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APPLIED ISSUES

Effects of temperature and ultraviolet radiation on microbial foodweb structure: potential responses to global change

SUMMARY

1. A series of growth experiments were conducted with natural plankton communities from a lake and river in northern Quebec, to evaluate the response of microbial foodweb structure to changes in ambient temperature and solar ultraviolet radiation (UVR).
2. Bioassays were incubated for 6 days at two temperatures (10 and 20 °C) and three near-surface irradiance conditions [photosynthetically active radiation (PAR) + UVA + UVB, PAR + UVA, and PAR only).
3. The concentration of total bacteria showed no net response to temperature, but the percentage of actively respiring bacteria, as measured with the cellular redox probe 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), was up to 57% higher at 20 °C relative to 10 °C. Chlorophyll a concentrations in the < 2 μm size fraction also reacted strongly to temperature, with a net increase of up to 61% over the temperature range of 10-20 °C. 4. The UVR effects were small or undetectable for most of the measured variables; however, the percentage of actively respiring bacteria was significantly inhibited in the presence of UVR at 20 °C, decreasing by 29-48% on day 6 in the lake experiments and by 59% on day 2 in one river experiment.
5. The results show differential sensitivity to temperature among organisms of microbial communities in subarctic freshwaters, and a resilience by the majority of microorganisms to their present UVR conditions. Microbial foodwebs in northern freshwaters appear to be relatively unresponsive to short-term (days) changes in UVR. However, the observed responses to temperature suggest that climate change could influence community structure, with warmer temperatures favouring picoplanktonic phototrophs and heterotrophs, and a shift in nanoplankton species composition and size structure.

Keywords: arctic, global change, picoplankton, protozoa, temperature, ultraviolet.

Introduction

The northern high latitude environment is currently subject to long-term shifts in ambient temperature associated with climate change (Maxwell, *1997),* and in the incident flux of solar ultraviolet radiation (UVR) associated with changes in strato

spheric ozone concentration (Rex *et* al., 1997). Changes in such environmental conditions have the potential of altering the productivity and reproduction of individual species in arctic and subarctic regions, with consequent repercussions at the community and ecosystem levels. Experiments manipulating variables such as temperature, light

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and nutrients have been conducted on northern terrestrial ecosystems and have shown that while there can be striking responses by certain plants and vegetation growth forms, these are restricted to only a few species and translate into relatively small ecosystem-level effects (Chapin *et al.,* 1995; Björn *et al.,* 1997). Aquatic ecosystems may also be impacted by changing climatic factors (Schindler *et al.,* 1990; Yan *et al.,* 1996), but information equivalent to that now available for terrestrial environments is lacking. Freshwater ecosystems are a major component of the northern landscape and may be particularly sensitive to environmental change (Vincent & Pienitz, 1996). Ultraviolet radiation effects on aquatic environments may be additionally controlled by vegetation shifts in the catchment area, which, in turn, affect the input of chromophoric dissolved organic matter (Schindler, 1998). Northern freshwaters contain these UVscreening compounds within a concentration range where small changes give rise to large variations in underwater UVR and spectral balance (Laurion, Vincent & Lean, 1997).

Despite the considerable literature concerning temperature effects and UVR impacts on micro-organisms, the combined effects of these two variables are not well known. Roos & Vincent (1998) demonstrated that UVR inhibition of growth in a matforming cyanobacterium was greater at low ambient temperature, consistent with the hypothesis that cellular damage by UVR is largely temperature independent, while biological repair processes are temperature dependent. They also showed, however, that cyanobacterial populations had the capacity to acclimatize to their ambient UVR field.

The central aim of the present study was to test the hypothesis of differential response to combinations of temperature and solar UVR by organisms in natural freshwater microbial communities; for example, there is some evidence that cyanobacteria are more strongly stimulated by warm temperatures than other algal groups (Robarts & Zohary, 1987). Heterotrophs, such as bacteria, are likely to be more responsive to temperature than lightlimited phytoplankton. The central hypothesis of the present study was that contrasting reactions of this type would lead to an altered community structure (abundance and species composition) over timescales of more than one generation. Outdoor microcosm experiments were used to assess this hypothesis on plankton communities from two contrasting freshwater environments in subarctic Quebec.

Materials and methods

Study sites

Sampling was conducted at sites near Kuujjuaraapik (latitude 55°17'N, longitude 77°46'W), in the subarctic of northern Quebec, from June to August 1995. Lake Kayouk is a shallow (maximum depth ~2.0 m), polymictic, coastal lake in a forest-tundra catchment, and the Great Whale River is a major inflow into Hudson Bay. The river was sampled 3 km upstream of its mouth to avoid the inclusion of marine and estuarine organisms. Water column profiles of temperature and irradiance (PAR and UVR) were measured on several dates during the summer, using a PUV-500 profiler (Biospherical Instruments Inc., San Diego, CA, U.S.A.). Water for the 6-day outdoor incubation experiments was pumped from a depth of 0.35 m at a distance of ~5 m from shore, and was obtained from each site twice during the summer. The Lake Kayouk experiments are referred to as L1 (started 27 June 1995) and L2 (24 July 1995), and the Great Whale River experiments as R1 (5 July 1995) and R2 (7 August 1995).

