Experimental whole-lake increase of dissolved organic carbon concentration produces unexpected increase in crustacean zooplankton density

PATRICK T. KELLY[1], NICOLA CRAIG[2], CHRISTOPHER T. SOLOMON[2], BRIAN C. WEIDEL[3], JACOB A. ZWART[1] and STUART E. JONES[1]

[1]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA, [2]Department of Natural Resource Sciences, McGill University, St. Anne de Bellevue, QC H9X 3V9, Canada, [3]U.S. Geological Survey, Great Lakes Science Center, Lake Ontario Biological Station, Oswego, NY 13126, USA

Abstract

The observed pattern of lake browning, or increased terrestrial dissolved organic carbon (DOC) concentration, across the northern hemisphere has amplified the importance of understanding how consumer productivity varies with DOC concentration. Results from comparative studies suggest these increased DOC concentrations may reduce crustacean zooplankton productivity due to reductions in resource quality and volume of suitable habitat. Although these spatial comparisons provide an expectation for the response of zooplankton productivity as DOC concentration increases, we still have an incomplete understanding of how zooplankton respond to temporal increases in DOC concentration within a single system. As such, we used a whole-lake manipulation, in which DOC concentration was increased from 8 to 11 mg L^{-1} in one basin of a manipulated lake, to test the hypothesis that crustacean zooplankton production should subsequently decrease. In contrast to the spatially derived expectation of sharp DOC-mediated decline, we observed a small increase in zooplankton densities in response to our experimental increase in DOC concentration of the treatment basin. This was due to significant increases in gross primary production and resource quality (lower seston carbon-to-phosphorus ratio; C:P). These results demonstrate that temporal changes in lake characteristics due to increased DOC may impact zooplankton in ways that differ from those observed in spatial surveys. We also identified significant interannual variability across our study region, which highlights potential difficulty in detecting temporal responses of organism abundances to gradual environmental change (e.g., browning).

Keywords: cyclopoid copepods, *Daphnia*, dissolved organic carbon, ecosystem manipulation, north temperate lakes, resource stoichiometry, zooplankton

Introduction

Surveys of freshwater systems across the Northern Hemisphere have identified a pervasive increase in dissolved organic carbon (DOC) concentrations, or "browning", over the past two decades. For example, between 1988 and 2003 the DOC concentration of the Hudson River doubled (Findlay, 2005) and 72% of surveyed lakes and streams in Scandanavia, the UK, and eastern North America have positive trends in DOC concentration since 1990 (Monteith *et al.*, 2007). Hypothesized mechanisms for this recent increase in DOC concentration via elevated terrestrial organic matter (t-OM) loads include changes in climate, nitrogen deposition, land-use change, and reduced sulfate deposition (Garnett *et al.*, 2000; Freeman *et al.*, 2004; Findlay, 2005; Monteith *et al.*, 2007). While all of these

mechanisms may be important at particular locations or time scales, the leading hypothesis for the observed decadal-scale increases in DOC concentration is reductions in atmospheric sulfate deposition from industrial regulations. This decrease in sulfate deposition and corresponding recovery from acidification has potentially led to large-scale increases in soil pH and a reduction in multivalent ions reducing the ionic strength of soils, and subsequently an increase in DOC solubility (Monteith *et al.*, 2007; Clark *et al.*, 2010). Although research continues on the hypothesized drivers of DOC increases, the response of lake food webs to this global change is of major importance, yet has received much less attention.

Increases in DOC concentration could have strong negative effects on lake food web productivity. Terrestrially derived DOC is dominated by humic substances (Jones, 1992). These high-molecular weight compounds attenuate light, which can influence the

Correspondence: Patrick T. Kelly, tel./fax 513 529-3628, e-mail: kellypt2@miamioh.edu

heat distribution in the water column (Read & Rose, 2013) and reduce mixed-layer depth (Houser, 2006). Shading from chromophoric DOC can also significantly reduce autotroph biomass and primary productivity through light limitation (Ask *et al.*, 2009; Jones *et al.*, 2012; Godwin *et al.*, 2014). Finally, the high-molecular weight and complex chemical structure of these compounds suggests that they are recalcitrant in nature, but indeed consumed by heterotrophic bacteria (Tranvik, 1988; Berggren *et al.*, 2010).

Increases in DOC concentration also impact lakes in ways that may hypothetically lead to increases in consumer productivity. Terrestrially derived organic carbon may support >50% of consumer biomass in some lakes (Cole *et al.*, 2011; Solomon *et al.*, 2011; Karlsson *et al.*, 2012), potentially indicating it serves as a subsidy for consumer production. Although poor nutritional composition of terrestrial material suggests the direct subsidy of consumers is unlikely (Brett *et al.*, 2009; Kelly *et al.*, 2014), indirect impacts of greater DOC concentration may increase the availability and nutrient and biochemical quality of basal resources. Increased DOC may directly stimulate greater bacterial production, which may serve as a significant source of energy for zooplankton (Hessen & Anderson, 1990). Additionally, loads of DOC-associated nutrients (Lennon & Pfaff, 2005) may reduce nutrient limitation for primary producers and bacteria, increasing food availability for zooplankton (Cottingham & Narayan, 2013). Light attenuation from elevated DOC concentration, in addition to inputs of DOC-associated nutrients may influence the light-to-nutrient ratio, thereby altering autochthonous basal resource stoichiometry, and decreasing the ratio of carbon-to-phosphorus in the seston (Sterner *et al.*, 1997). There is also evidence for an increase in essential fatty acid content of basal resources in lakes with higher DOC concentration (Gutseit *et al.*, 2007), potentially reducing fatty acid limitation of consumers.

To date, understanding of the implications of lake browning for aquatic consumers is drawn from spatial surveys of lakes that span broad gradients of DOC concentrations (Karlsson *et al.*, 2009, 2015; Kelly *et al.*, 2014; Craig *et al.*,2015). Yet previous work in ecosystem ecology suggests that patterns observed in comparative studies do not always translate to ecosystem change through time (Kratz *et al.*, 2003). Because a multitude of other factors covary with DOC in these cross-lake surveys (e.g., hydrologic setting, nutrient loads, and lake size), space may not adequately substitute for time, and we remain uncertain about how increasing DOC will influence consumer biomass and production in a given lake (Solomon *et al.*, 2015). This uncertainty necessitates the use of whole-ecosystem experiments to identify the

temporal dynamics of ecosystem change. We assessed how adult crustacean zooplankton responded to an increase in lake DOC concentration using a whole-lake ecosystem manipulation in which DOC was experimentally increased in one basin of a divided lake. Our manipulation also allowed us to explore the effect of interannual variation in weather on our ability to detect gradual responses of zooplankton density to global change processes. Despite meaningful interannual variation in weather across our experiment, we detected a weak positive response of zooplankton density to increased DOC concentration, which contrasted strongly with the large decline in zooplankton density that would have been predicted based on previous comparative studies along DOC gradients.

