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DOES ECOSYSTEM SIZE DETERMINE AQUATIC BACTERIAL RICHNESS?

ISABEL RECHE,[1,2] ELVIRA PULIDO-VILLENA,[2] RAFAEL MORALES-BAQUERO,[1] AND EMILIO O. CASAMAYOR[3,4]

[1]*Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, Granada 18071, Spain*
[2]*Instituto del Agua, Universidad de Granada, Granada 18071, Spain*
[3]*Unidad de Limnología, Centro de Estudios Avanzados de Blanes-CSIC, 17300 Blanes, Girona, Spain*

Abstract. With the advent of DNA-based molecular technologies, microbial ecologists now have the tools to test whether general ecological patterns apply to microorganisms. In this study, we selected 11 high-mountain lakes from Sierra Nevada (Spain) to test the predictions of island-biogeography theory in relation to ecosystem size and isolation, and to assess the influence of other factors (i.e., ecosystem productivity, resource richness, and biotic interactions) on bacterial community structure. Bacterial operational taxonomic units (OTUs), generated by denaturing-gradient gel electrophoresis of polymerase chain-reaction-amplified 16S rRNA genes, were used as a surrogate of predominant ''biodiversity units.'' OTU composition among lakes was heterogeneous, and the number of site-specific OTUs was near 50%. Lake remoteness did not affect the number of bacterial OTUs although the spatial distribution of the lakes significantly influenced bacterial composition. Lakes that were closer together had more similar bacterial fingerprints. We found a consistent positive association between bacterial OTU richness and lake area. The slope of this relationship (0.161 ± 0.026, including literature data) was similar to slopes obtained for organisms with high dispersion rates.

Key words: bacteria; bacterial community structure; dispersion; diversity; ecosystem size; island biogeography; richness.

INTRODUCTION

Species richness and diversity in biological communities appear to be controlled by several, non-exclusive factors such as biological interactions (competition and predation), ecosystem productivity, and both habitat size and heterogeneity (Tonn and Magnuson 1982, Chesson 2000, Mittelbach et al. 2001). The relationship between species richness and habitat size has become one of the most consistent of all ecological patterns (Rosenzweig 1995) and it appears to be one of the few laws in ecology (Lawton 1999). Within this context, the ''theory of island biogeography'' proposed by MacArthur and Wilson (1967) offers two major predictions. First, larger islands will support more species than smaller islands. Second, the number of species will decline with the increasing remoteness of an island. These predictions are based on variable dispersal success, depending on island size and remoteness, and variable rates of extinction and speciation.

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[4] Corresponding author: E-mail: casamayor@ceab.csic.es

Prior to the application of genetic tools to study microbial diversity, biogeography (the study of the distribution of species) was mostly focused on macroorganisms and microorganisms were largely ignored. The traditional hypothesis among microbiologists, ''Everything is everywhere, but the environment selects'' (Baas-Becking 1934), presumes ubiquity based on the high dispersal rates for microorganisms. This hypothesis has been reinforced by field studies in eukaryotic microorganisms (Finlay and Clarke 1999, Finlay 2002). In contrast, recent studies suggest that some microbial taxa can exhibit geographical isolation and marked distribution patterns (Whitaker et al. 2003, Papke et al. 2003). Therefore, there is an active debate on the existence or absence of bacterial biogeographical patterns (Fenchel 2003, Horner-Devine et al. 2004*a*).

Lakes and ponds can be considered as islands within a ''sea'' of land (Dodson 1992). In particular, high mountain lakes exemplify remote systems usually far from human impact where aquatic biota undergo extreme physical conditions such as low temperatures and high UV exposure. In addition, these lakes are ice covered most of the year and are very sensitive to environmental changes (Sommaruga and Psenner 2001). Therefore, high-mountain lakes appear to be suitable

TABLE 1. Location, catchment area, landscape heterogeneity (LH), morphometry, lake absorption coefficients at 320 nm (a_{320}), chlorophyll-*a* (Chl. *a*), total phosphorus (TP), dissolved organic carbon (DOC), bacterial richness (BR), and bacterial diversity (BD) in the study lakes.