Temperature and UVR manipulation protocol

The lake or river water was incubated in 20L polyethylene containers (Cubitainers) that transmitted 85% of incident UVA (380 nm) and 65% of UVB (320 run). Lakes in the region of Kuujjuaraapik are phosphorus (P) limited (Bergeron,1995), and previous microcosm experiments have shown that planktonic cell abundance decreases rapidly in the absence of nutrient stimulation (Bergeron & Vincent, 1997). Therefore, in the present study, inorganic P(K_2HPO_4 at a final concentration of 1 µg P $L^{-1)}$ was added to each Cubitainer at 2-day intervals (following subsampling) for the duration of each experiment. The Cubitainers were placed in two water baths (2.4 m *x* 0.6 m *x* 0.3 m) that were partitioned into individual chambers (0.3 m *x* 0.6 m *x* 0.3 m). Each chamber was covered with one of three filters: UVT Plexiglas (transmits all wavelengths of PAR and

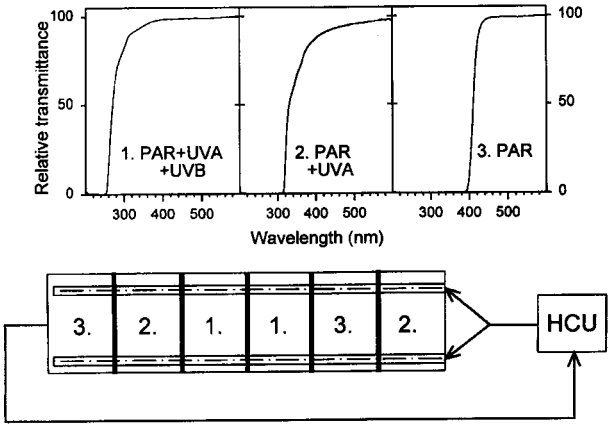

Fig. 1 Plan view of the incubation system developed for the experiments (one of two identical water baths is shown), with transmission qualities of the Plexiglas filters used. Each water bath was divided into six sections with 'double venetian blind' separators, which allowed water but not light to transfer between the compartments. Two inflow hoses perforated with holes ran the length of the water baths and brought temperature-controlled water into the system, while one outflow returned water to the heating/cooling unit (HCU). The Plexiglas filters were affixed to the top of the compartments, which each held one Cubitainer. The linear sequence of Cubitainers was placed randomly, and repositioned (with their respective filter) randomly every 2 days after sampling.

UVR), Dupont Mylar (transmits PAR and UVA) and UF3 Plexiglas (transmits PAR only; Fig. 1). Transmission qualities were confirmed using a Hewlett-Packard diode array spectrophotometer (model HP-8452A). Temperature control was obtained by recirculating water through high-volume heating/ cooling units (model VWR 1174), which maintained 10 and 20 °C in each water bath, respectively. These temperatures were chosen from within the summer temperature range of Lake Kayouk (Rae & Vincent, 1998) and were used in the river experiments for purposes of comparison. Two replicates for each temperature-irradiance treatment resulted in a total of twelve Cubitainers per incubation experiment. Although the experimental set-up was not truly replicated *(sensu* Hurlbert, 1984) with respect to the temperature treatment (only one temperature control unit per temperature), the large temperature difference between incubators was closely maintained throughout the experiments and no other confounding variables were apparent.

Cubitainers were filled in the evening and the water within them was allowed to equilibrate in the water baths for 12 h; day 0 subsampling was begun the morning following water collection. The Cubitainers were sampled on days 0, 2, 4 and 6, for chlorophyll a (Chl *a,* µg L^{-1}), total bacteria, actively respiring bacteria, picocyanobacteria and plankton > 2 µm in size. Throughout each of the four experiments, ambient irradiance (PAR, UVA at 380 run, and UVB at 320 nm) was measured four times daily at 09.00, 12.00, 15.00 and 18.00 h using the PUV-500 2 *1t* sensor. Water incubated for experiment L1 was unfiltered, but for subsequent experiments water was pre-filtered using a 200-µm Nitex screen to remove macrozooplankton, thereby eliminating confounding effects of grazing by these organisms.

Sample analyses

Dissolved organic carbon (DOC) was analysed in samples from the first date of sampling of each environment. The lake or river water was filtered through a 0.45-gm Sartorius cellulose acetate filter and stored in glass in the dark and cold (4 °C). Dissolved organic carbon concentration was determined by high temperature oxidation with a Shimadzu TOC analyser (model 5050).

For chlorophyll a analysis, 100-250 mL of water were filtered through MFS GF75 glass fibre filters (GF/F equivalents) and the filters stored frozen in the dark for 2-4 weeks. Chlorophyll a concentration was determined by extracting the filtered material in boiling 95% ethanol (Nusch, 1980) and measuring the extract fluorescence with a Sequoia Turner fluorometer (model 450) using NB440 (blue excitation) and SC665 (red emission) filter blocks. A standard solution of chlorophyll a from *Anacystis nidulans* (Sigma Biochemical Co.) was used for calibration. The extracts were measured before and after the addition of 0.2 mL of 0.1 N HCl to correct for phaeopigments.

Following cell preservation for microscopy, all samples were stored in darkness at 4 °C for 2-6 months. Microscope analysis of samples was performed using a Zeiss Axiovert 100 inverted fluorescence microscope. Bacteria samples were preserved in 4% (final concentration) formaldehyde. Sample preparation for microscopy involved the staining of 1-2 mL of sample with 0.05 µg mL^{-1} of the fluorochrome 4',6-diamidino-2-phenylindole

(DAPI) for 20 min, followed by filtration through a 0.2-µm black Nuclepore membrane (Hobbie, Daley & Jasper, 1977; Porter & Feig, 1980). Filters were mounted on slides and examined at 1000X magnification, using a 365 nm excitation UV filter block. At least 400 cells were counted in five to fourteen randomly selected fields.