Materials and methods

Lake manipulation experiment

We conducted a whole-lake ecosystem manipulation experiment in Long Lake at the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan, USA (89°32′W, 46°13′N). The purpose of the experiment was to observe the impact of increased DOC concentrations on lake food webs. Long Lake is an 8.1 ha seepage lake with a mean depth of 3.8 m and a maximum depth of 14 m occurring at both ends of an hourglass-shaped lake. A previous experiment observed the the DOC concentration increased in the eastern basin of Long Lake after the lake was divided with a plastic cruatin as part of a food web manipulation experiment (Carpenter *et al.*, 1995; Christensen *et al.*, 1996).

We used a Before-After Control-Impact (BACI) design to assess the effect on the lake food web of an increase in DOC concentration. We initiated the experiment in May 2011, installing a 1/16th inch nylon mesh curtain (Memphis Net & Twine Co., Inc., Memphis, TN, USA) to divide the lake into an eastern "treatment" basin and a western "reference" basin of approximately equal size and bathymetry (3.18 and 4.87 ha, respectively, with mean depth of approximate 4 m and max depth of approximately 14 m in each basin). We monitored the zooplankton populations and other food web members of each basin during the summers of 2011 and 2012. Then, in September 2012, we replaced the permeable mesh curtain with an impermeable plastic curtain made from a continuous piece of high-density polyester weave (Curry Industries, Ltd., Winnipeg, MB, Canada). The impermeable curtain was weighed with sand bags on the lake sediments and extended approximately 30 cm above the lake surface by floats. The curtain was extended at least 15 m onshore to ensure lake division with fluctuating water levels. Because the vast majority of the DOC load (but a small fraction of the hydrologic load) to the lake enters via a small intermittent inlet to the treatment basin (mean stream DOC concentration of 75.0 mg C L^{-1}), installing the plastic curtain concentrated the DOC load in the treatment basin and increased the DOC concentration there from ~8 mg L^{-1} premanipulation to ~11 mg L^{-1} during the first

year postmanipulation and thereafter (Fig. 1). Concurrently, there was a smaller decline in DOC concentration in the reference basin from approximately 8 to 6 mg L^{-1}. After installing the plastic curtain, we continued to monitor the zooplankton populations and the rest of the food web of each basin for an additional two summer seasons (2013 and 2014). Premanipulation, the treatment and reference basins were chemically similar with identical food web structure. The primary planktivore in Long Lake is the invertebrate predator *Chaoborus*, because abundant mature *Micropterus salmoides* (largemouth bass) maintain low minnow and young-of-year bass densities.

Sample collection

Zooplankton samples were collected each week from late May through mid-August in all four years of the experiment. Duplicate vertical tows were taken with an 80 μm mesh zooplankton net (Aquatic Research Instruments, Hope, ID, USA) from 2 m above the sediments to the surface at the approximate deepest location of each basin. Tows were combined and preserved with either Lugol's solution (2011 in both basins) or 70% ethanol (2012–2014 in both basins) immediately after sampling. Although a majority of the water column was sampled, it is possible that *Chaoborus* were under-sampled using this method as they may be close to sediments during the daytime. However, this method was used consistently across both basins, therefore we feel as though it would not affect the interpretation of the results. Integrated water samples were taken from the upper mixed layer for analysis of water chemistry characteristics and bacterial production. Total phosphorus was measured following persulfate digestion using a colorimeteric assay (Menzel & Corwin, 1965) and dissolved organic carbon was analyzed on filtrate after passing through a 0.7 μm GF/F filter, using a Shimadzu TOC-V total organic carbon analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). Seston carbon-to-phosphorus stoichiometry was determined by capturing particulate matter from approxi-
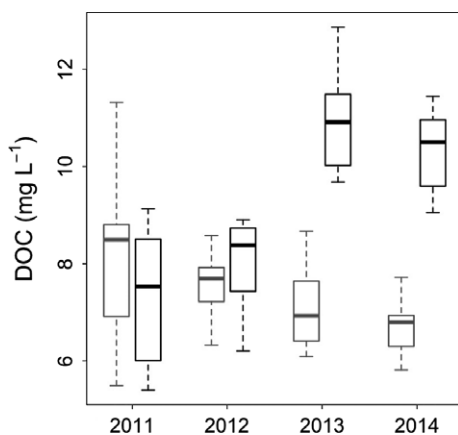
mately 200 mL of water from the upper mixed layer on precombusted 0.7 μm GF/F filters. Particulate organic matter was analyzed for carbon content using an elemental analyzer (Costech, Valencia, CA, USA). Prior to particulate phosphorus analysis, filters were combusted at 450°C for 2 h to remove excess carbon. Combusted filters were combined with 30 mL of milli-reverse osmosis water, and analyzed colorimetrically after persulfate digestion (Menzel & Corwin, 1965). Bacterial production was assayed in 2011–2013, but not 2014, following the ^{3}H leucine microcentrifuge method (Smith & Azam, 1992) on water from the upper mixed layer. Water temperature profiles were also measured in each basin using a fixed temperature chain (Onset HOBO pendants; Onset Computer Corporation, Bourne, MA, USA), and meteorological data were taken from a floating platform on the reference basin (Onset HOBO Met station; Onset Computer Corporation). Dissolved oxygen (DO) was measured in each basin at a fixed depth (0.7 m) in the epilimnion at 10-minute intervals using a DO sonde (YSI 6600 V2 Sonde; YSI Incorporated, Yellow Springs, OH, USA). We estimated rates of GPP by fitting a maximum likelihood metabolism model to the high frequency DO cycles as described by Solomon *et al.* (2012). Mixed-layer depth (Z_{mix}) was calculated as the shallowest depth at which the temperature gradient exceeded 1°C m^{-1} (Fee *et al.*, 1996) in weekly temperature profiles measured with a DO/temperature profiler (Yellow Springs Instruments, Yellow Springs, OH, USA).

Zooplankton density estimation

Adult crustacean zooplankton were subsampled and counted according to the following taxonomic classifications: calanoid copepods, cyclopoid copepods, *Daphnia spp.*, *Holopedium gibberum*, and *Bosmina spp.* using a Bogorov counting tray and stereomicroscope. One thousand individuals, or all individuals if less than one thousand were present, were counted from each sample. Subsamples were extrapolated to estimate whole-sample abundance of each taxon. We did not classify zooplankton by developmental stages, rather copepod nauplii were excluded from the analysis and copepodites were classified as "adult" cyclopoid copepods.