Lake (lake code)	Location UTM (30S)†	Altitude (m above sea level)	Catchment area (ha)	Watershed and aspect‡	LH§
Río Seco (RS)	VG694009	3020	9.9	RS-S	M/IO
Caldera (CA)	VG708012	3050	23.5	CA-S	R
Aguas Verdes (AV)	VG674006	3050	12.8	AV-S	M/IO
Yeguas (YE)	VG662013	2880	50.0	V-N	R
Virgen Superior (VS)	VG665008	2950	25.1	V-N	M
Virgen Media (VM)	VG664009	2940	31.2	V-N	M
Laguna 2 (L2)	VG735014	3020	27.4	7L-S	M
Laguna 4 (L4)	VG737012	2970	57.1	7L-S	M
Laguna 5 (L5)	VG734009	2980	50.9	7L-S	M
Laguna 7 (L7)	VG739004	2890	154.6	7L-S	M/IO
Peñón Negro (PN)	VF738983	2820	28.2	PN-S	M

† Universal Transverse Mercator coordinate system, zone 30S.
‡ Watersheds and faces: RS (Río Seco), CA (Caldera), AV (Aguas Verdes), V (Virgen), 7L (Siete lagunas), PN (Peñón Negro); S (southern face), N (northern face).
§ Landscape heterogeneity: M, partially surrounded by meadows; R, rocky terrain; IO, presence of inlets and/or outlets.
‖ Data from Morales-Baquero et al. (1999).

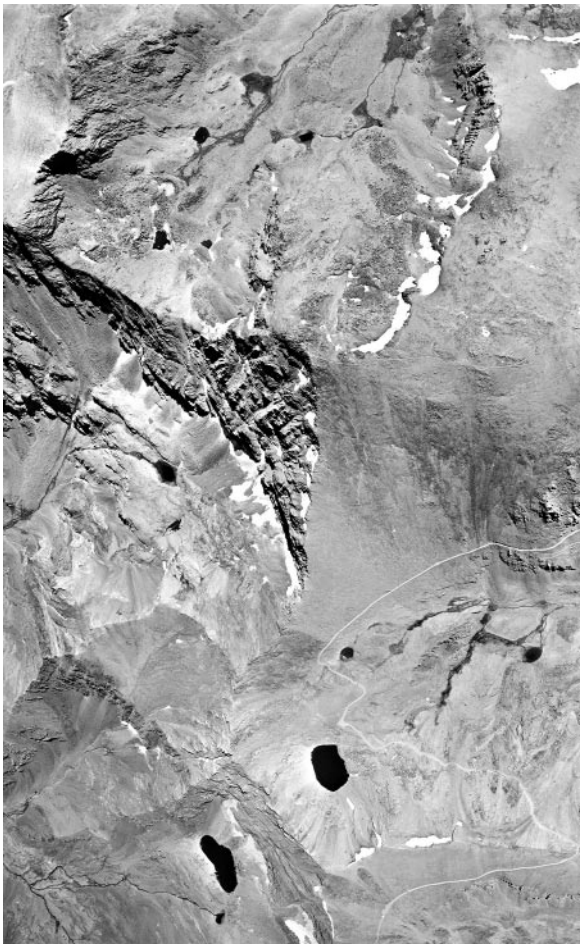

PLATE 1. Aerial photograph (scale 1:17 000) showing some of the high-mountain lakes studied in the Sierra Nevada (Spain) on both sides of the Mulhacen Peak (3482 m above sea level), like islands surrounded by a ''sea'' of land. Photo credit: Instituto de Cartografía de Andalucía.

systems to test island-biogeography predictions for microorganisms.