The number of actively respiring bacteria was determined using the protocol outlined in Lovejoy *et al.* (1996). Aliquots of water (20 mL) from the subsampling of each Cubitainer were incubated with 0.23 mg mL^{-1} (final concentration) of the tetrazolium salt 5-cyan-2,3-ditolyl tetrazolium chloride (CTC) in darkness in the water baths for 2 h, and then the bacteria were preserved with 1% (final concentration) formaldehyde. When reduced, CTC forms crystals which fluoresce bright red under both green (546 nm) and blue (450-490 nm) excitation (Lovejoy *et* a1.,1996). Preparation for microscopy involved filtration of 2-5 mL of preserved sample through black Nuclepore membranes, and subsequent examination at 1000X to count 300-400 cells in ten to forty random fields. Prepared slides of both total and actively respiring bacteria were kept frozen and in darkness for up to 2 weeks before cell counting.

Picocyanobacteria were preserved in 1% (final concentration) paraformaldehyde (Hall & Vincent, 1990). Samples were prepared for microscopy by filtering 10-15 mL through a 0.2-µm black Nuclepore membrane. The filters were examined at 1000 x immediately following filtration. Red autofluorescence of phycocyanin-containing (PC) cells was observed under green excitation, and yellow-orange autofluorescence of phycoerythrin-containing (PE) cells was observed under blue excitation. At least 400 cells were counted in 20-100 fields for each sample. Picoeukaryotes, recognized by their bright red fluorescence of chlorophyll a under both green and blue excitation, were also counted. However, this component was always less than 1% of the total counts and was not therefore included in the subsequent data analysis.

Plankton > 2 µm were preserved in 1% glutaraldehyde and 0.1% paraformaldehyde (Tsuji & Yanagita, 1981) and were subsequently examined using the FNU method that combines fluorescence, Nomarski and Utermôhl techniques (Lovejoy *et al.,* 1993). Samples (100 mL) were allowed to sediment into a chamber (3 mL volume) for 20 h, and were then left to stain with 0.3 µg mL^{-1} DAPI for a further 2 h. Samples were enumerated using DAPI fluorescence and Nomarski optics to locate and identify flagellated and ciliated protozoa, and other plankton > 2 um in size. For each sample, three to four transects were enumerated to provide a final count of 350-400 cells. Macrozooplankton remaining in the Cubitainers on day 6 of experiment L1 were concentrated, preserved in formalin and counted using a dissecting microscope.

Statistical analysis

Each biological variable measured during the 6-day experiments was tested statistically for effects of temperature, UVR and temperature-UVR interaction over time, using a repeated measures ANoVA. Assumptions of normality, equal variance and sphericity were verified and met in all analyses. For the plankton sized > 2 µm data, only day 6 samples were analysed, and a two-way ANOVA was used. All statistical analyses were performed using SAS 6.12.

Results

Physical and chemical measurements

The depth of 1% irradiance for UVR at 320 nm was the same at both sites (Fig. 2), consistent with their similar values of DOC (Table 1). However, UVA at 380 nm and PAR were both attenuated more rapidly in the Great Whale River than in Lake Kayouk, reflecting the higher concentrations of suspended particulate matter in the river.

Temperature profiles showed that diurnal stratification occurred in Lake Kayouk but frequent mixing events prevented the formation of a major summer thermocline (Fig. 2); in the Great Whale River temperature profiles were consistently isothermal and indicative of continuous mixing throughout the water column. Ambient water temperature in Lake Kayouk (recorded continuously at 35 cm depth over a 7-week period; details are given in Rae & Vincent, 1998) was variable, while discrete measurements in the river showed that temperatures in this environment were comparatively steady (Table 1). Water temperature at time of sampling for L1 and L2 was 15 and 14.5 °C,

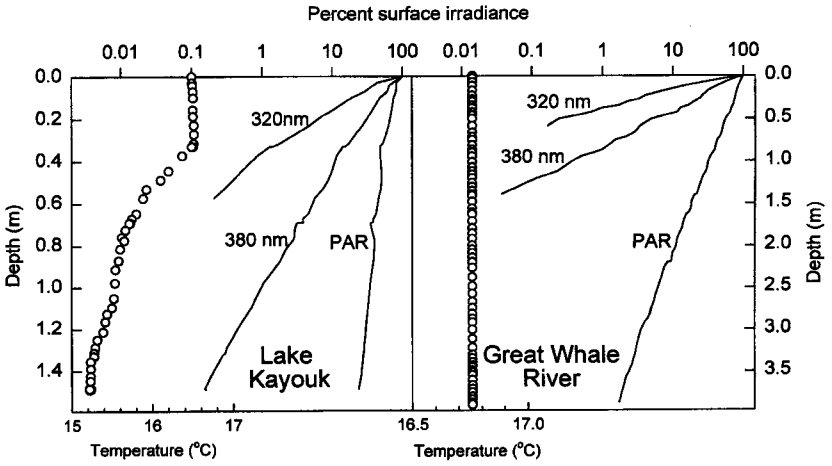

Fig. 2 Penetration of photosynthetically available radiation (PAR: 400–700 nm) and ultraviolet radiation (UVR) at 320 nm (UVB) and 380 nm (UVA) in the water columns of Lake Kayouk and the Great Whale River in July 1995. Profiles were taken within 2 h of midday under cloudless conditions. Symbols show the thermal structure in each water column. Note the difference in depth scales between the two graphs.

