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INDEPENDENT GRADIENTS OF PRODUCER, CONSUMER, AND MICROBIAL DIVERSITY IN LAKE PLANKTON

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Abstract. Interactions between trophic levels during food web assembly can drive positive correlations in diversity between producers, consumers, and decomposers. However, the contribution of trophic interactions relative to local environmental factors in promoting species diversity is poorly understood, with many studies only considering two trophic levels. Here we examine correlations in diversity among zooplankton, phytoplankton, and bacteria in the pelagic zone of 31 lakes in British Columbia, Canada. We sampled species diversity of zooplankton and phytoplankton through morphological identification, and bacterial genetic diversity was estimated by denaturing gradient gel electrophoresis (DGGE) of 16S rDNA polymorphisms. We looked for correlations in diversity that were independent of the abiotic environment by statistically controlling for 18 limnological variables. No significant correlations were found between the diversity of zooplankton, phytoplankton, and bacteria. In addition, the physical factors that were associated with species composition in one trophic level were independent of those that were important for another. Our results provide no support for the importance of direct feedbacks between producers, consumers, and decomposers in maintaining diversity. Zooplankton, phytoplankton, and bacterial diversity and composition are regulated independently from one another and respond to different environmental variables. These results suggest that species of lake plankton show loose trophic associations with one another due to broad diets in consumers and decomposers.

Key words: *bacteria; coexistence; community structure; denaturing gradient gel electrophoresis (DGGE); food webs; microbial diversity; pelagic community; phytoplankton; species interactions; zooplankton.*

INTRODUCTION

The question of how species coexist in ecosystems is a persistent issue in community ecology. G. E. Hutchinson (1959) first pointed out that Gause's (1934) axiom of competitive exclusion is in contradiction with the high diversity found in apparently homogeneous habitats such as the pelagic zones of lakes and oceans. He termed this contrast the "paradox of the plankton" (Hutchinson 1961). Numerous solutions to the paradox have been proposed, mostly dealing with the ways in which natural systems violate the assumptions of simplified models predicting competitive exclusion. Tilman and Pacala (1993) summarize the alternative solutions to Hutchinson's paradox. The theories fall into several categories: (1) population limitation by multiple resources or local physical factors; (2) temporal or spatial heterogeneity in resources and local physical factors; (3) interspecific trade-offs between competitive and colonization abilities; (4) nonequilibrium population dynamics; and (5) interactions between trophic levels. Here we focus on the last category, the role of facilitation between organisms

at different trophic levels and the reciprocal effects of diversity between producers, consumers, and decomposers.

Diversity maintenance via multitrophic interactions can occur when a number of conditions are met. First, predators may promote diversity among competitors (keystone predation; Paine 1966) when their impact is greatest for dominant competitors and thereby prevents exclusion (Leibold 1996). Second, a diverse resource base can increase the potential for niche partitioning and coexistence among multiple competitors (Tilman 1988, Siemann et al. 1998, Interlandi and Kilham 2000). According to these mechanisms, the number of potentially coexisting competitors is their number of limiting resources plus predators, provided that predators are differentiated in their prey preference. Third, positive feedbacks in diversity between consumers and prey depend on sequential assembly rules (Grover 1994). That is, invasion of producers allows for colonization by specialized consumers, which promotes further invasion by competing producers through keystone predation. A potentially unlimited number of consumers and resources can persist if each colonizing prey is followed by a consumer that weakens its effects on later competitors (Grover 1994). If these mechanisms are important for maintaining diversity in nature, then we expect to

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observe correlations in diversity between producers and consumers.

Positive feedbacks in diversity may also arise via facilitation between producers and decomposers (Van der Heijden et al. 1998, Reynolds et al. 2003). Decomposer taxa may vary in their ability to mineralize organic compounds found in different plant species. Producers and microbes may therefore be joined in mutualisms mediated by nutrient recycling. A diverse decomposer community can increase the rate of nutrient cycling via the variable ability of different microbes to mineralize different organic compounds; therefore high producer diversity may depend on high decomposer diversity (Naeem et al. 2000). The reverse may also be important, where high producer diversity provides more opportunity for niche specialization among decomposers. Van der Heijden et al. (1998) observed positive feedbacks between decomposer and primary producer diversity in microcosms simulating European grasslands. Mycorrhizal fungi diversity promoted the maintenance of plant diversity. Microbes may also act as pathogens or consumers of plants and thereby drive negative feedbacks that maintain diversity among producers via keystone predation (Bever 1994). Interactions between plants and soil microbes thereby show both positive (via microbially mediated resource partitioning) and negative (via soil borne plant pathogens) feedbacks that can influence the maintenance of large-scale temporal and spatial gradients of species richness (reviewed by Reynolds et al. [2003]). However, decomposers in many aquatic systems are difficult to identify as a group. Prokaryotes mineralize organic compounds but also act as pathogens of producers and consumers and competitors for nutrients with phytoplankton (Wetzel and Likens 1991). Although feedbacks in diversity across trophic levels are a plausible mechanism for species coexistence, the importance of such feedbacks relative to other drivers such as abiotic control is poorly understood in most ecosystems.

If trophic level interactions are important for promoting species coexistence, then diversity should be positively correlated across trophic levels. That is, high diversity of producers should coincide with high diversity among consumers and decomposers. Most studies of diversity patterns across trophic levels come from terrestrial systems and largely focus on primary producer and herbivore interactions (Murdoch et al. 1972, Siemann et al. 1998, Haddad et al. 2001, Hawkins and Porter 2003). Most terrestrial studies have found positive correlations in diversity across trophic groups. By contrast, similar studies in aquatic ecosystems have found weak and often variable patterns (Allen et al. 1999, Shurin and Allen 2001, Irigoien et al. 2004, Declerck et al. 2005). Consequently, empirical correlations in diversity across trophic levels have provided mixed support for the importance of feedbacks in maintaining diversity.