Data analysis

We used a Welch *t* test to compare differences in zooplankton density in paired sampling events during a pre- and postmanipulation period in the treatment and reference basin. Unlike randomized intervention analysis, the Welch *t* test is robust to large differences in means and variances in the pre- vs. postmanipulation periods, as was seen in our experiment, and therefore has been recommended for use in whole-ecosystem manipulation experiments (Stewart-Oaten *et al.*, 1992; Stewart-Oaten, 1996; Stewart-Oaten & Bence, 2001). In addition to total zooplankton density, a Welch *t* test was also used to identify statistically significant changes in taxon-specific (*Daphnia*, *Holopedium*, and cyclopoid copepods) density in response to our DOC treatment, as well as for changes in zooplankton community composition (% Cladocera). Finally, we tested for

Fig. 1 Boxplot of annual epilimnetic dissolved organic carbon concentration in the reference (grey boxes) and treatment (black boxes) basins (measure weekly from May through September). Boxplot gives the 5th, 25th, 50th, 75th, and 95th percentile of the distribution of lake DOC concentration.

changes in environmental covariates that may have been impacted by the DOC treatment and that were hypothesized to impact zooplankton denisties directly, including particulate C:P, GPP, Z_{mix}, and chlorophyll *a* concentration (see Zwart *et al. in press* for discussion of GPP and Z_{mix}). Weekly zooplankton, chlorophyll *a*, and C:P data and difference between basins were not consistently temporally autocorrelated (Table S1), and all were normally distributed after log transformation.

To identify potential proximate mechanisms for observed changes in zooplankton density in response to our DOC manipulation, we compared the seasonal mean of weekly differences in density between the treatment and the reference basins to the seasonal mean of weekly differences in environmental covariates between the treatment and the reference basin (GPP, C:P, chlorophyll *a*, *Chaoborus* biomass, bacterial production, and Z_{mix}). Comparing the mean differences is directly analogous to the Welch *t* test results and removes the effect of interannual climatic differences.

We observed substantial interannual variation in zooplankton densities across the experiment. We hypothesized these may have resulted from changes in weather patterns or ice-off differences among years. As such, we compared zooplankton densities to average air temperature and precipitation data taken from meteorological data for Land O'Lakes, WI and ice-off dates from nearby Sparkling Lake, a focal site of the North Temperate Lakes LTER (North Temperate Lakes LTER: Ice duration – Trout Lake Area 1981 – current).

Because the reference basin declined slightly in DOC concentration in response to the manipulation, we also compared our treatment and reference basins to a set of seven lakes in the region for which there was monthly zooplankton data over the experimental period. Zooplankton densities were taken from the seven northern lakes in the North Temperate Lakes Long Term Ecological Research (NTL-LTER) dataset for 2011–2014 (North Temperate Lakes LTER: Zooplankton – Trout Lake Area 1982 – current). We filtered data to use similar classifications for adult crustacean zooplankton, excluding rotifers and copepod nauplii from the analyses. As LTER data were monthly rather than weekly, for this analysis we reduced our observations from Long Lake to monthly using data from the sample dates closest to sample dates of the NTL-LTER lakes. To compare our treatment response to the NTL-LTER lakes, we performed a Welch *t* test on the difference between the monthly zooplankton densities in the treatment basin and the mean monthly densities of the NTL-LTER (Stewart-Oaten & Bence, 2001).

In addition to providing additional references for our treatment basin, the NTL lakes allowed us to explore the detectability of subtle directional change in zooplankton densities in the context of heterogeneous responses to regional drivers, such as interannual weather variation. Previous theoretical work has highlighted the importance of similar response to regional drivers between treatment and reference ecosystems, or coherence, for detection of whole-ecosystem experimental treatment or gradual long-term change (Carpenter *et al.*, 1998; Osenberg *et al.*, 2006). We quantified overall coherence of zooplankton densities amongst lakes in the

region using the interclass correlation coefficient (r_i; Rusak *et al.* 1999; Rusak *et al.*, 2008), calculated as:

$$r_i = \frac{MS_m - MS_E}{MS_m + (n-1)MS_E} \quad (1)$$

where MS_m and MS_E are the mean squares for month-year and error, respectively, from a two-way ANOVA without replication. Zooplankton abundances were z-scored to zero mean and standard deviation of one for the analysis.

We were also interested in the importance of coherence between each individual reference lake and the treatment basin in detecting change in zooplankton densities. We used the Pearson's correlation coefficient (*r*) for monthly abundances in the treatment basin compared to each reference lake as our measure of coherence. To assess the effect of coherence on detectability of gradual change in zooplankton densities, we compared our measure of coherence to *p*-values of the Welch *t* tests for each treatment-reference pair. All statistical analyses were performed with R statistical software (R Core Development Team, 2015).

Results

Zooplankton responses to temporal change in DOC

Total zooplankton density increased in the treatment basin relative to the reference in response to our whole-lake DOC manipulation (Welch's $t = -2.97$, df = 30.15, $P = 0.005$; Fig. 2). Total zooplankton density was 36% greater in the reference basin in the two premanipulation years (mean in treatment = 54997 ind. m^{-2} and reference = 86242 ind. m^{-2}; Table S2), but 12% greater in the treatment basin postmanipulation (mean in treatment = 15174 ind. m^{-2} and reference = 13250 ind. m^{-2}; Table S2). While zooplankton densities were greater in the treatment basin relative to the reference basin postmanipulation, zooplankton densities decreased more than 80% in both basins between the pre- and postmanipulation years. This decrease was associated with lower spring temperatures (mean 0°C in 2011–2012 and −4°C in 2013–2014) and later ice-off dates (day of year 115 and 79 in 2011 and 2012; 129 and 127 in 2013 and 2014).

