Lett. **13:** 453–463, doi:10.1111/j.1461-0248.2009.01438.x

Sommaruga, R., R. Psenner, E. Schafferer, K. A. Koinig, and S. Sommaruga-Wograth. 1999. Dissolved organic carbon concentration and phytoplankton biomass in high-mountain lakes of the Austrian Alps: Potential effect of climatic warming on UV underwater attenuation. Arct. Antarct. Alp. Res. **31:** 247–253, doi:10.2307/1552253

Vinebrooke, R. D., and P. R. Leavitt. 1998. Direct and interactive effects of allochthonous dissolved organic matter, inorganic nutrients, and ultraviolet radiation on an alpine littoral food web. Limnol. Oceanogr. **43:** 1065–1081, doi:10.4319/lo.1998.43.6.1065

Weyhenmeyer, G. A., and J. Karlsson. 2009. Nonlinear response of dissolved organic carbon concentrations in boreal lakes to increasing temperatures. Limnol. Oceanogr. **54:** 2513–2519, doi:10.4319/lo.2009.54.6_part_2.2513

Williamson, C. E., W. Dodds, T. K. Kratz, and M. A. Palmer. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Front. Ecol. Environ. **6:** 247–254, doi:10.1890/070140

———, D. P. Morris, M. L. Pace, and O. G. Olson. 1999. Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. Limnol. Oceanogr. **44:** 795–803, doi:10.4319/lo.1999.44.3_part_2.0795

———, C. Salm, S. L. Cooke, and J. E. Saros. 2010. How do UV radiation, temperature, and zooplankton influence the dynamics of alpine phytoplankton communities? Hydrobiologia **648:** 73–81, doi:10.1007/s10750-010-0147-5

———, J. E. Saros, W. F. Vincent, and J. P. Smol. 2009. Lakes and reservoirs as sentinels, integrators, and regulators of climate change. Limnol. Oceanogr. **54:** 2273–2282, doi:10.4319/lo.2009.54.6_part_2.2273

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