Table 1 Physical and chemical measurements from Lake Kayouk and the Great Whale River during the period 24 June to 16 August 1995

	Lake Kayouk	Great Whale River
Water temperature (°C)		
Minimum	8.6	16.0
Maximum	21.4	17.5
Mean	15.6	16.6
SD	2.6	0.6
Attenuation coefficient (m^{-1})		
PAR	1.0	1.1
UVA (380 nm)	4.6	5.2
UVB (320 nm)	11.1	13.1
Dissolved organic carbon ($mg\,L^{-1}$)		
DOC	4.2	3.6

respectively, and for R1 and R2 was *17* and 16 °C, respectively.

Weather conditions varied over the course of each incubation, resulting in differences in PAR and UVR exposure by the organisms. Cumulative exposure at the mid-point of the Cubitainers for the 2-day period preceding each sampling date varied from *87* to 651 kJ *cm* $^{-2}$ nm $^{-1}$ of UVA *(380* nm), and from *8* to *77* kJ *cm*$^{-2}$ nm $^{-1}$ of UVB (320 nm). Biologically effective doses of UVR were three to four times higher in the UVT (UVA and UVB) compared to the Mylar (UVA) treatments (biological weighting function for UV-photoinhibition of algal photosynthesis; Cullen, Neale & Lesser, 1992).

Biological measurements

Lake Kayouk and the Great Whale River are both oligotrophic systems with less than 2 µg L^{-1} of chlorophyll *a*. The < 2 µm fraction contributed up to 50% of the chlorophyll *a*, indicating the prevalence of small cells in these freshwaters. Photosynthetic picoplankton (cyanobacteria < 2 µm) were in the range of 10^6-10^7 cells L^{-1}, and were dominated by forms containing the pigment phycocyanin (PC), although phycoerythrin-containing (PE) picocyanobacteria were also present, particularly in the river community. Heterotrophic picoplankton (bacteria < 2 µm) averaged 10^9 cells L^{-1}, but less than 10% of these were metabolically active as measured with CTC.

The concentration of plankton > 2 *µm* in both the lake and river was 10^6 cells L^{-1} throughout the period of sampling. The majority of these cells were in the nanoplankton size range (2-20 µm; Table 2) and netplankton (> 65 µm) represented less than 1.5% of the total counts. Chlorophytes and chrysophytes were dominant taxa in both environments, but Lake Kayouk had more cyanobacteria (dominated by *Merismopedia* sp.) than the river, which had a larger representation of pennate diatoms. Ciliated protozoa were low in abundance, while flagellated cells represented up to *33%* of total plankton > 2 µm counts.

UVR effects

The effects of ultraviolet radiation on the microbial communities were generally small and varied among experiments. Chlorophyll *a* in all fractions (total community, > 2 µm, < 2 µm) showed no significant

differences among solar radiation treatments over the course of each incubation experiment. Picocyanobacteria from the UF3 treatment (no UVR) in R1 were more abundant by 38 and 40% at days 2 and 6 than in either of the other two treatments. This UVR response was restricted to the PC-containing picocyanobacteria ($F = 4.7$, P = 0.02), while the PE-containing picocyanobacteria showed no significant difference with and without UVR (F = 2.1, P = 0.15). This effect on picocyanobacteria was not observed in experiments L1, L2 or R2.

Although the total heterotrophic bacterial abundance was unresponsive to different solar radiation treatments, the number of actively respiring cells (ARCs; i.e. cells that took up and reduced CTC) as a percentage of total bacteria responded significantly to UVR in three of four experiments (Fig. 3). In L1, %ARCs at 20°C on day 6 were 48% lower in the presence of UVA and UVB (F = 5.9, P = 0.02) and in L2 were 29% inhibited by UVB (F = 4.3, P = 0.02). Also on day 6 of L2, %ARCs at 10 °C increased by 43% when UVB was removed but decreased again when UVA was removed as well (Fig. 3b). ARCs in both river experiments increased by day 2 and then declined substantially by day 6. On day 2 of R1, %ARCs were greatest at 20°C in the absence of all UVR (F = 5.6, P = 0.009), representing a 47% inhibition by UVA and a further 29% inhibition by UVB (Fig. 3c). Statistically significant differences among UVR treatments were not detected in R2.

Plankton > 2 μm in size were analysed on day 6 of experiments L2 and R1. There was no significant UVR effect on any of the taxa within the river plankton. In the lake experiment, there was a significant UVA effect on the total diatom abundance, which was twice as high under the PAR-only filter relative to the two other treatments (F = 7.7, P = 0.02).