The DOC-mediated increase in total zooplankton density in the treatment compared to the reference was largely due to increased *Daphnia* and cyclopoid copepod densities (Fig. 2). However, there was a similar increase in *Holopedium* in 2014 (Fig. 2). Despite high week-to-week variability in area-specific density of individual taxa and total zooplankton, the observed increases in the treatment basin were marginally significant for both *Daphnia* ($t = -1.99$, df = 40.08, $P = 0.05$; Table S2) and cyclopoids (Welch's $t = -1.90$, df = 39.70, $P = 0.06$; Table S1), but less so for *Holopedium* (Welch's $t = -1.68$, df = 21.95, $P = 0.10$; Table S2). There was no significant change in percent Cladocera between the
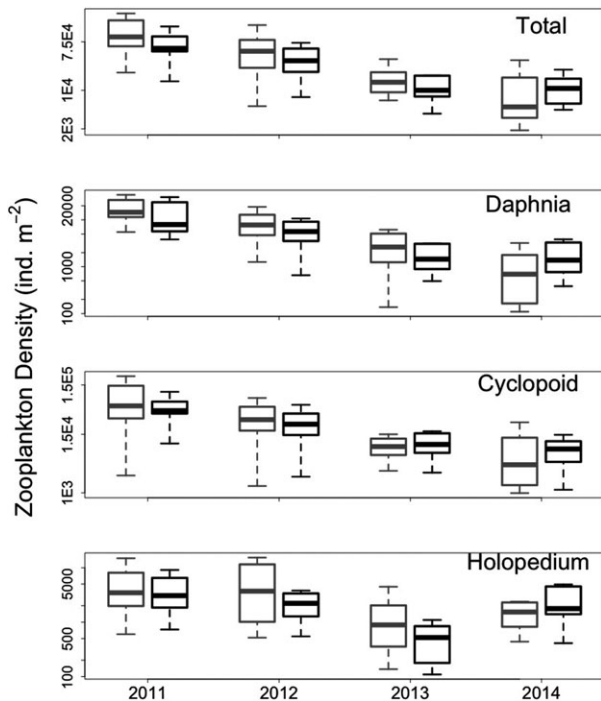
Fig. 2 Boxplots of annual zooplankton densities in the reference (grey boxes) and treatment basins (black boxes) across the experiment (measured weekly from May through September). The boxplots give the 5th, 25th, 50th, 75th, and 95th percentile of the distribution of zooplankton density for each year. Outliers (points outside 1.5 times the interquartile range) were excluded from the plots ($N = 48$ across the entire study).

basins in the pre- vs. postmanipulation years (Welch's $t = -0.07$, df = 39.97, $P = 0.94$).

Drivers of zooplankton biomass response

Hypothesized proximate drivers of zooplankton density also responded strongly to the DOC manipulation (Fig. 3; means ± SE in Table S3). The increase in DOC had resource- and nonresource-mediated impacts on the lake, as seston carbon-to-phosphorus stoichiometry was significantly lower in the treatment basin relative to the reference (Welch's $t = 2.66$, df = 42.02, $P = 0.01$; Fig. 3a), while GPP (Welch's $t = -6.60$, df = 403.67, $P < 0.001$; Fig 3b; Zwart *et al. in press*) and chlorophyll *a* concentration significantly increased (Welch's $t = -2.89$, df = 50.86, $P = 0.005$; Fig. 3c). Lower treatment basin seston C:P postmanipulation was due primarily to increased concentrations of particulate phosphorus (Welch $t = -3.40$, $P = 0.001$; Fig. 4c), not to decreases in particulate carbon concentrations (Welch's $t = -1.36$, df = 50.04, $P = 0.19$; Fig. 4a). Additionally, the DOC manipulation significantly reduced mixed-layer depth by approximately 0.3 m (Welch's $t = 77.94$, df = 34629.05, $P < 0.001$; Zwart *et al. in press*, Fig. 3d). There

was no change in bacterial production in response to the manipulation (Welch t test $t = -0.79$, df = 29.92, $P = 0.43$ for two premanipulation years and one post manipulation year; Fig. S1); this response variable was not measured in the final postmanipulation year. In general, *Chaoborus* biomass was variable across basin-years, declining in both basins in 2013 and 2014 compared to premanipulation years (Fig. 3e). *Chaoborus* biomass did not systematically vary with the DOC manipulation (Welch's $t = -0.79$, df = 38.54, $P = 0.43$), but was greatest in the reference basin in 2011–2013, and more similar between basins in 2014.

The difference in weekly zooplankton density between the treatment basin and the reference basin was associated with greater differences in gross primary production and chlorophyll *a*, and lower seston C:P in the treatment relative to the reference (Fig. 5a–c). There was no pattern between the difference in zooplankton biomass and the difference in Z_{mix} or *Chaoborus* abundance between basins (Fig. 5d,e).

Impact of coherence on detectability of change

Amongst all reference lakes, coherence of monthly zooplankton densities was moderate ($r_i = 0.29$). Perhaps owing to this moderate coherence of the references and/or reduced statistical power as a result of using monthly observations, the positive effect of our DOC manipulation on treatment basin zooplankton densities was not statistically significant when compared to all eight reference systems (reference basin + NTL-LTER lakes; Welch's $t = -1.21$, df = 13.45, $P = 0.24$). We did, however, observe a positive effect of coherence between a specific reference system and the treatment basin on the detectability of the subtle positive response in zooplankton abundance to the manipulation (Fig. 6), with the reference basin having the highest estimate of coherence in monthly zooplankton densities compared to the treatment ($r = 0.94$). Furthermore, regardless of whether we used the reference basin of Long or one of the NTL-LTER lakes as the statistical reference, the comparison with the treatment basin of Long always indicated either a significant increase or no significant change in zooplankton density in the treatment basin. This contrasted with the significant decrease in treatment basin zooplankton density that we expected based upon previous comparative studies (Kelly *et al.*, 2014).

Discussion

We used a whole-lake manipulation to test the expectation, derived from surveys of lakes with different DOC concentrations, that temporal increase of the DOC concentration in a single lake would significantly reduce
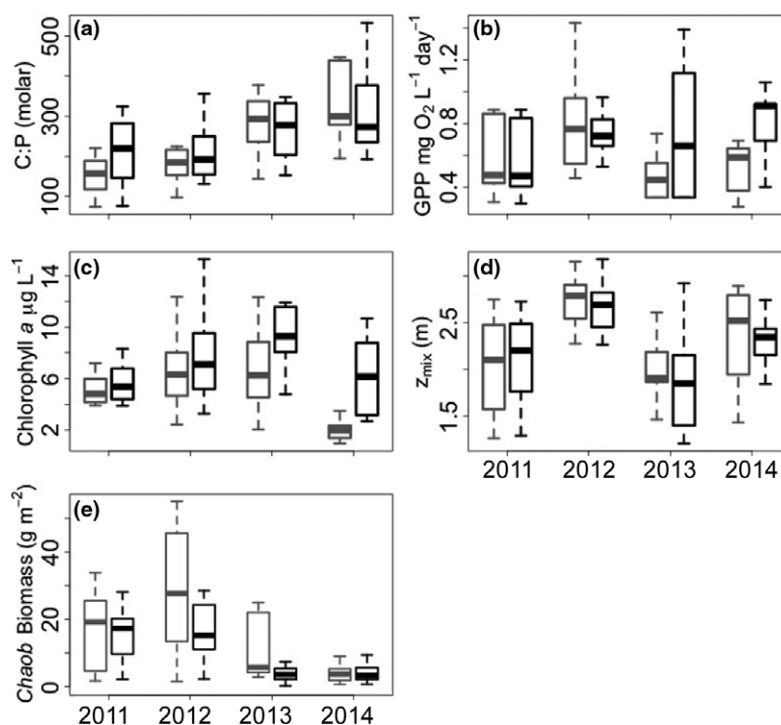
Fig. 3 Boxplots of annual (a) seston carbon-to-phosphorus stoichiometry, (b) gross primary production, (c) chlorophyll a concentration, (d) mixed layer depth, and (e) Chaoborus in the DOC treatment basin (black boxes) and reference basin (grey boxes); response variables were measured weekly from May through September, except primary production, which was measured daily. The boxplots give the 5th, 25th, 50th, 75th, and 95th percentile of the distribution of drivers for each year.
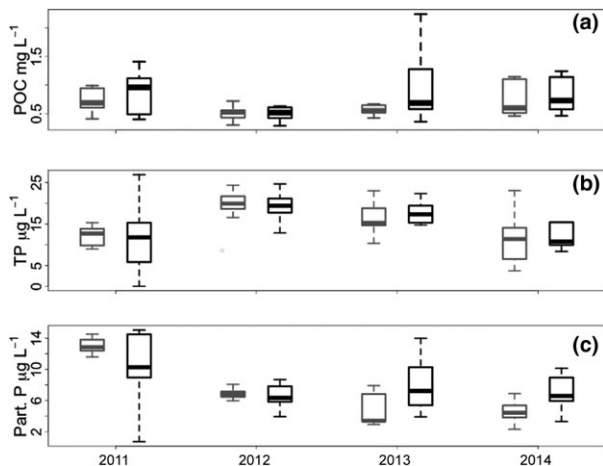