In this study we selected 11 lakes located in remote areas of Sierra Nevada (southeastern Spain) at elevations of 2800–3100 m above sea level (see Plate 1). We used a 16S rRNA gene-based fingerprinting technique (Muyzer 1998) to determine bacterial community structure (richness, diversity, and composition). Fingerprints were banding patterns where each band was related to one single population and translated to one operational taxonomic unit (OTU) that was considered as a surrogate of the predominant bacterial ''species'' present. Our specific goals were: (1) to test the island-biogeography predictions for effect of lake size and isolation and (2) to investigate other biotic and abiotic predictors of bacterial richness, diversity, and composition.

MATERIALS AND METHODS

Study sites

The study lakes are located above tree line, have small catchments, and receive low inputs of mineral and organic nutrients (Morales-Baquero et al. 1999, Reche et al. 2001). They are of glacial origin, small and shallow, and ice covered for 8–9 months every year. The following lakes were selected from different watersheds of varying aspect to represent the regional heterogeneity (Table 1): Caldera (CA), Yeguas (YE), Rio Seco (RS), Aguas Verdes (AV), Virgen Superior (VS), Virgen Media (VM), Laguna 2 (L2), Laguna 4 (L4), Laguna 5 (L5), Laguna 7 (L7), and Peñón Negro (PN). Chemical and biological details from these lakes can be found elsewhere (Morales-Baquero et al. 1999, Morales-Baquero and Conde-Porcuna 2000, Reche et al. 2001, Pulido-Villena et al. 2003).

Sampling and chemical analyses

Both space and time scales affect species richness estimates (Rosenzweig 1995). In this study, all sam-

TABLE 1. Extended.

Lake‖							
Max. depth (m)	Size (ha)	a_{320} (m^{-1})	Chl. *a*‖ (μg/L)	TP (μmol/L)	DOC (μmol/L)	BR	BD
2.0	0.42	1.65	0.5	0.47	119	8	1.47
7.0	2.10	1.08	0.2	0.08	113	6	1.20
2.8	0.19	2.90	0.6	0.59	102	6	0.89
2.5	0.33	0.92	2.2	0.04	78	6	1.66
1.3	0.08	1.09	0.4	0.17	47	6	1.10
0.8	0.01	5.68	2.2	0.76	223	4	0.57
3.5	0.34	8.54	0.5	1.10	300	9	1.98
0.4	0.19	2.24	0.7	0.24	75	5	1.13
2.0	0.18	2.65	1.7	0.43	91	9	1.98
0.8	0.53	1.67	0.8	0.30	47	7	1.24
2.0	0.67	3.27	4.1	1.18	106	8	1.44

pling was carried out within four days to minimize time variations. Samples from the pelagic zone were taken at one single point during the ice-free period of 2000 for analysis of nucleic acids, dissolved organic carbon (DOC), absorbance, total phosphorus (TP), and other bacterial parameters published elsewhere (Pulido-Villena et al. 2003). Because these high-mountain lakes are very small, shallow, and well mixed by wind, we assumed low within-lake variability and we took only one sample at the center of the lake as representative of the pelagic zone. Recent studies show that the greatest portion of the bacterial sample variation is associated with the among-lake variability (Casamayor et al. 2000, Yannarell and Triplett 2004). Duplicate samples for TP were analyzed following Murphy and Riley (1962) after digestion. For DOC concentration, duplicate samples were filtered through precombusted Whatman GF/F glass fiber filters and analyzed in a Shimadzu TOC analyzer (Model 5000) (Duisburg, Germany) equipped with a Shimadzu platinized-quartz catalyst for high-sensitivity analysis (Benner and Strom 1993). Triplicate samples for water absorbance were filtered through Whatman GF/F filters and absorbance at the specific wavelength of 320 nm was measured in a spectrophotometer and expressed as Napierian absorption coefficients (a_{320}) in m^{-1} (Miller 1998). This coefficient is significantly related to UV transmission into the water column (Laurion et al. 2000).