Table 2 Cell abundance of plankton > 2 μm on day 0 (n = 4) for experiments L2 (Lake Kayouk) and R1 (Great Whale River). Values are 10^5 cells L^{-1} (SE)

	Lake Kayouk	Great Whale River
Total cells	9.79 (0.84)	9.77 (0.38)
Ciliates	0.07 (0.04)	0.12 (0.08)
Nanoflagellates	3.26 (0.39)	2.06 (0.27)
Other	6.47 (0.66)	7.59 (0.35)
Size fractions		
2–5 μm	3.86 (0.36)	5.09 (0.51)
5–10 μm	2.22 (0.47)	1.51 (0.22)
10–20 μm	2.62 (0.41)	1.39 (0.46)
20–65 μm	1.03 (0.13)	1.68 (0.19)
> 65 μm	0.05 (0.01)	0.11 (0.07)
Algal taxa		
Cyanophyta	2.61 (0.39)	0.03 (0.02)
Chlorophyta	3.25 (0.39)	3.39 (0.23)
Euglenophyta	0.11 (0.14)	0.02 (0.02)
Chrysophyta	2.19 (0.45)	1.13 (0.21)
Cryptophyta	0.39 (0.10)	0.24 (0.16)
Pyrrophyta	0.14 (0.03)	0.53 (0.21)
Bacillariophyta	0.61 (0.14)	3.24 (0.36)
Unknown	0.43 (0.23)	1.06 (0.47)

Temperature effects

Temperature significantly affected the net growth of several microbial foodweb components during the experimental incubations. The general pattern for chlorophyll a in all fractions and all but one experiment (L1) at 20°C was an initial phase of rapid increase in concentration followed by a stabilization

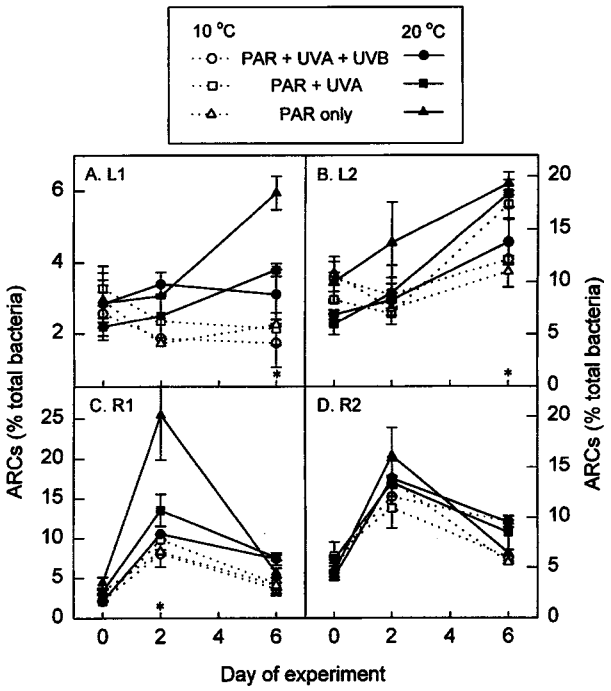

Fig. 3 Actively respiring cells (ARCs) as a percentage of total bacterial abundance over the course of four 6-day incubation experiments. Cells were incubated under combinations of two temperatures and three solar radiation conditions. Values represent means (n = 2) ± 1 SE. * indicates a significant ultraviolet radiation (UVR)–temperature interaction effect for L1 and R1, and a significant UVR treatment effect for L2 (P < 0.05).

or decline beyond day 4 of the incubation (Fig. 4). Chlorophyll *a* concentration at 10'C responded more gradually and converged with the 20'C-values towards the end of three of the experiments. The <2μm chlorophyll *a* fraction showed a greater response to temperature increase than the > 2 gm fraction, with up to 61% greater concentration at 20°C relative to 10 °C (Fig. 5).

Picocyanobacterial abundance changed little at either temperature over the course of both lake incubations (L1 and L2); an order of magnitude difference in absolute abundance was observed between the experiments (10^6 *vs.* 10^7 cells L^{-1}; Fig. 6a, b). In the river experiments, R1 and R2, picocyanobacterial abundance on day 2 was 48 and 31% higher at 20'C relative to 10'C (R1: F=25.6, P=0.0001; R2: F=21.3, P=0.0001), but then declined (Ri) or remained relatively constant (R2) over the final 4 days of the experiment (Fig. 6c, b). Abundance at 10'C changed little over time in R1 and declined sharply in R2.

Total bacteria concentrations remained near the day 0-values (L1) or declined steadily over time (L2, R1, R2; Fig. 6e-h) and were not significantly affected by temperature. The pattern of change by the percentage of actively respiring bacteria in the lake experiments was one of increase by up to 62% over time at 20'C, and less (L2) or no (L1) change at 10'C. In both river experiments, % ARCs increased by 100-400% over the first 2-day interval, and then declined over the subsequent 4 days. Averaged over all four experiments, %ARCs on days 2 and 6 were 40-60% higher at 20 relative to 10 °C (Fig. 5).

A detailed analysis of the plankton community sized > 2 um from experiments L2 and R1 showed that there were net changes in abundance from day 0 to day 6 for most organisms. This change was generally one of increase, but in some cases was a decrease over time (Fig. 7). Examination of the > 2 gm plankton in R1 revealed no significant effects of temperature on functional size or taxonomic groupings. The analysis of the L2 experiment showed that temperature effects were apparent in the > 2 um lake plankton community. There was an order of magnitude greater rise in the number of ciliated protozoa at 20 compared to 10 °C (F = 20.3, P = 0.004; Fig. 7a), although the overall