We measured the diversity of phytoplankton, zooplankton, and pelagic bacteria in 31 lakes in southwestern British Columbia, Canada, to test for correlations in diversity. The lakes represented a broad range of environmental variables (e.g., productivity, salinity, pH) but were similar in surface area (within the same order of magnitude). Phytoplankton and zooplankton composition and diversity were measured by morphological identification, while bacterial diversity was determined by amplification of 16S rDNA followed by denaturing gradient gel electrophoresis (DGGE).

If positive feedbacks between trophic levels maintain diversity in these communities, then we expect to find positive correlations in diversity between trophic levels. However, such associations may also arise due to correlated responses of different taxa to shared environmental gradients. That is, if the same limiting abiotic factors influence different trophic levels, then lakes with similar physical conditions may contain similar numbers of species. To control for variation in abiotic conditions, we measured a suite of limnological variables in each lake. In this way we were able to test for correlations in diversity among trophic levels that were independent of environmental gradients. We looked for correlations in diversity after accounting for variation that could be explained by measured abiotic variables. Such correlations imply a direct effect of diversity in one trophic level on that of another. Alternatively, the absence of such correlations suggests that feedbacks through facilitation between trophic levels play no detectable role in maintaining diversity. Finally, we asked whether the environmental variables that were strongly correlated with community composition were similar among trophic levels.

METHODS

Study region

We sampled 31 lakes throughout an area of ~30 000 km² in the southern interior and the mainland coast of southwestern British Columbia (Fig. 1). The lakes ranged in surface area from 20 to 395 ha. Lakes were selected to represent the full range of local environmental conditions to produce broad diversity gradients in the organisms sampled. Each lake was sampled on one occasion during the day between late May and early September 2004. At each site we measured physical (dissolved oxygen, pH, conductivity, temperature, light, Secchi depth), chemical (chlorophyll *a*, total phosphorus, total nitrogen, total organic carbon, dissolved organic carbon), morphometric (elevation, latitude, longitude, surface area, maximum depth), and biological (zooplankton, phytoplankton, pelagic bacteria) variables. See Appendix A for a list of lakes with corresponding variables.

Physical and chemical variables

A depth profile was established for temperature, dissolved oxygen, pH, conductivity, and light (PAR) measured at 1-m intervals from just below the surface to

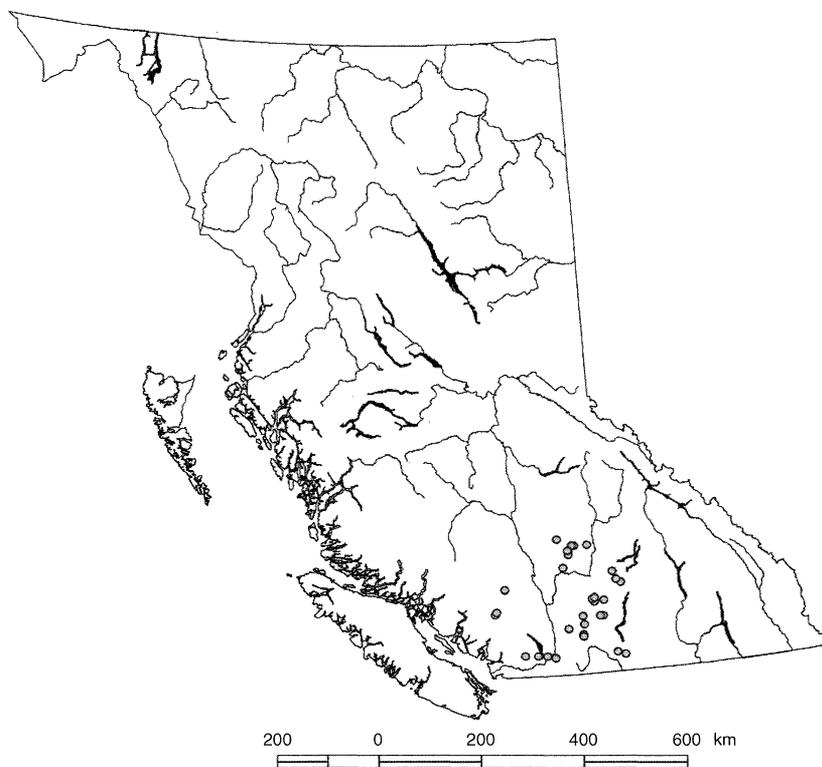


FIG. 1. Map of British Columbia, Canada, with sites sampled (gray circles).

the metalimnion. Vertical light attenuation for each lake was determined as the slope of \log_e irradiance (μmol) vs. depth (m). Water samples were collected at 1-m intervals from the subsurface to the metalimnion using a tube sampler (3.5 cm diameter). Samples were pooled across depths in the field and subsampled for total organic carbon (TOC), dissolved organic carbon (DOC), total phosphorus (TP), total nitrogen (TN), and chlorophyll *a* analyses. TOC and DOC (filtered through Whatman GF/F filters) samples were collected in dark, acid-washed glass jars and immediately stored on ice. TP and TN samples were collected in acid-washed Nalgene bottles and frozen immediately after collection. Chlorophyll *a* samples were collected by filtering lake water through Whatman GF/F filters, which were then wrapped in foil and frozen for later analysis, which occurred within 14 days. Both TP and chlorophyll *a* concentrations were determined using standard limnological methods (Wetzel and Likens 1991). TOC and TN concentrations were determined directly from liquid samples using a Shimadzu TOC-VCSH analyzer (Shimadzu, Kyoto, Japan) with a detection limit 4 $\mu\text{g C/L}$. DOC (mg C/L) concentrations were determined using a Dohrmann Phoenix 8000 UV-Persulfate analyzer (Tekmar Dohrmann, Cincinnati, Ohio, USA) with a detection limit of 0.1 mg C/L.