Fig. 4 Boxplots of annual (a) particulate organic carbon (POC), and (b) total (TP) and (c) particulate phosphorus concentrations in the treatment basin (black boxes) and the reference basin (grey boxes) showing medians and quantiles (measured weekly from May through September). The boxplots give the 5th, 25th, 50th, 75th, and 95th percentile of this distribution for each year.

zooplankton density (Kelly *et al.*, 2014). Surprisingly, we observed a small increase in zooplankton density in the treatment basin relative to the reference in response to increasing DOC concentration, which sharply contrasted with the significant decline expected from a previous comparative survey (Kelly *et al.*, 2014). This increase in zooplankton density in response to the manipulation appeared to be due to increased phosphorus supply owing to DOC-associated phosphorus (Lennon & Pfaff, 2005; Cottingham & Narayan, 2013), which enhanced primary production and resource quality (carbon-to-phosphorus stoichiometry) in the treatment basin.

Response of zooplankton to elevated DOC

Greater zooplankton density in the treatment basin was associated with an increase in GPP and chlorophyll *a*. Greater primary production has been repeatedly shown to stimulate herbivore production (Coe *et al.*, 1976; McNaughton *et al.*, 1989), especially in systems controlled by bottom-up processes (McCauley & Kalff, 1981). In our experiment, greater GPP in the treatment basin relative to the reference postmanipulation seems to be related to an increase in phosphorus concentration in the postmanipulation years in the treatment basin (Welch's $t = -2.17$, $P = 0.035$). Since there is often DOC and TP co-export from the terrestrial landscape (e.g., Dillon & Molot, 1997, 2005), increased stream water and solute load to a lake will likely result in increased
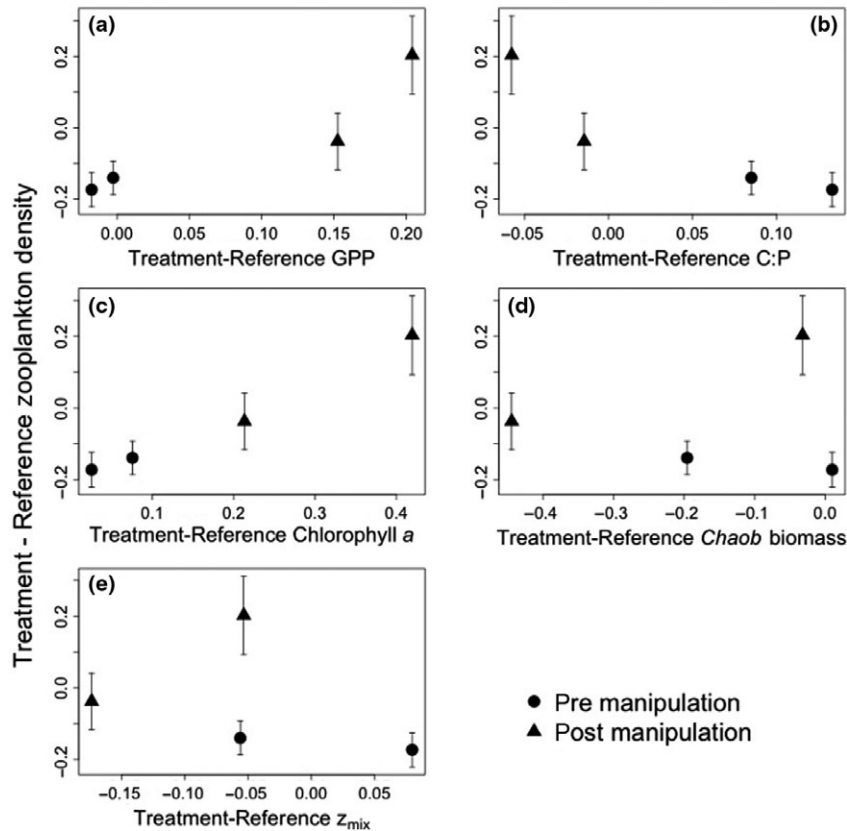
Fig. 5 Scatter plots of the mean difference in log-transformed biomass between the treatment and the reference (treatment-reference) and the mean difference in log-transformed environmental covariates that were hypothesized to impact zooplankton biomass in response to the manipulation. (a) GPP, (b) seston C:P, (c) Chlorophyll a, (d) Chaoborus biomass, and (e) mixed layer depth.

DOC and TP concentrations. Despite increased light attenuation by the increased DOC, the light climate of the treatment and reference basins remained identical. This was because of the reduced mixed-layer depth in the treatment basin, resulting in essentially no change in the average amount of light in the upper mixed layer (Fee *et al.*, 1996; Jones, 1998; Zwart *et al.*, in press).