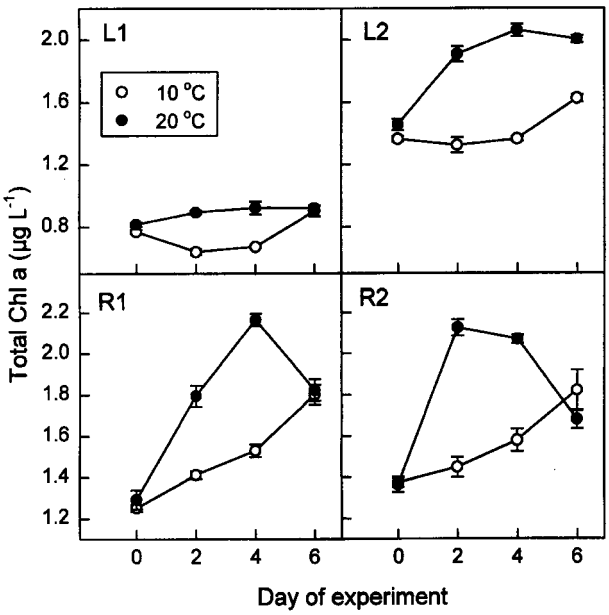

Fig. 4 Change in the total chlorophyll *a* concentration over time at two temperatures, in each of four incubation experiments. Values are means ($n = 2$) ± 1 SE.

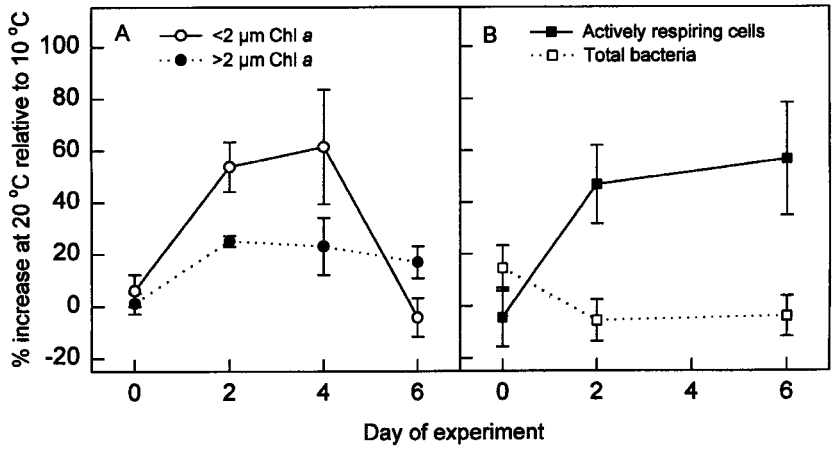

Fig. 5 The response of chlorophyll *a* fractions (a) and bacteria (b) at 20 °C relative to 10 °C. Values represent the means ($n = 4$) ± 1 SE, for all data pooled from the four experiments.

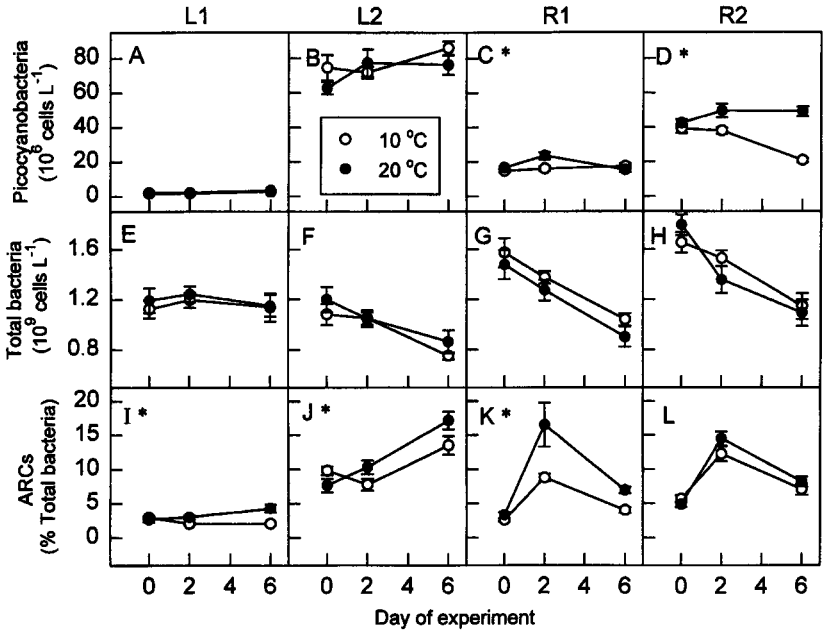

Fig. 6 Change in abundance of picocyanobacteria (a–d), heterotrophic bacteria (e–h), and actively respiring cells (ARCs) as a percentage of total bacterial abundance (i–l) over time at two temperatures, in each of four incubation experiments. * indicates a significant difference over time between temperatures. Values represent means ($n = 6$) ± 1 SE.

contribution of ciliates to total > 2 um plankton abundance remained small (<5%). Flagellated cells responded conversely with greater final abundance at 10 °C (F = 6.9, P = 0.04). The analysis according to size revealed that 2-5 gm cells increased over time at 10 but not at 20°C (F=64.8, P=0.0002), while the 10-20 um cells showed the reverse pattern (F=21.3, *P* = 0.004; Fig. 7b). Chrysophyte abundance responded positively at the lower temperature (F = 23.1, P = 0.003) and the Pyrrophyta increased over time at both temperatures but with four times greater abundance at 20°C on day 6 (F=35.0, P=0.001; Fig. 7c). Other components of the > 2 gm plankton changed over time (i.e. net growth or loss) but the changes were not statistically different between temperatures. Experiment L1 macrozooplankton, which was dominated numerically by the calanoid copepod *Leptodiaptomus minutus,* had 38% greater abundance at 20'C by the end of the experiment *(t = 7.9,* P = 0.001).