Plankton community richness

Phytoplankton (see Plate 1) and zooplankton richness were determined using morphological identification,

while bacterial richness was determined with molecular methods. Phytoplankton were collected by pumping water samples, ~ 1 L per depth interval, through tubing lowered at 1-m intervals through the photic zone. Samples were pooled across depths and thoroughly mixed. A 500-mL subsample was then collected and immediately fixed in Lugol's iodine solution. Phytoplankton were identified to genus or species under an inverted microscope at 100 \times , 200 \times , or 400 \times magnification (depending on sample density) after settling samples in 25-mL counting chambers for ~ 24 h. In most cases between 200 and 500 individual cells were counted. Zooplankton were collected by vertically hauling a plankton net (30-cm diameter opening, 1 m long, 54 μm Nitex mesh) through the water column to the surface from 1 m above the lake bottom. The concentrated sample was fixed in Lugol's solution. Zooplankton were most often identified to species, but sometimes only to genus. Crustaceans and rotifers were identified and enumerated under a 60 \times stereomicroscope. All individuals were counted if the sample contained fewer than ~ 250 animals. When more individuals were encountered, subsamples were taken with a Folsom plankton splitter (Van Guelpen et al. 1982).

Phytoplankton communities were sampled from the epilimnion, whereas zooplankton were collected from the whole water column. Many zooplankton show pronounced daily vertical migrations (Neill 1992); therefore species found at depth during the day may

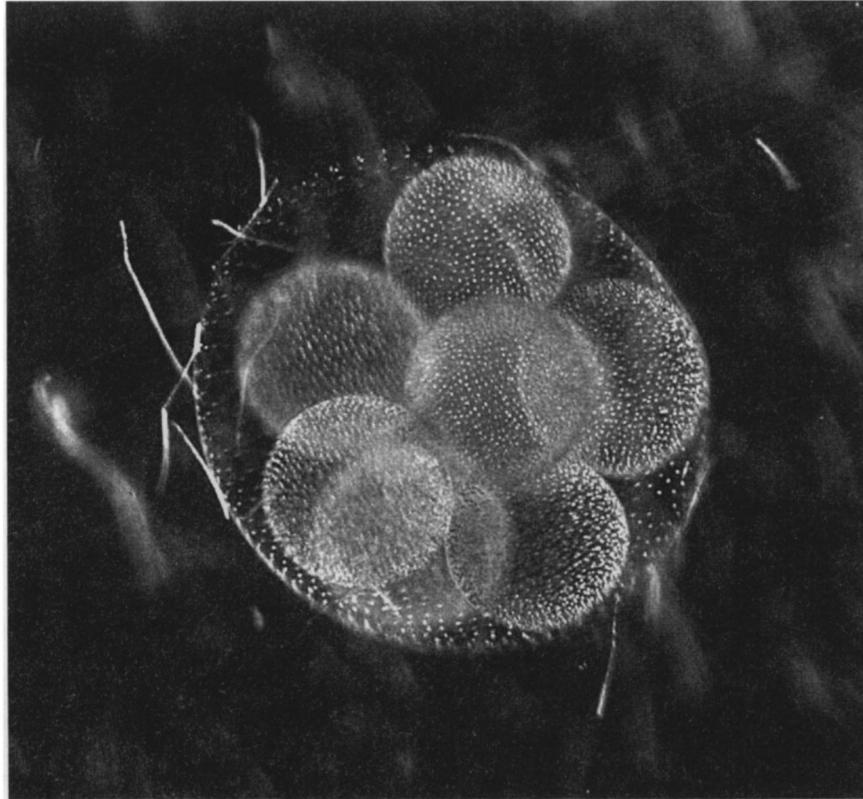


PLATE 1. Volvox colony (55 \times) collected from Salmon Lake, southwestern British Columbia, Canada, 2004. Photo credit: A. Longmuir.

have occupied the epilimnion at night. Our goal was to characterize the pelagic communities that coexist on a time scale of days. The bacterial community was sampled by lowering a length of weighted tubing, rinsed several times with site water, to the thermocline until 1 L was collected. This water was then filtered through a 0.42 μm -pore-sized nitrocellulose filter to remove larger particles. Bacterial cells were collected on a 0.2 μm -pore-sized nitrocellulose filter, which was immediately frozen in the field.

DNA isolation

Bacterial DNA was obtained with an extraction kit (MoBio Ultra Clean Soil DNA Kit # 12800-50; MO BIO Laboratories, Carlsbad, California, USA) and the resulting DNA was used as templates for the polymerase chain reaction (PCR). The 16S rDNA from each community was amplified with previously described primers (Muyzer et al. 1993). Four of the 31 bacterial samples did not successfully amplify. The amplified product was 200 base pairs in size and for this reason the primers used included a 40 bp GC clamp. The amplification was performed with a PTC-100 Programmable Thermal Controller (MJ Research, Watertown, Massachusetts, USA) and the following final volumes: 2 μL of DNA template, 0.125 μL of each primer, 2.5 μL of deoxyribonucleoside triphosphate, 2.5 μL of 10 \times PCR

buffer, and 2 units of Taq DNA polymerase (AmpliTaq, Roche Molecular Systems, Pleasanton, California, USA). The PCR began by incubating the samples for 7 min at 94 $^{\circ}\text{C}$ to denature the template DNA. The temperature was then lowered to an annealing temperature of 58 $^{\circ}\text{C}$ for 1 min. Primer extension was then performed at 72 $^{\circ}\text{C}$ for 3 min. The reactions ran for a total of 30 cycles. The presence of amplified products was confirmed by electrophoresis in 2% (mass/volume) agarose gels stained in ethidium bromide and viewed on a UV transilluminator.

Denaturing gradient gel electrophoresis

The richness of each bacterial community was determined by the number of bands resulting from denaturing gradient gel electrophoresis (DGGE). DGGE separates the 16S rDNA sequence polymorphs based on their tendency to dissociate at different concentrations of denaturants. This is a common technique for making comparisons between bacterial communities from environmental samples (Muyzer et al. 1993, Bell et al. 2005, Lyautey et al. 2005). DGGE was performed as described by Muyzer et al. (1993) using the D-Code Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, California, USA). Since more than one gel was required to run all the samples, an environmental sample (one of the sampled lakes), with a

broad enough band pattern to encompass the communities sampled, was used as a marker to standardize band patterns between gels. The gels used were 8% polyacrylamide with a denaturant gradient ranging from 20% to 55% (100% denaturant being 7 mol/L urea and 40% deionized formamide). Electrophoresis was run at a constant voltage of 200 V for three hours at 60°C in a buffer of 1× Tris-Acetate-EDTA (TAE). After electrophoresis, the gels were stained with 4 µL of 10000× SYBR Green I (Molecular Probes, Invitrogen, Carlsbad, California, USA) in 500 mL of 1× TAE and visualized by UV transillumination.