In addition to chlorophyll *a* and GPP stimulating greater zooplankton densities in the treatment basin, nutritional quality of resources increased in response to the DOC manipulation. Similar to increases in chlorophyll *a* and GPP, this relative decrease in seston C:P was also likely due to the greater total phosphorus concentration in the treatment basin. In aquatic systems, resource phosphorus concentration is tightly coupled to zooplankton growth and reproduction, suggesting that phosphorus limitation in these systems may regulate zooplankton biomass (Hessen, 2008). As phytoplankton carbon:nutrient stoichiometry can vary depending on nutrient availability (nonhomeostatic), zooplankton are often faced with resources that are stoichiometrically unbalanced (Urabe & Sterner, 1996;

Sterner *et al.*, 1997). Consumers using resources beyond these nutritional thresholds face stoichiometric constraints (approximate 230–300 for *Daphnia*; Urabe & Watanabe, 1992; Anderson & Hessen, 2005), needing to cope with excess carbon through excretion, molting, or respiration (Jensen & Hessen, 2007; He & Wang, 2008). Distance from the nutritional threshold is also critical as increasing seston C:P ratios will increasingly diminish zooplankton growth and reproduction. Therefore, although the difference in C:P was slight, the lower C:P in the treatment basin (306 vs. 329, on average) likely led to reduced stoichiometric constraints and contributed to increased zooplankton densities.

Other hypothesized drivers of zooplankton density changed throughout the experiment, but were either apparently unimportant in structuring zooplankton density, or difficult to evaluate. Kelly *et al.* (2014) suggested reductions in mixed-layer depth were the likely determinant of zooplankton productivity across a DOC gradient. Although there was a significant reduction in mixed-layer depth in the treatment basin compared to the reference after the manipulation, it may
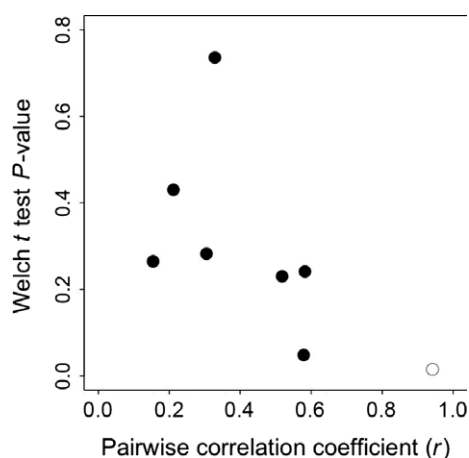
Fig. 6 Scatter plot of the Pearson's correlation coefficient (r) for monthly comparisons between treatment basin and reference systems (NTL-LTER lakes and reference basin) and the *P* value from Welch t tests for mean monthly differences in zooplankton density pre- and post-manipulation. Open circle represents *P* value of Welch t test comparing monthly differences between treatment and reference basin, and closed circles represent the NTL-LTER lakes.

have been offset by the concomitant changes in GPP and reduced nutritional constraints. Food web structure can be a strong determinant of both zooplankton density and community structure (Wellborn *et al.*, 1996; Carpenter *et al.*, 2001; Jansson *et al.*, 2010), and past research suggests changing water color can influence the relative importance of predation by the invertebrate predator *Chaoborus* (Boeing *et al.*, 2004). However, the relationship between *Chaoborus* biomass and zooplankton density was generally positively correlated within both basins. Additionally, there was no systematic relationship between yearly differences in *Chaoborus* biomass and zooplankton density (Fig. 4). In fact, zooplankton densities were higher in the reference basin in 2011 and 2012 despite greater *Chaoborus* biomass, suggesting predators may have had comparatively less impact on the difference in total zooplankton density between basins than resource-mediated influences. While it is possible *Chaoborus* densities are under-estimated given daytime sampling, sampling methods were consistent across basins and we therefore do not expect any bias toward one basin or the other. Additionally, high concentrations of UV radiation may alter zooplankton community composition and reduce biomass (Williamson *et al.*, 1999; Marinone *et al.*, 2006). While increases in water color and light extinction from DOC in the treatment basin may have reduced UV penetration through the water column, the initial DOC concentration of Long Lake attenuates sufficient UV radiation at the surface as to not significantly impact zooplankton (Williamson *et al.*, 1996).

Detecting gradual change in the context of variable temporal coherence among lakes

We know from previous work that weather can play a significant role in yearly zooplankton dynamics, although the direction and magnitude of the response appears to be variable. Changes in weather have been demonstrated to have a significant effect on plankton phenology and life histories (Adrian *et al.*, 1999, 2006; George & Hewitt, 2005), synchrony among lakes (Rusak *et al.*, 2008), as well as biomass and abundance (Preston & Rusack, 2010). Evidence suggests that weather patterns driving water temperature and thermal stratification of lakes are particularly important at key times throughout the growing season (Huber *et al.*, 2010), and data from our experiment indicate cold spring and late ice-off dates may generally reduce baseline abundance and annual productivity (Fig. 1). However, there is also evidence that this effect of weather can be decoupled across lakes, with differences in trophic status, plankton communities, and predator regimes having a significant influence on the relative role of weather in shaping zooplankton abundance in a given ecosystem (Anneville *et al.*, 2010; Fig. S2). As such, significant variability in lake zooplankton responses to interannual variability in weather within and across systems presents significant challenges for detecting subtle ecological responses to long, slow environmental changes like lake browning.

Additional zooplankton density data from lakes in our region (NTL-LTER lakes) allowed us to evaluate the effect of temporal coherence in response to variation in interannual weather on the detectability of subtle changes in zooplankton density. The moderate coherence of monthly zooplankton density from the NTL-LTER lakes and our treatment basin made the statistical detection of a significant treatment effect, and likely gradual change in zooplankton density over time, difficult (Stewart-Oaten, 1996; Stewart-Oaten & Bence, 2001) As such, our results provide empirical support for the theoretical assumption that coherence between reference and treatment systems is a prerequisite for detection of subtle and gradual ecological changes (Stewart-Oaten, 1996; Stewart-Oaten & Bence, 2001). This inference was further supported by the negative correlation we observed between coherence and detectability amongst the set of eight reference systems (Fig 6). However, it is most important to note that despite wide variation in the degree of temporal coherence in zooplankton densities amongst our treatment-reference pairs, all reference systems indicated a weak positive response or no change in zooplankton density to our DOC manipulation, which starkly contrasts with expectations set by recent comparative studies along DOC gradients (Kelly *et al.*, 2014).