Temperature-UVR interaction effects

The repeated-measures ANOVA analyses showed that there were no significant temperature-UVR interactions over the time course of the incubation experiments, with the exception of percentageARCs in L1 (day 6) and R1 (day 2), where the greatest increases were detected in the absence of UVR at 20°C.

Discussion

The experiments reported here show that the microbial foodwebs in two northern freshwater ecosystems have varied reactions to global change variables, with a general resilience to current UVR levels but sensitivity to change in ambient temperature. Subtle differences were observed in the ability of some populations to cope with UVR and also a stronger response to temperature, which could lead to some change at the community level. However, these results do not suggest a major ecosystem-level effect (e.g. a change in total community biomass) over the timescale of a few generations. Similar findings from terrestrial vegetation ecosystems showed that certain plant species and growth forms responded differently to environmental variables, but overall net community processes were not substantially altered (Chapin et al., 1995). The experiments are an aquatic parallel to the terrestrial ecosystem study, in that the effects of variables associated with global change were examined on whole communities at a timescale appropriate to the organisms studied.

The majority of micro-organisms examined in this study can be considered well adjusted to their present UVR environment. In the river, and in the lake ecosystem during wind events, cells are swept through gradients of underwater irradiance because of turbulent mixing processes, and therefore the

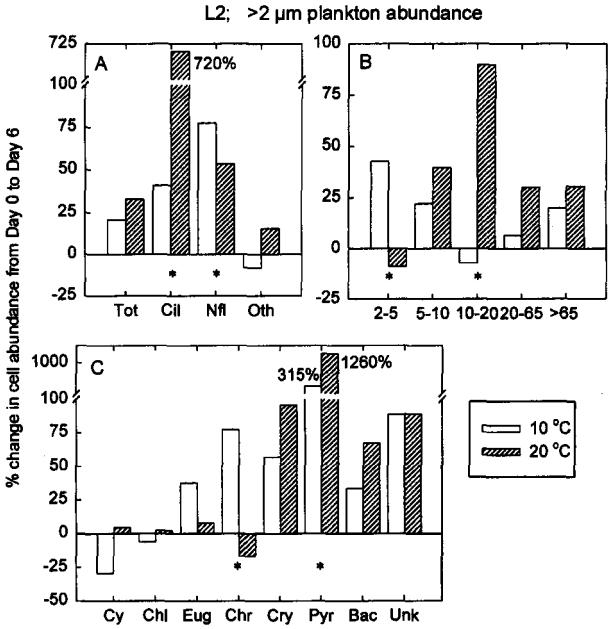

Fig. 7 The percentage change in cell abundance of >2 µm plankton components from day 0 ($n = 4$) to day 6 ($n = 6$) at two temperatures in experiment L2. (a) Changes in the total >2 µm community (Tot), the ciliated protozoa (Cil), the nanoflagellated cells (Nfl) and other non-ciliated and non-flagellated cells (Oth = diatoms, desmids and cyanobacteria). (b) Changes in size distribution. The five size fractions are in units of µm. (c) Changes in taxonomic composition: Cyanophyta (Cy); Chlorophyta (Chl); Euglenophyta (Eug); Chrysophyta (Chr); Cryptophyta (Cry); Pyrrophyta (Pyr); Bacillariophyta (Bac) and unknown or unidentifiable (Unk). * indicates a significant difference between temperatures on day 6.

micro-organisms must adjust to frequent changes in their ambient light environment. The static nature of these experiments provided a severe test of UVRsensitivity, but mimicked conditions where organisms are retained in the near-surface diurnal thermoclines, which have been shown to occur in subarctic lakes (Milot-Roy & Vincent, 1994; Scully & Vincent, 1997; this study). Greater UVR exposures may be experienced by these ecosystems in the future in association with the continuing degradation of the ozone layer (increasing UVB) and climate-related effects on DOC input to lakes (changing UVA and UVB).

Ambient UVR had an impact on the abundance of metabolically active bacteria in these experiments, particularly at the higher temperature. This effect was primarily induced by UVA, although some UVB suppression was also observed. UVR has been shown to have direct negative effects on bacterial production (Herndl, Müller-Niklas & Frick, 1993; Müller-Niklas et al., 1995) and the latter rate variable has been shown to correlate with actively respiring cells (ARCs; Lovejoy *et al.,* 1996; del Giorgio, Prairie & Bird, 1997). The results obtained by the present study show that under current UVR levels, the respiring bacterial community can be suppressed. This observation implies that UVR, and especially UVA, exerts a controlling influence on carbon flux through the ecosystem, because bacteria are a key link in the recycling of carbon back into the microbial foodweb via the protozoa which feed on them (Sherr & Sherr, 1991). However, it should be noted that the tetrazolium salt (5-cyano-2,3-ditolyl tetrazolium chloride; CTC) measurement of ARCs is a new technique in aquatic microbial ecology, and requires further study to define more fully its ecophysiological meaning (Lovejoy *et al.,* 1996).

Ultraviolet A inhibition of picocyanobacteria in one experiment and of diatoms in another provided additional evidence that UVA is capable of damaging micro-organisms, and is consistent with observations elsewhere (Kim & Watanabe, 1993; Quesada & Vincent, 1997). Several diatom species have been shown to be sensitive to UVR in laboratory cultures (e.g. Hazzard, Lesser & Kinzie, 1997; Nilawati, Greenberg & Smith, 1997), and in the lake experiment reported here this group was also found to be more sensitive than other algal divisions. However, this effect was not replicated in the river experiment and emphasizes the differences in sensitivity among communities. Ultraviolet radiation-induced species shifts among algae have been suggested from experimental results on natural communities (Worrest, Thomson & van Dyke, 1981; Wangberg, Selmer & Gustavson, 1996) but observations by the present study suggest that such impacts on phytoplankton community structure will be minor in subarctic lakes.