Bacteria richness

Taxonomic richness of the bacterial communities was estimated by the number of bands present in the DGGE, and the relative abundance of each taxonomic unit was estimated by the relative intensity of each band. GelCompar II software (Applied Maths, Austin, Texas, USA) was used to identify bands and estimate their intensity. A number of assumptions are necessary when using DGGE banding patterns to estimate taxa richness and abundance. The first is that each band represents one distinct sequence polymorphism in the rDNA. Cases may exist where a band contains DNA from several taxa, or a single taxa contributes more than one band (Sekiguchi et al. 2001). These issues may lead to errors where DGGE either over- or underestimates the genetic richness of the bacterial community. The second issue is that PCR bias may lead to differences in band intensity due to variation in the tendency of different sequences to amplify (von Wintzingerode et al. 1997). Band intensity may therefore be an imprecise reflection of the relative abundance of different groups (Eichner et al. 1999, Diez et al. 2004). However, all the issues with using DGGE to estimate community diversity have analogs in morphological indices (cryptic species, taxonomic uncertainty due to phenotypic plasticity, variable sampling efficiency for different species). DGGE offers one consistent measure of the genetic diversity of the bacterial community for comparison with other groups with many of the same limitations as other diversity metrics.

Richness and diversity metrics

The association between the richness of each trophic level and the matrix of environmental variables was tested by multiple regression analysis using SAS, Version 5.1 (SAS 2001). A Kolmogorov-Smirnov test was used to test variables for normality. Variables that significantly deviated from normality were transformed (\log_e or square-root transformations) to minimize the deviations. The models to predict species richness were constructed by first testing each environmental variable for relationships with richness in simple regression models including both the first- and second-order terms to allow for the possibility of nonlinear responses. Sampling date was also included as an independent variable to test for seasonal patterns in diversity and

richness. Environmental variables that had P values <0.10 in individual models were then entered together into the multiple regression model. A backward selection procedure was used to select the best model to explain species richness in zooplankton, phytoplankton, and bacterial communities. To test for correlations in richness across trophic levels that are independent of environmental variability, richness of the two other guilds was entered after the selection procedure had chosen the best model based on environmental variables. We also tested for correlations using the Shannon-Weiner diversity index to account for differences in abundance among taxa. The Shannon-Weiner index for each trophic level was analyzed in the same manner as richness in the multiple regression models.

Univariate analyses were used to identify associations between species richness and environmental gradients, and correlations in richness across trophic levels. We used multivariate gradient analyses to test for associations between community composition and abiotic factors, and to ask whether composition of the three trophic levels responded to similar abiotic factors. All multivariate analyses were performed with CANOCO 4.5 (ter Braak 1988). Direct gradient analyses were used to examine the relationship between community matrices and transformed environmental variables. To determine the most appropriate ordination method (unimodal or linear) a detrended correspondence analysis was completed for each species \times lake matrix (zooplankton, phytoplankton, and bacteria). The gradient length term measures the beta diversity in community composition. A gradient length between 3.0 and 4.0 indicates that either linear or unimodal methods are reasonable, while a gradient value >4.0 indicates that a unimodal method is appropriate (Lepš and Šmilauer 2003). For both zooplankton and phytoplankton the largest gradient length of the first four DCA axes was >3.0 , while the largest gradient for the bacteria matrix was >6.0 . We therefore used a unimodal ordination method, canonical correspondence analysis (CCA), for all three groups. CCA is an ordination analysis that uses the variation in the environmental matrix to explain the variation in the biotic matrix. The manual selection and random permutation procedures (reduced model, 999 random permutations; ter Braak and Šmilauer 1998) in CANOCO were used to choose the most parsimonious ordination for each trophic group. A biplot of sites and environmental gradients was created to visualize the results from the CCA. The importance of each environmental variable can be inferred from correlations between environmental variables and the species axes. To avoid the destabilization that can occur as a result of strong correlations among environmental variables when using canonical coefficients, inter-set correlations are reported here (Srivastava 1995). To test whether trophic levels were affected by similar abiotic factors, we tested for correlations between the loadings of each environmental variable on the species data across