Nucleic acids, DGGE, and band-pattern analyses

A variable volume (200 to 2500 mL) of lake water was pre-filtered through a 52-μm mesh net to eliminate large zooplankton and algae, and then filtered through 0.2-μm polycarbonate filters (Nuclepore Corporation, Pleasanton, California, USA) until filter clogging decreased flow rate to <1 mL/min. Because differences up to one order of magnitude were found in cell abundance among lakes (Pulido-Villena et al. 2003), filter clogging gave an indication that we processed a similar number of cells for each sample. Filters were stored frozen at −70°C until treatment. DNA extraction, bacterial 16S rRNA genes PCR-amplification, denaturing-gradient gel electrophoresis (DGGE), and image analysis were carried out as previously described (Casamayor et al. 2000). Universal bacterial primers 341f and 907r were used (Casamayor et al. 2000) and about 600 ng of polymerase chain reaction (PCR)-amplified DNA was added to each lane. The intensity of each band was measured by integrating the area under the peak and was expressed as percentage of the total area in the lane. The error measured among analytical replicates was <4%. A band was defined as a stain signal with intensity >0.2% of the total intensity for each lane. Bands occupying the same position in the different lanes were identified, and a qualitative matrix (presence/absence) was constructed. The binary data set was used to calculate a dissimilarity matrix with the Jaccard's coefficient. The number of bands and the intensity of each band were used to calculate the most widely used diversity index, Shannon-Weaver (H'), as follows:

$$H' = \sum_{i=1}^{i=n} p_i \ln p_i \quad (1)$$

where n is the number of bands in the sample of each lake and p_i the relative intensity of the ith band. From here on and for convenience, we use the term ''OTU'' (operational taxonomic unit) for each DGGE band and the term ''OTU composition'' for the DGGE band pattern.

Testing the predictions of island-biogeography theory

The two main predictions of island-biogeography theory were tested. First, the number of species on an island (or in a lake) is predicted by a power function (Eq. 2) where A is the island (lake) size (in square meters), S is the number of species (DGGE bands), C is a parameter that depends on the taxon, biogeographical region, and population density (the intercept in the log-transformed function, Eq. 3), and z is a parameter that changes very little among taxa (the slope of the log-transformed function, Eq. 3):

$$S = CA^z \quad (2)$$

$$\log_{10} S = \log_{10} C + z \log_{10} A. \quad (3)$$

We determined the area–bacterial OTUs relationships specifically for the study lakes and also including data from other lakes of different geographical regions (Lindström and Leskinen 2002, Zwart et al. 2002).

The second prediction is that more remote lakes will have lower species richness. Lake remoteness (how grouped or dispersed is a lake in the geographical space) was determined by using an index of habitat connectivity proposed by Hanski (1994) and simplified by us for lakes as

$$\Gamma_i = \sum_{j \neq i} e^{-d_{ij}} A_j \quad (4)$$

where Γ_i is the connectivity of lake i with respect to the remaining lakes ($j \neq i$) of Sierra Nevada, d_{ij} is the Euclidean distance (in kilometers) between lake i and the remaining lakes (including also those that were not sampled in the present study), and A_j is the area (in square kilometers) of each lake different from lake i ($j \neq i$). A high value of Γ_i indicates that lake i is surrounded by many and/or relatively large lakes, so that the likelihood of being colonized by a given species is high. By contrast, a low value of Γ_i indicates that lake i is surrounded by few and/or relatively small lakes, so that the likelihood of being colonized is low.