Temperature responses by the micro-organisms of Lake Kayouk and the Great Whale River generally followed the pattern of greater increase in chlorophyll a biomass and cell abundance at 20°C relative to 10°C. These observations are consistent with the general acceleration of photosynthesis, respiration and biosynthetic processes at warmer temperatures (e.g. Robarts & Zohary, 1987; Sosik & Mitchell, 1994). It can be inferred from chlorophyll a measurements that net growth of the <2 um phototrophs reacted more strongly to temperature than that of the > 2 um fraction. This was consistent across the four experi-

ments, each with a distinct microbial community, and is in accordance with results of photosynthesis experiments that have shown smaller phytoplankton cells to have a greater response to increasing temperature than larger cells (Andersson, Haecky & Hagstrôm, 1994; Rae & Vincent, 1998). The < 2 μm fraction in Lake Kayouk and the Great Whale River is dominated by picocyanobacteria, with a very small representation by picoeukaryotes (< 1% total picophytoplankton abundance). Cyanobacteria are known to be particularly responsive to temperature (Robarts & Zohary, 1987; Tang, Tremblay & Vincent, 1997), which may explain the faster net growth of the < 2 μm chlorophyll a fraction in the present experiments. Cell counts for picocyanobacterial abundance did not reveal results consistent with those of the < 2 μm chlorophyll a, and indicate temperature-dependent variation in the amount of chlorophyll a per picocyanobacterial cell and/or a differential response by different genetic components of the picocyanobacterial community.

By the end of most experiments at 20 °C, there was a decline or plateau in population densities, indicating a decrease in the ratio of gains to losses. This effect was not observed in the majority of 10 °C incubations and is likely to be the result of nutrient exhaustion in the faster growing cultures at warmer temperature. Because of the phosphorus limitation in lakes of the Quebec subarctic (Bergeron & Vincent, 1997), the containers were supplemented with inorganic phosphorus, but the rapid initial growth at 20 °C may have depleted a secondary nutrient, such as nitrate. Nitrate concentrations in freshwaters of this region have been reported at 10 μg N L^{-1} (Bergeron,1995).

Total heterotrophic bacteria showed no significant response to temperature, with a general decline in abundance during each experiment. This latter effect is probably a result of increased grazing pressure by ciliates and nanoflagellates, both of which increased in abundance during the incubations. The positive effect of temperature on the percentage of actively respiring cells (%ARCs) was not exhibited by the total bacterial population, and provides support to the observation that DAPI-counts of bacterial abundance probably include non-functional and 'ghost' cells (Zweifel & Hagstrom,1995). The smaller responses of %ARCs at 10°C are consistent with a decreased affinity by bacteria for substrates at low temperatures (Nedwell & Rutter, 1994). The interaction effect between high temperature and UVR found in two of the experiments reported here is contrary to the hypothesis of less UVR damage at higher temperature because of faster repair mechanisms. However, the responsiveness of ARCs to temperature suggests that, with climate warming, metabolically active bacteria could become a larger component of the microbial community.

Net growth over time of the total > 2 μm plankton communities in the lake and river was unresponsive to temperature, suggesting that environmental warming or cooling will not have substantial impacts at the ecosystem-level. However, some populations reacted strongly to temperature, leading to detectable community-level shifts. The ciliated protozoan were strongly stimulated at higher temperature and the potential result of an increase in grazing pressure on small cells (Rassoulzedegan, Laval-Peuto & Sheldon, 1988) may have contributed to the reduction of the 2-5 μm size class at 20 °C. The Pyrrophyta and Chrysophyte also responded (in opposite direction) to a temperature increase, indicating that warming could lead to an altered representation of algal taxa in the community. Cyanobacteria > 2 μm were abundant (27% of total cell counts) in Lake Kayouk, but contrary to expectations did not respond significantly to higher temperature, which is perhaps a reflection of increased grazing pressure. The difference in copepod abundance between temperatures at the end of the first lake experiment indicated a temperature-dependence of development time and/or food supply (Williams & Jones, 1994). An intrinsic characteristic of experiments using natural communities, such as in the present study, is the large amount of withintreatment variability (see also Kelley et al., 1997; Laurion, Lean & Vincent, 1998), which masks any subtle effects associated with treatment conditions. However, assays as conducted here, with multiple trophic levels, offer a more realistic evaluation of whole community responses to different environmental factors than do single-species laboratory experiments, albeit within the limitations imposed by containers such as an artificial hydrodynamic regime and modified loss processes (see Bergeron & Vincent, 1997). The responses observed represent the net effect of temperature or UVR on gain and loss processes and cannot be interpreted in direct physiological terms. As such, however, the experiments are more relevant than single-species assays to address the question of

how microbial foodwebs respond to changes in their ambient environment.

Many of the planktonic organisms examined in this study are resistant to their present UVR environment but show varied responses to temperature. The results imply that climate change would favour shifts in the size structure and composition of the plankton community, but that alterations to underwater UVR would primarily affect bacterial metabolism. These potential responses to global change processes could ultimately impact on higher trophic levels through the resultant shifts in food quality and quantity.

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