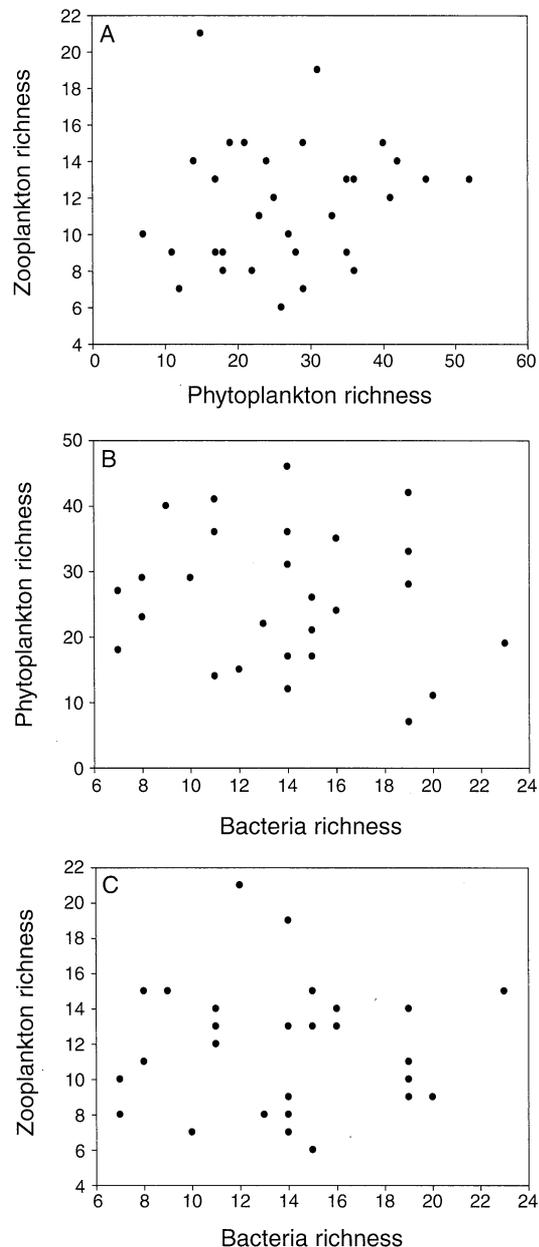


FIG. 2. Correlations in species richness between zooplankton, phytoplankton, and pelagic bacteria communities.

groups. A large loading indicates a strong association between the species and abiotic variable. If different trophic levels respond to the same limnological factors, then the loadings of predictor variables should be positively correlated between zooplankton, phytoplankton, and bacteria.

RESULTS

The lakes are characterized by a broad range of local environmental conditions (Appendix A). For example, lakes ranged in elevation from 50 to 1450 m. Lake trophic status ranged from oligotrophic to eutrophic

with broad concentrations of total nitrogen (<0.001 – 3.7 mg/L, mean = 0.45), chlorophyll *a* (7.0 – 1287.7 $\mu\text{g/L}$, mean = 116.0), total organic carbon (TOC, 0.61 – 69.77 mg/L, mean = 12.62), pH (6.54 – 9.17 , mean = 7.96), and conductivity (7.9 – 874 $\mu\text{S/cm}$, mean = 200.8).

A total of 40 crustacean and rotifer zooplankton species were identified (richness range across lakes = 6–21; median = 13) with the cladoceran *Daphnia pulex* present in 24 of the 31 lakes, often dominating the zooplankton community. Algal community richness totaled 156 taxa with individual lake samples containing 7–52 taxa (median = 26). Small flagellates dominated most communities. The range of bacterial taxonomic richness across lakes was 7–23 (median = 14) and the total number of bands was 60.

Richness of zooplankton, phytoplankton, and bacteria were uncorrelated among lakes (all $P > 0.2$; Fig. 2). Shannon-Weiner diversity index also showed no correlation between the three groups (Appendix B).

Models predicting species richness

Abiotic variables accounted for 29–48% of the variation in bacterial, phytoplankton, and zooplankton richness (Table 1). The variables that best predicted zooplankton richness included the first- and second-order terms for elevation, dissolved oxygen, and TOC ($R^2 = 0.40$, $P = 0.034$). Phytoplankton richness was best predicted by the first- and second-order term for Secchi depth ($R^2 = 0.29$, $P = 0.006$). The multiple regression model for bacterial richness was the only model improved by adding richness of either of the other two guilds. Bacteria richness was best predicted by the second-order term for light extinction, and the first- and second-order terms for dissolved oxygen and phytoplankton richness ($R^2 = 0.48$, $P = 0.013$). After accounting for the environmental variables, the relationship between bacteria and phytoplankton richness was nonlinear with a negative first-order term and positive second-order term. Of all the important environmental variables, dissolved oxygen was the only one common to more than one trophic level. However, zooplankton and bacteria showed opposite correlations with dissolved oxygen: the relationship between dissolved oxygen and zooplankton richness was positive, while that with bacterial richness was negative. Second-order terms were included in the significant predicting variables for each model, suggesting that community responses were often nonlinear. Sampling date was never a significant predictor of richness or diversity of any of the three groups (all $P > 0.15$); therefore, the patterns we found were not driven by differences in the timing of sampling of the different lakes.

Community composition

Relationships between the composition of each pelagic community and the environmental variables were tested using canonical correspondence analysis (CCA). The initial analyses included all lakes. However,

TABLE 1. Multiple regression models for richness in zooplankton, phytoplankton, and bacteria.

Model and source	Parameter value	Partial R^2	df	F	P
Zooplankton richness ($R^2 = 0.3982$)					
Elevation	-1.021	0.136	1, 29	5.63	0.026
Elevation ²	0.016	0.093	1, 29	3.81	0.062
DO	5.876	0.087	1, 29	3.62	0.069
DO ²	-0.391	0.084	1, 29	3.47	0.074
TOC	19.274	0.142	1, 29	5.89	0.023
TOC ²	-4.928	0.124	1, 29	5.13	0.032
Phytoplankton richness ($R^2 = 0.2915$)					
Secchi	77.998	0.255	1, 29	10.78	0.003
Secchi ²	-19.736	0.277	1, 29	11.74	0.002
Bacteria richness ($R^2 = 0.4775$)					
Light ²	3.38	0.247	1, 25	9.94	0.005
DO	-5.832	0.171	1, 25	6.89	0.016
DO ²	0.369	0.131	1, 25	5.27	0.032
Phytoplankton richness	-1.164	0.232	1, 25	9.31	0.006
Phytoplankton richness ²	0.02	0.197	1, 25	7.93	0.01

Notes: All variables left in the models were significant at $P < 0.10$ following backward stepwise selection procedure. Key to abbreviations: DO, dissolved oxygen; DOC, dissolved organic carbon; TOC, total organic carbon. The model for bacterial richness had fewer lakes because the bacterial DNA from four lakes failed to amplify in the PCR.

the relationship between community composition and the environmental gradients in the resulting ordinations were strongly influenced by Cultus Lake, a highly eutrophic lake (chlorophyll *a* concentration of 1287 $\mu\text{g/L}$) with an algal community dominated by colonial and filamentous cyanobacteria. The disproportionate effect of Cultus Lake obscured the relationships between the abiotic variables and the species matrix; therefore, it was removed from the analyses in the results that follow. The biplot for zooplankton shows an apparent outlier at the top center of the ordination. The removal of this community (Green Lake) did not significantly change the relationships so it was left in the analysis. The manual selection procedure was used to find the most important environmental variables explaining community composition. The ordination axes explained a moderate amount of variation for each community. The total amount of variation explained by the first four ordination axes for zooplankton, phytoplankton, and bacteria communities is 24.2%, 28.6%, and 32.8%, respectively. The variables most strongly correlated with the first axis were temperature ($r = -0.63$) in the case of zooplankton, elevation ($r = -0.51$) and temperature ($r = 0.47$) for phytoplankton, and Secchi depth ($r = -0.45$) for bacteria (Fig. 3).