Other potential controlling factors of bacterial community structure

Lake productivity, resource richness, and biological interactions were assessed as potential controlling factors of OTU richness and diversity using multiple regressions. Concentrations of chlorophyll-*a* (data from Morales-Baquero et al. 1999), and DOC and TP were used as surrogates of lake productivity and resource richness, respectively. Regulation by biological interactions was assessed by relating bacterial richness to rotifer richness (as a surrogate of congruence). Usually, a high level of congruence among taxa exists for endemic or rare species (Balmford and Long 1996). Therefore, high species richness in a given taxon would be associated with high species richness in another taxon. Data on the richness of rotifers were obtained from Morales-Baquero (1985). All variables were $\log_{10}$-transformed to fit normality assumptions. To determine the controlling factors of bacterial composition in the lakes, standard and partial Mantel tests were performed using the PASSAGE software for PC (Rosenberg 2001). The standard Mantel test is used to compare two independent dissimilarity matrices describing the same set of entities and to test whether the association is stronger than one would expect from chance (Sokal and Rolf 1995). The partial Mantel test is used to determine the relationship between two matrices while holding another one constant (Smouse et al. 1986). Dissimilarity matrices were calculated using the coefficient of Euclidean distances for quantitative data. The controlling factors tested were: lake spatial distribution, physical constraints, resources, and biotic interactions. The spatial-distribution matrix was obtained from the distances between pairs of lakes. The physical-constraints matrix included: a_{320} (an index of ultraviolet radiation stress), catchment area, maximum depth, and lake area. The resources matrix included: chlorophyll-*a*, TP, and DOC. Finally, the dissimilarity matrix of biotic interactions was built using rotifers species composition in the lakes using the Jaccard's coefficient (data from Morales-Baquero 1985).

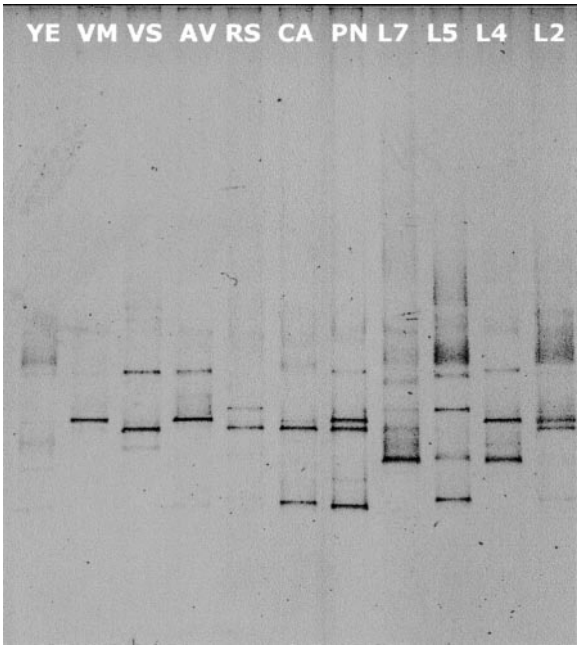

FIG. 1. Results of denaturing-gradient gel electrophoresis (DGGE) for bacterial 16S rRNA genes in the 11 high-mountain lakes from Sierra Nevada (Spain). Each DGGE band is an ''operational taxonomic unit'' (OTU). Lake codes are indicated in Table 1.

RESULTS

Fig. 1 shows the DGGE (denaturing-gradient gel electrophoresis) gel obtained for bacterial 16S rRNA genes after DNA extraction and PCR (polymerase chain reaction) amplification. The OTU's (operational taxonomic unit's) richness ranged from 4 (VM) to 9 (L2 and L5) (Table 1) and bacterial assemblages were very heterogeneous among lakes. We observed 26 different OTUs in the gel, but we did not detect any OTU that occurred in the complete set of lakes. Only one OTU was found in 10 of the 11 lakes (YE was the exception), although this OTU was a minor component with relative intensities ranging from 0.8% to 8.7%. Conversely, we found 12 bacterial OTUs that were site specific.