Spatial vs. temporal responses of zooplankton to increased DOC concentration

Although the positive response of zooplankton density to our DOC treatment was subtle and difficult to detect in the context of weak coherence amongst lakes in the region, the response was tremendously significant from an ecological perspective. The response of zooplankton to our DOC manipulation contrasted strongly with what was observed in a spatial comparison of lakes that varied in DOC concentration (Kelly *et al.*, 2014). Using the relationship between DOC and zooplankton densities from Kelly *et al.* (2014), we would expect an approximate 22% decrease in *Daphnia* and copepod densities with an increase from 8 to 11 mg L^{-1} in DOC concentration. Additionally, due to the decline in DOC concentration in the reference basin from 8 to 6 mg L^{-1}, we would have expected an approximate 12% increase in zooplankton density in the reference basin, and an even wider gap (34%) between the treatment and reference basin after the manipulation. Therefore, although the greater taxon-specific zooplankton densities in the treatment basin were marginally significant, the null expectation was for a significant decline in taxa-specific and total zooplankton in response to the DOC increase. Our results suggest that a more nuanced view of the effects of DOC increase on aquatic consumers is required. Further, we will require conceptual and quantitative models that simultaneously consider the negative (shading, habitat reduction, etc.) and positive (associated mineral nutrients, bacterial resource, etc.) effects of DOC on zooplankton to make robust predictions about the impact of spatiotemporal variation in DOC on lake food webs.

Acknowledgements

References

Adrian R, Walz N, Hintze T, Hoeg S, Rusche R (1999) Effects of ice duration on plankton succession during spring in a shallow polymictic lake. *Freshwater Biology*, **41**, 621–632.

Adrian R, Wilhelm S, Gerten D (2006) Life-history traits of lake plankton species may govern their phenological response to climate warming. *Global Change Biology*, **12**, 652–661.

Anderson TR, Hessen DO (2005) Threshold elemental ratios for carbon versus phosphorus limitation in Daphnia. *Freshwater Biology*, **50**, 2063–2075.

Anneville O, Molinero JC, Souissi S, Gerdeaux D (2010) Seasonal and interannual variability of cladoceran communities in two peri-alpine lakes: uncoupled response to the 2003 heat wave. *Journal of Plankton Research*, **32**, 913–925.

Ask J, Karlsson J, Persson L, Ask P, Byström P, Jansson M (2009) Terrestrial organic matter and light penetration: effects on bacterial and primary production in lakes. *Nature*, **54**, 2034–2040.

Berggren M, Ström L, Laudon H *et al.* (2010) Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. *Ecology Letters*, **13**, 870–880.

Boeing WJ, Leech DM, Williamson CE, Cooke S, Torres L (2004) Damaging UV radiation and invertebrate predation: conflicting selective pressures for zooplankton vertical distribution in the water column of low DOC lakes. *Oecologia*, **138**, 603–612.

Brett MT, Kainz MJ, Taipale SJ, Seshan H (2009) Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 21197–21201.

Carpenter SR, Christensen DL, Cole JJ *et al.* (1995) Biological control of eutrophication in lakes. *Environmental Science and Technology*, **29**, 784–786.

Carpenter SR, Cole JJ, Essington TE, Hodgson JR, Houser JN, Kitchell JF, Pace ML (1998) Evaluating alternative explanations in ecosystem experiments. *Ecosystems*, **1**, 335–344.

Carpenter SR, Cole JJ, Hodgson JR *et al.* (2001) Trophic cascades, nutrients, and lake productivity: whole-lake experiments. *Ecological Monographs*, **71**, 163–186.

Christensen DL, Carpenter SR, Cottingham KL *et al.* (1996) Pelagic responses to changes in dissolved organic carbon following division of a seepage lake. *Limnology and Oceanography*, **41**, 553–559.

Clark JR, Bottrell S, Evans CD *et al.* (2010) The importance of the relationship between scale and process in understanding long-term DOC dynamics. *Science of the Total Environment*, **408**, 2768–2775.

Coe MJ, Cumming DH, Phillipson J (1976) Biomass and production of large African herbivores in relation to rainfall and primary production. *Oecologia*, **22**, 341–354.

Cole JJ, Carpenter SR, Kitchell J, Pace ML, Solomon CT, Weidel B (2011) Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 1975–1980.

Cottingham KL, Narayan L (2013) Subsidy quantity and recipient community structure mediate plankton responses to autumn leaf drop. *Ecosphere*, **4**, 1–18.

Craig N, Jones SE, Weidel BC, Solomon CT (2015) Habitat, not resource availability, limits consumer production in lake ecosystems. *Limnology and Oceanography*, **60**, 2079–2089.

Dillon PJ, Molot LA (1997) Effect of landscape form on export of dissolved organic carbon, iron, and phosphorus from forested catchments. *Water Resources Research*, **33**, 2591–2600.

Dillon PJ, Molot LA (2005) Long-term trends in catchment export and lake retention of dissolved organic carbon, dissolved organic nitrogen, total iron, and total phosphorus: the Dorset, Ontario, study, 1978–1998. *Journal of Geophysical Research-Biogeosciences*, **110**, 1–7.

Fee EJ, Hecky RE, Kasian SEM, Cruikshank DR (1996) Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. *Limnology and Oceanography*, **41**, 912–920.

Findlay SEG (2005) Increased carbon transport in the Hudson River: unexpected consequence of nitrogen deposition? *Frontiers in Ecology and the Environment*, **3**, 133–137.

Freeman C, Fenner N, Ostle NJ *et al.* (2004) Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature*, **430**, 195–198.

Garnett MH, Ineson P, Stevenson AC (2000) Effects of burning and grazing on carbon sequestration in a Pennine blanket bog, UK. *The Holocene*, **10**, 729–736.

George D, Hewitt DP (2005) The impact of year-to-year changes in the weather on the dynamics of Daphnia in a thermally stratified lake. *Aquatic Sciences*, **40**, 33–47.

Godwin SC, Jones SE, Weidel BC, Solomon CT (2014) Dissolved organic carbon concentration controls benthic primary production: results from in situ chambers in north-temperate lakes. *Limnology and Oceanography*, **59**, 2112–2120.

Gutseit K, Berglund O, Granéli W (2007) Essential fatty acids and phosphorus in seston from lakes with contrasting terrestrial dissolved organic carbon content. *Freshwater Biology*, **52**, 28–38.

He X, Wang W-X (2008) Stoichiometric regulation of carbon and phosphorus in P-deficient Daphnia magna. *Limnology and Oceanography*, **53**, 244–254.

Hessen DO (2008) Efficiency, energy and stoichiometry in pelagic food webs; reciprocal roles of food quality and food quantity. *Freshwater Reviews*, **1**, 43–57.

Hessen DO, Anderson T (1990) Bacteria as a source of phosphorus for zooplankton. *Hydrobiologia*, **206**, 217–223.

Houser JN (2006) Water color affects the stratification, surface temperature, heat content, and mean epilimnetic irradiance of small lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 2447–2455.

Huber V, Adrian R, Gerten D (2010) A matter of timing: heat wave impact on crustacean zooplankton. *Freshwater Biology*, **55**, 1769–1779.

Jansson M, Jonsson A, Andersson A, Karlsson J (2010) Biomass and structure of planktonic communities along an air temperature gradient in subarctic Sweden. *Freshwater Biology*, **55**, 691–700.