Species composition in zooplankton, phytoplankton, and bacteria responded to independent environmental gradients. The loadings of the 18 environmental variables were unrelated between trophic levels (all absolute r values < 0.143 , $P > 0.572$; Fig. 4). Although the strength of association between species and environmental gradients is not correlated across trophic levels, some variables emerged as important for multiple groups. Temperature showed a strong loading for all groups. Chlorophyll *a* also showed a strong loading for both zooplankton and phytoplankton composition,

while maximum lake depth shows a strong loading for phytoplankton and bacteria (Fig. 4).

DISCUSSION

Our survey of British Columbian lakes found that the richness and composition of zooplankton, phytoplankton, and bacterial communities are independent of one another and respond to different environmental variables. Correlations in diversity across trophic levels can arise either through feedbacks involving consumers that prevent competitive exclusion, niche differentiation of consumers to utilize different resources, mutualisms between producers and decomposers through nutrient recycling, or common responses to the same environmental gradients. Our results provide no evidence for any of these mechanisms in the maintenance of species richness. Instead, the patterns indicate that independent processes control the richness of zooplankton, phytoplankton, and bacteria. The environmental gradients associated with species richness were different for each trophic level. The only exception was dissolved oxygen, which was positively correlated with zooplankton richness and negatively with bacterial richness. Previous studies have shown positive correlations between zooplankton abundance and dissolved oxygen as low oxygen concentrations reduce recruitment (Roman et al. 1993). The negative relationship between bacterial richness and dissolved oxygen may reflect high diversity of mixotrophic and anoxia-tolerant microbes under low oxygen. The limnological variables associated with community composition also showed no consistencies across the three guilds (Fig. 3). These patterns agree with others that suggest that diversity accumulates independently in different parts of planktonic food webs (Allen et al. 1999, Irigoien et al. 2004, Declerck et al. 2005), and

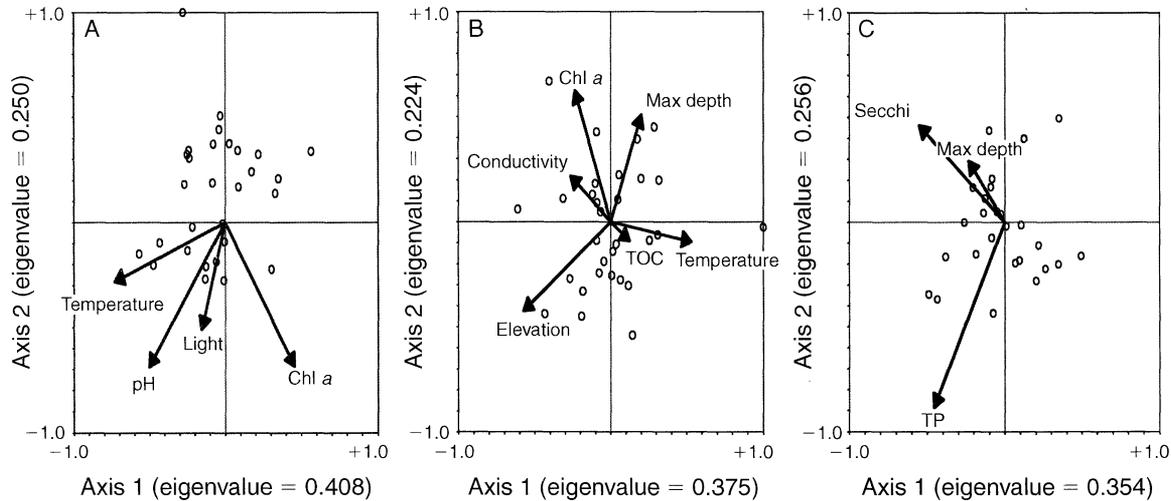


FIG. 3. Biplots of the first two CCA axes for the lake communities. The biplots show associations between the environmental gradients and species composition in each community (A, zooplankton; B, phytoplankton; C, bacteria). Individual lakes are represented by the open circles. Arrows represent environmental variables that predicted variation in composition based on permutation tests. Arrow length indicates the strength of correlation between the predictor variable and the species data. Abbreviations: TOC, total organic carbon; TP, total phosphorus.

that species diversity at one trophic level has little direct effect on diversity at other trophic levels.

Trophic complexity has been proposed as a potential solution to the paradox of species coexistence (Hutchinson 1961, Tilman and Pacala 1993). Previous studies have found support for mechanisms of intertrophic facilitation of species diversity. Interlandi and Kilham (2000) found that diversity of lake phytoplankton was closely associated with the evenness of four abiotic resources (light, N, P, and Si). The contrast with our results may arise because resource diversity for heterotrophs (mesozooplankton and bacteria) is more difficult to quantify than that for primary producers. Mineral nutrients and light are likely to represent essential resources for phytoplankton; however different phytoplankton taxa may be a substitutable resource for zooplankton that package nutrients in different ratios (Rothhaupt 2000). As a result, heterotroph diversity may be less closely tied to autotroph diversity than are autotrophs and their abiotic resources.