To test the first prediction of island-biogeography theory, we determined the area–bacterial OTUs relationships specifically for the study lakes and literature

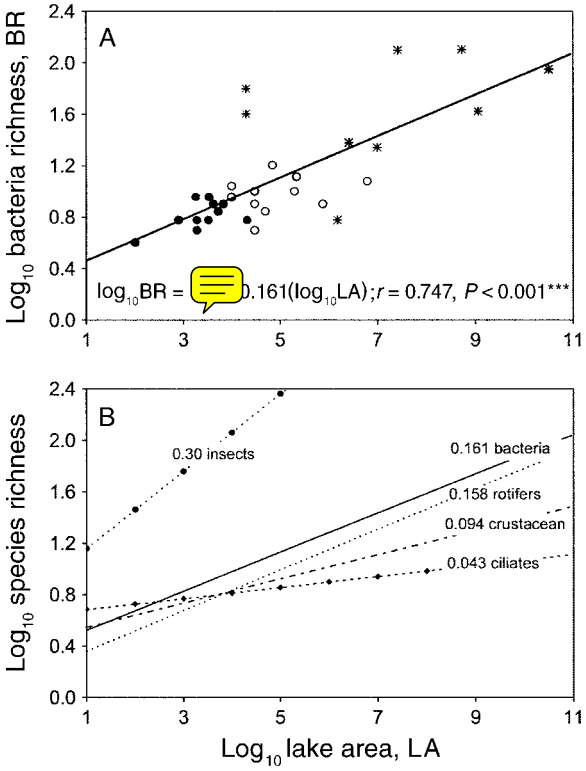

FIG. 2. (A) Relationship between bacterial OTU richness and lake area, including data from literature (solid circles correspond to this study, open circles to Lindström and Leskinen [2002], and asterisks to Zwart et al. [2002]). (B) Comparison of the relationship between species richness and ecosystem size among taxa (lake area was measured in square meters). Note that numbers on the lines indicate the slope values.

data (Lindström and Leskinen 2002, Zwart et al. 2002). For Sierra Nevada lakes, a positive and marginally significant (n = 11 lakes, r = 0.562, P = 0.072) relationship was observed between log lake area (in square meters) and bacterial OTU richness. The slope (z) of this relationship was 0.104 ± 0.051 (mean ± 1 SE). For literature data alone we found a similar relationship (n = 21 lakes, r = 0.643, P = 0.002) with z value of 0.152 ± 0.042. The slope of this second relationship was not significantly different (df = 28, t = 0.727, P = 0.473) from the slope obtained specifically for the Spanish lakes. Overall, the area–bacterial OTU richness relationship including literature data (Fig. 2A) was positive and significant (n = 32 lakes, r = 0.747, P < 0.001) with z value of 0.161 ± 0.026.

To test the second prediction of island-biogeography theory, we related lake connectivity (Γ_i) to bacterial OTU richness and diversity. No significant relationships were observed between Γ_i and bacterial OTU richness (n = 11 lakes, r = 0.261, P = 0.438, power = 0.197) or diversity (n = 11 lakes, r = 0.268, P = 0.425, power = 0.203). To assess other potential controlling factors of OTU richness and diversity we performed multiple-regression analyses selecting chlorophyll-*a* concentration (as surrogate of lake productivity), DOC and TP (as surrogates of resource richness), and rotifer species richness (as surrogate of biological interactions) as independent variables. No significant results were obtained either for bacterial OTU richness (P = 0.569) or diversity (P = 0.772). To assess the major factors controlling bacterial community composition, the similarity matrix obtained from the DGGE banding pattern was compared to matrices of spatial distribution, physical constraints, resources, and biotic forces using Mantel tests (Table 2). The composition of the bacterial assemblages appeared to be significantly influenced by the spatial distribution of the lakes in Sierra Nevada (r = 0.29, P = 0.025). The shorter the distance between two lakes, the more alike the bacterial fingerprints. To determine if there was another potential controlling factor while the spatial distribution of lakes is fixed we performed partial Mantel tests. No significant results were found for any of the factors considered (Table 2).