Jensen TC, Hessen DO (2007) Does excess dietary carbon affect respiration of Daphnia? *Oecologia*, **152**, 191–200.

Jones RI (1992) The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia*, **229**, 73–91.

Jones RI (1998) Phytoplankton, Primary Production, and Nutrient Cycling. In: *Aquatic Humic Substances: Ecology and Biogeochemistry* (eds Hessen DO, Tranvik LJ), pp. 145–175. Springer-Verlag, Berlim, Germany.

Jones SE, Solomon CT, Weidel BC (2012) Subsidy or subtraction: How do terrestrial inputs influence consumer production in lakes? *Freshwater Reviews*, **5**, 37–39.

Karlsson J, Byström P, Ask J, Ask P, Persson L, Jansson M (2009) Light limitation of nutrient-poor lake ecosystems. *Nature*, **460**, 506–509.

Karlsson J, Berggren M, Ask J, Byström P, Jonsson A, Laudon H, Jansson M (2012) Terrestrial organic matter support of lake food webs: evidence from lake metabolism and stable hydrogen isotopes of consumers. *Limnology and Oceanography*, **57**, 1042–1048.

Karlsson J, Bergström AK, Bystrom P, Gudasz C, Rodriguez P, Hein C (2015) Terrestrial organic matter input suppresses biomass production in lake ecosystems. *Ecology*, **96**, 2870–2876.

Kelly PT, Solomon CT, Weidel BC, Jones SE (2014) Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. *Ecology*, **95**, 1236–1242.

Kratz TK, Deegan LA, Harmon ME, Lauenroth WK (2003) Ecological variability in space and time: Insights gained from the US LTER program. *BioScience*, **53**, 57–67.

Lennon JT, Pfaff LE (2005) Source and supply of terrestrial organic matter affects aquatic microbial metabolism. *Aquatic Microbial Ecology*, **39**, 107–119.

Marinone MC, Marque SM, Suárez DA *et al.* (2006) UV rRadiation as a potential driving force for zooplankton community structure in Patagonian lakes. *Photochemistry and Photobiology*, **82**, 962–971.

McCauley E, Kalff J (1981) Empirical Relationships Between Phytoplankton and Zooplankton Biomass in Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 458–463.

McNaughton SJ, Oesterheld M, Frank DA, Williams KJ (1989) Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. *Nature*, **341**, 142–144.

Menzel D, Corwin N (1965) The measurement of total phosphorous in seawater based on the liberation of organically bound fractions by persulfate oxidation. *Limnology and Oceanography*, **10**, 280–282.

Monteith DT, Stoddard JL, Evans CD *et al.* (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–540.

North Temperate Lakes LTER: Ice duration – Trout Lake Area (1981) Current, North Temperate Lakes Long Term Ecological Research program (http://lter.limnology.wisc.edu), NSF, Center for Limnology, University of Wisconsin – Madison.

North Temperate Lakes LTER: Zooplankton – Trout Lake Area (1982) Current, North Temperate Lakes Long Term Ecological Research program (http://lter.limnology.wisc.edu), NSF, Center for Limnology, University of Wisconsin – Madison.

Osenberg CW, Bolker BM, White JS, St. Mary CM, Shima JS (2006) Statistical issues and study design in ecological restorations: Lessons learned from marine reserves. In: *Foundations of Restoration Ecology* (eds Falk DA, Palmer MA, Zedler JB), pp. 280–302. Islan Press, Washington, DC, USA.

Preston ND, Rusack JA (2010) Homage to Hutchinson: does inter-annual climate variability affect zooplankton density and diversity? *Hydrobiologia*, **653**, 165–177.

R Core Team (2015) *R: A Language and Environmental for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Read JS, Rose KC (2013) Physical responses of small temperate lakes to variation in dissolved organic carbon concentrations. *Limnology and Oceanography*, **58**, 921–931.

Rusak JA, Yan ND, Somers KM, McQueen DJ (1999) The temporal coherence of zooplankton population abundances in neighboring North-Temperate lakes. *The American Naturalist*, **153**, 46–58.

Rusak JA, Yan ND, Somers KM (2008) Regional climatic drivers of synchronous zooplankton dynamics in north-temperate lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **65**, 878–889.

Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. *Marine Microbial Food Webs*, **6**, 107–114.

Solomon CT, Bruesewitz DA, Richardson DC, et al. (2012) Ecosystem respiration: drivers of daily variability and background respiration in lakes around the globe. *Limnology and Oceanography*, **58**, 849–866.

Solomon CT, Carpenter SR, Clayton MK *et al.* (2011) Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. *Ecology*, **92**, 1115–1125.

Solomon CT, Jones SE, Weidel BC *et al.* (2015) Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: Current knowledge and future challenges. *Ecosystems*, **18**, 376–389.

Sterner RW, Elser JJ, Fee EJ, Guildford SJ, Chrzanowski TH (1997) The light: nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. *The American naturalist*, **150**, 663–684.

Stewart-Oaten A (1996) Problems in the analysis of environmental monitoring data. In: *Detecting Ecological Impacts* (eds Schmitt RJ, Osenberg CW), pp. 109–132. Academic Press Inc, San Diego, CA, USA.

Stewart-Oaten A, Bence JR (2001) Tempoal and spatial variation in environmental impact assessment. *Ecological Monographs*, **71**, 305–339.

Stewart-Oaten A, Bence JR, Osenberg CW (1992) Assessing effects of unreplicated perturbations: no simple solutions. *Ecology*, **73**, 1396–1404.

Tranvik LJ (1988) Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microbial Ecology*, **16**, 311–322.

Urabe J, Sterner RW (1996) Regulation of herbivore growth by the balance of light and nutrients. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 8465–8469.

Urabe J, Watanabe Y (1992) Possibility of N or P limitation for planktonic cladocerans: An experimental test. *Limnology and Oceanography*, **37**, 244–251.

Wellborn GA, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, **27**, 337–363.

Williamson CE, Stemberger RS, Morris DP, Frost TM, Paulsen SG (1996) Ultraviolet radiation in North American lakes: Attenuation estimates from DOC measurements and implications for plankton communities. *Limnology and Oceanography*, **41**, 1024–1034.

Williamson CE, Hargreaves BR, Orr PS, Lovera PA (1999) Does UV play a role in changes in predation and zooplankton community structure in acidified lakes? *Limnology and Oceanography*, **44**, 774–783.

Zwart JA, Craig N, Kelly PT, Sebestyen SD, Solomon CT, Weidel BC, Jones SE (In press) Metabolic and physiochemical responses to a whole-lake experimental increase in dissolved organic carbon in a north-temperate lake. *Limnology and Oceanography*.