The lack of correlations in diversity between trophic levels may also reflect differences in taxonomic resolution and sampling efficiency, or trophic complexity within the three groups of organisms. Zooplankton and phytoplankton were consistently identified to genus or species; however, genetic diversity was measured in bacteria. Genetic diversity may be unrelated to phenotypic or functional diversity. However, species diversity and genetic diversity are often controlled by the same processes (i.e., drift, immigration, response to habitat heterogeneity) and show positive correlations in nature (Vellend 2005). Our measure of bacterial genetic diversity may therefore reflect morphological or functional variability of the microbial community. In addition, zooplankton include both herbivores and

predators of bacteria and other mesozooplankton, while bacteria include autotrophs, mixotrophs, detritivores, and pathogens. Bacteria are particularly functionally diverse; the groups represented by our DGGE bands may include autotrophs (cyanobacteria and anoxygenic phototrophs), chemotrophs (nitrifiers, colorless sulfur bacteria, and methanogens), and both aerobic and anaerobic heterotrophs. It is possible that diversity within the different functional forms of prokaryotes responds to that of zooplankton and phytoplankton; however methods such as ours would not detect such associations. Our taxonomic designation of "trophic levels" may be too coarse to detect the role of trophic facilitation of coexistence.

Our results suggest that correlations of autotroph and heterotroph diversity may be consistently stronger in terrestrial than aquatic ecosystems. Haddad et al. (2001) and Siemann et al. (1998) found positive correlations between species richness of terrestrial plants and insects in experimental grasslands on scales of 10 m². Hawkins and Porter (2003) found positive correlations between butterfly and plant diversity between bioregions in California, USA, although the relationship disappeared after accounting for variation due to productivity and topographic gradients. Their study suggested that correlations in diversity across trophic levels were driven by a correlated response to the environment rather than by direct causal links. Positive feedbacks have also been found between the diversity of soil microbes and plant diversity in grassland microcosms (Van der Heijden et al. 1998). Our results agree with the weak or absent richness correlations found in several other aquatic systems (Allen et al. 1999, Irigoien et al. 2004, Declerck et al. 2005; but see Shurin and Allen [2001]). This general contrast in richness patterns between terrestrial and

aquatic systems implies that the mechanisms of assembly and community structure operate differently in the two systems.

Several possible explanations may account for the contrasting patterns of diversity between terrestrial and aquatic ecosystems. First, consumers may be less specialized in aquatic environments than on land (Strong 1992, Irigoien et al. 2004, Declerck et al. 2005). Many planktonic herbivores are filter feeders and therefore may lack the strong associations that terrestrial plants and herbivores have as a consequence of specialized diets or structural complexity. Second, Declerck et al. (2005) suggests that differences of scale may explain the resulting inconsistencies between aquatic and terrestrial systems. Many experimental terrestrial studies have made comparisons on relatively small local scales of experimental plots rather than regional or global scales. However, it is interesting to note that the large spatial scale of the study by Hawkins and Porter (2003) did not obscure the positive correlations between California butterfly and plant diversity.

Our results provide no indication for a direct or consistent effect of consumer diversity on producer coexistence. This result does not imply that consumers have no impact on the diversity of producers, rather that consumers and primary producers in lakes are not linked in tight pair-wise associations as envisioned by Grover (1994). A number of other studies have found strong predator effects on prey diversity that range from positive to negative depending on habitat characteristics such as connectivity or productivity (Chase et al. 2002). For example, Shurin (2001) found that fish and insect predators reduced zooplankton diversity in isolated pond communities, but increased it when immigration from the regional pool was allowed. Proulx and Mazumder (1998) used meta-analysis to show that predators tend to reduce prey diversity at low productivity and increase it in eutrophic systems. Similarly, Worm et al. (2002) found that the effects of consumers on macroalgae diversity in rocky shore communities shifted from negative to positive as productivity increased. Our results suggest that although particular predators may have major effects on prey coexistence, the number of predator taxa per se does not consistently enhance or reduce prey diversity.

Our results also suggest that genetic diversity of the bacterial community responds to different gradients in the physical environment than either zooplankton or phytoplankton richness. Horner-Devine et al. (2004) argued that patterns of bacterial diversity across environmental gradients "may be qualitatively similar to those observed for plants and animals." We found that while the general processes that structure bacterial communities may be similar to those of eukaryotes, the specific environmental factors are different in lake plankton.

Bacterial diversity in our lakes was most strongly associated with indicators of productivity (light attenu-

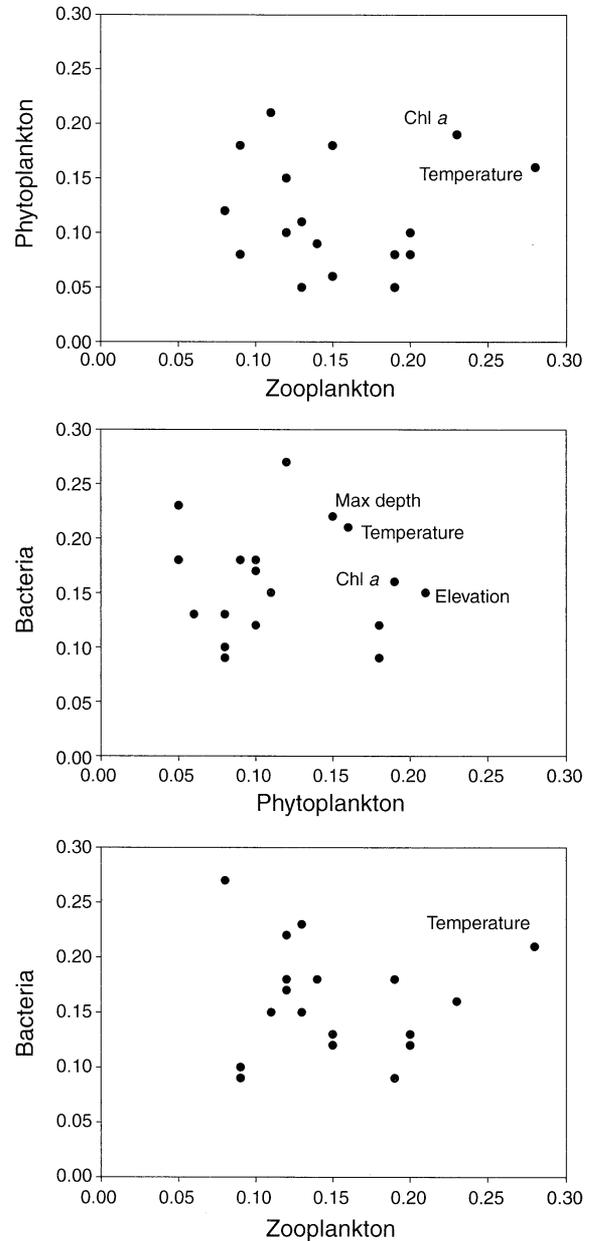


FIG. 4. Correlations of environmental loadings between communities. Each point is the variance explained by a particular environmental variable. Variables with large loadings in two trophic levels are labeled.