DISCUSSION

The ''theory of island biogeography'' proposes that size and remoteness of an island determine its species richness. The positive relationship observed in this study between lake area and bacterial OTU (operational taxonomic unit) richness is consistent with the first prediction of this theory (Fig. 2A). In general, the existence of more species on large islands than small islands may be related to (1) lower extinction rates and higher immigration rates and/or (2) the presence of

TABLE 2. Results of Mantel test comparison between bacterial-composition matrix and spatial-distribution (distance between pairs of lakes), physical-constraints (a_{320}, catchment area, maximum depth, lake area), resources (chlorophyll-*a*, total phosphorus, dissolved organic carbon), and rotifers-composition matrices.

Matrix type	Mantel			Partial Mantel†		
	Z	r	P	Z	r	P
Spatial distribution	350 351	0.292	0.025*	…	…	…
Physical constraints	316	−0.025	0.511	0.11	0.00	0.98
Resources	7327	−0.225	0.961	−206	−0.21	0.20
Rotifers	54	0.060	0.301	0.06	0.04	0.41

* P < 0.05.
† The partial Mantel test holds spatial distribution constant.

more available niches (Angermeier and Scholosser 1989). Microorganisms usually have vast population sizes and high dispersion rates, most populations are probably cosmopolitan, and extinctions and allopatric speciations are expected to be rare (Fenchel 1993, 2003). Therefore, the balance among extinction, immigration, and speciation rates likely plays a minor role in determining the number of bacterial OTUs in lakes.

Higher niche availability seems then to be more plausible for the case of microorganisms, using either the Eltonian (i.e., what a species is doing) or Hutchinsonian (i.e., interaction of multiple resources and factors) views of the niche (Schoener 1989). In fact, more complex (longer) food webs have been observed in larger lakes (Post et al. 2000). Therefore, lake size could have influenced the bacterial richness observed here more through food-web complexity than through effects of target size for colonization. If large lakes are more heterogeneous in their niche availability, then we have to consider whether collecting a single sample per lake, as we did here, could have led to a spurious correlation. The range of lakes was relatively small, shallow, nonstratified and well lit, probably reducing effects of lake size on within-lake heterogeneity at the bacterial level. The similarity in regression slopes for Sierra Nevada lakes (Fig. 2A: solid circles) and data from other larger lakes sampled with different protocols (e.g., composite samples from different sites, Lindström and Leskinen 2002) suggests that the sampling strategy used here did not bias the observed pattern. In any case, a potential underestimation of bacterial richness in the large lakes would not generate a positive relationship between species richness and lake size such as we observed.

The slope of bacterial OTU richness–lake area relationship was close to the slopes found for other planktonic organisms (Fig. 2B) such as crustacean zooplankton ($z = 0.094$, data from Dodson [1992]) or rotifers ($z = 0.158$, data from Dodson et al. [2000]); slightly higher than that for benthic ciliates ($z = 0.043$, data from Finlay et al. [1998]) or for bacteria from saltmarsh sediments ($z = 0.040$, data from Horner-Devine et al. [2004*b*]) and far from the high slope ($z = 0.31$) observed in macroorganisms such as insects (Gaston 1992). All the slopes obtained for planktonic or sediment microorganisms are at the lowest limit of the range (0.1–0.5) reported for very diverse taxa by Connor and McCoy (1979). In general, a low slope in the species richness–ecosystem area relationship is consistent with (1) high dispersion rates and low extinction rates due to vast population sizes (Connor and McCoy 1979), and (2) presence of continental islands (e.g., lakes, high mountains, fragmented habitats), which usually present lower slopes (range 0.12–0.17) compared with oceanic terrestrial islands where recolonization is more difficult (Wilson 1961). Therefore, we found a very consistent pattern between bacterial OTU richness and lake area with a z value typical of planktonic organisms that have high dispersion rates. This rationale is also indirectly supported by the lack of influence of lake connectivity (how grouped or dispersed is a lake, see Eq. 4) on bacterial OTU richness. In this study, the number of bacterial OTUs in a lake appears to be independent of its remoteness, contradicting the second prediction of the island-biogeography theory.

Despite the absence of effect of lake connectivity on the number of bacterial OTUs, the spatial distribution of the high-mountain lakes in Sierra Nevada (a matrix reflecting the proximity between pairs of lakes) did significantly affect the composition of the bacterial assemblages (Table 2). A plausible explanation for this apparent contradiction is that recolonization of a lake by microorganisms from adjacent lakes is more frequent than from more remote lakes, leading to similar species composition in neighbor lakes irrespective of the total number of OTUs present that appears to be controlled by lake size. In general, ecosystem productivity and resource richness appear to affect species richness, although positive (based on species-energy theory), negative (based on both the paradox of enrichment and the cultural-eutrophication evidence), and unimodal (species richness is the highest at intermediate levels of productivity) relationships have all been observed in natural systems (Currie 1991, Rosenzweig 1971, Dodson et al. 2000). This array of patterns depends on both the scale and the taxonomic group, and is yielded by complex and variable mechanisms (Waide et al. 1999, Mittelbach et al. 2001, Chase and Leibold 2002). However, in our study, concentration of chlorophyll-*a*, and DOC and TP did not appear to influence bacterial community composition, OTU richness, or diversity. The wide metabolic repertoires available in the prokaryotic world and the complex (scale and taxa dependent) influence of ecosystem productivity on species richness (Waide et al. 1999, Chase and Leibold 2002) may explain the absence of any relationship between productivity, resource richness, or biotic interactions and bacterial OTU richness. Further, the relation between ecosystem productivity and bacterial richness appears to also vary among the different bacterial lineages (e.g., Cytophaga-Flavobacteria-Bacteroides; α-Proteobacteria; β-Proteobacteria) (Horner-Devine et al. 2003). The use of finer-resolution markers (such as DGGE with taxon-specific primers, e.g., Nübel et al. 1999) will likely reveal new patterns in these systems.

Microbial ecologists can use 16S rRNA gene sequences to analyze bacterial community structure in natural ecosystems despite the difficulty in establishing an appropriate unit of diversity for bacteria (Nübel et al. 1999, Rosselló-Mora and Amann 2001, Rodríguez-Valera 2002). The ribosomal genes represented here by DGGE bands (each band is one OTU) means that microbial diversity studies are carried out with entities that are not strictly species. Diversity can be studied using any kind of unit, as long as the definition is clear and used consistently in all the study systems (Hughes

et al. 2001). However, some DGGE limitations should be briefly considered here. First, the band number gives an indication of those targeted populations with abundance above 0.3–0.4% of the total targeted cells (Casamayor et al. 2000, 2002 and references therein). Since rare species in a community have little effect on the overall flux of energy and matter, diversity indices weighted by their proportional abundances may be more relevant than the number of distinct populations itself. Second, although PCR biases could also alter the proportions of the original 16S rRNA gene pool, careful handling such as reducing the number of PCR cycles and processing all the samples simultaneously can substantially reduce biases. A more detailed discussion on potentials and limitations of DGGE can be found in Casamayor et al. (2000, 2002 and references therein).

Overall, we found a solid pattern between lake area and bacterial OTU richness that fits the first prediction of the island-biogeography theory. The bacterial richness–area relationship showed a low slope value as is common for organisms with high dispersion rates. Consequently, lake remoteness did not appear to affect the number of bacterial OTUs although the distribution of lakes in the geographical space of Sierra Nevada influenced bacterial communities yielding similar OTUs composition in nearby lakes. Studies such as ours and Horner-Devine et al. (2004*b*) corroborate the species–area relationship as one of the most solid patterns in general ecology, and will help to bridge the existing gap between ecologists of macro- and micro-organisms.

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