ation and dissolved oxygen; Table 1). Total phosphorus was also one of the variables describing variation in bacterial community composition (Fig. 3). These results agree with a number of other studies. Lindstrom (2000) found that bacterial composition in Swedish lakes was most closely associated with the biomass of microzooplankton, cryptophytes, chrysophytes, and trophic status. Yannarell and Triplett (2005) found that bacterial richness was associated with pH and Secchi depth in Wisconsin, USA, lakes. However, Horner-

Devine et al. (2003) found that the relationship between productivity and diversity varied among bacterial lineages. Kent et al. (2004) found that bacterial richness declined in the summer when mixotrophic and heterotrophic flagellates became most abundant, suggesting that consumer pressure reduces diversity in bacteria. Reche et al. (2005) found that lake area was related to bacterial richness (also measured by the number of DGGE bands), in contrast with our results. Their lakes were smaller than ours but covered a similarly small size range (0.01–2.1 ha vs. 20–395 ha). Overall, bacterial community composition and diversity appear to be most strongly associated with lake productivity although area and consumer pressure may also be important.

Discrepancies between our study and other bacterial diversity relationships may reflect methodological or taxonomic differences. Different methods of DNA-based community fingerprinting (ARISA, T-RFLP, DGGE) are commonly used to determine bacterial diversity. Method-specific bias may contribute to contrasting results. Methods like DGGE that consider all taxa present (i.e., do not discriminate between active and dormant taxa) may not accurately represent the functionally active community. Finally, bacteria as a group may not represent a single trophic level, as prokaryotes can function as decomposers, pathogens, or autotrophs (Wetzel and Likens 1991). The lack of consistent correlations between bacterial and eukaryotic diversity may reflect a combination of positive- and negative-feedback mechanisms in the interactions between these organisms.

Models to predict zooplankton or phytoplankton species richness were not significantly improved by including richness from the other two trophic levels. By contrast, bacteria richness was negatively related to phytoplankton richness after accounting for variation due to light and dissolved oxygen. This result contrasts with Declerck et al. (2005) who found no associations in richness between these two trophic levels. Although the association we observed is weak (partial $R^2 = 0.232$), the negative relationship implies that a mechanism other than facilitative nutrient cycling may be important. For example, heterotrophic or mixotrophic bacteria can compete with phytoplankton for dissolved mineral nutrients (Rothhaupt and Güde 1992). Our measure of prokaryotic diversity does not separate the autotrophic and heterotrophic portions of the bacterial community; therefore bacteria and phytoplankton may interact through competition, nutrient recycling, and predation.

Different limnological variables were associated with richness in phytoplankton, zooplankton, and bacteria. Dissolved oxygen, total organic carbon, and elevation best predicted zooplankton richness, while Secchi depth was most important for phytoplankton richness, and light, dissolved oxygen, and phytoplankton richness for bacteria. In contrast, previous studies have found pH to be an important controlling factor of zooplankton (Yan et al. 1996, Shurin et al. 2000, Arnott et al. 2001),

phytoplankton (Arnott et al. 2001), and bacterial richness (Yannarell and Triplett 2005, Fierer and Jackson 2006). Lake area was not a strong predictor of local richness in contrast to several previous studies (Dodson 1992, Allen et al. 1999), perhaps due to the smaller range that we sampled. Indicators of productivity were often associated with zooplankton, phytoplankton, or bacterial richness (Table 1), and often showed unimodal patterns similar to other studies (Mittelbach et al. 2001, Declerck et al. 2005). The variation in environmental control between trophic levels suggests that the important niche axes for coexistence differ between the three groups. Opportunities for differentiation include inorganic compounds and light in phytoplankton (Interlandi and Kilham 2000, Stomp et al. 2004), organic molecules in heterotrophic bacteria (Naeem et al. 2000), and habitat partitioning (Leibold 1991), as well as particles of different size and composition in zooplankton (Sommer 1989). The lack of association in diversity between trophic levels may arise because the potential axes for coexistence through ecological specialization show little overlap.

Conclusions

We found no evidence for associations in diversity between producers, consumers, and microbes in lakes. These patterns are consistent with other studies of lakes (Allen et al. 1999, Declerck et al. 2005) and marine plankton (Irigoien et al. 2004), but contrast with studies of terrestrial plants and insects (Murdoch et al. 1972, Siemann et al. 1998, Haddad et al. 2001, Hawkins and Porter 2003). Understanding the control of diversity in natural systems is complicated by the range of processes and interactions that affect coexistence. The lack of strong richness associations across trophic levels does not imply that trophic interactions are unimportant as agents of diversity. Rather, the results suggest that tight pair-wise associations between consumers, producers, and decomposers do not drive positive feedbacks in community assembly. Pelagic food webs may not be assembled through sequential invasion of specialist consumers and resources (Grover 1994). Instead, a few strongly interacting grazers, producers, and decomposers may play dominant roles in driving diversity patterns. Other solutions to the paradox of the plankton (spatial or temporal heterogeneity, nonequilibrium dynamics) may explain the high diversity observed in the pelagic zones of lakes.

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APPENDIX A

A table of the lakes sampled for this study, and their major physical characteristics (*Ecological Archives* E088-097-A1).

APPENDIX B

Correlations in Shannon-Weiner diversity between zooplankton, phytoplankton, and pelagic bacteria communities (*Ecological Archives* E088-097-A2).