

Responses of heterotrophic bacteria to solar irradiance in the eastern Pacific Ocean

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ABSTRACT: We investigated the effects of sunlight on bacterial ³H-leucine (Leu) and ³H-thymidine (TdR) incorporation at 12 locations, from 41° S to 4° N in the eastern Pacific Ocean, during July and August 2000. Surface water samples amended with Leu and TdR were incubated under ambient sunlight using optical filters corresponding to the following wavebands: UVB+UVA+PAR, UVA+PAR, long-wavelength UVA+PAR, and PAR. Incorporation rates of Leu and TdR in dark controls were statistically compared to rates in the light treatments to determine the effect of solar irradiance on bacterial production at each station. We observed robust photo-stimulation of TdR incorporation with UVA+PAR and long-UVA+PAR treatments at 17° S and in all light treatments at 13° S. PAR stimulation of Leu incorporation occurred over much of the south to north survey. Bacterial community structure analyses indicated the presence of 4 communities that exhibited unique responses to ambient solar irradiance. Between 21° S and 4° N, the $\delta^{13}\text{C}_{\text{DIC}}$ of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) was significantly and inversely correlated with UVB, UVA, PAR, and dark Leu incorporation, but not to sea surface temperature, or concentrations of nitrate and chlorophyll. Our results demonstrate widespread direct dependence on solar irradiance, especially longer wavelengths, for bacterial production in surface water of the southeastern Pacific Ocean. Additionally, our data suggest that latitudinal trends in $\delta^{13}\text{C}_{\text{DIC}}$ are strongly associated with trends in solar UVB and bacterial production in upwelling waters, with implications for carbon cycling in tropical and subtropical waters.

KEY WORDS: Microbial diversity · Pacific Ocean · Ultraviolet radiation · Bacterioplankton

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INTRODUCTION

Heterotrophic bacteria play a central role in the biogeochemistry and trophic dynamics of marine ecosystems (Goldman et al. 1985, Thingstad et al. 1993, Azam 1998, Tortell et al. 1999, Strom 2000). Concerns over stratospheric ozone depletion have stimulated research on the effects of ultraviolet radiation (UVR; 280–400 nm)

on the growth and metabolism of heterotrophic bacteria (see reviews by Harm 1980, Herndl 1997, Jeffrey et al. 2000, Moran & Zepp 2000). Previous investigations, however, focused on processes operating on limited temporal (d) and spatial (<1 to 10 km²) scales. These limitations have made the development of global-scale hypotheses and ecological forecasting models difficult (Jeffrey et al. 2000, Moran & Zepp 2000).

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The intensity of UVR and photosynthetically-active radiation (PAR; 400–700 nm), and the ratio of UVB (280–320 nm) to UVA (320–400 nm) vary with latitude and season due to geographic variations in total column ozone concentrations and solar zenith angle (Whitehead et al. 2000). Less predictable short term (hourly) variations in solar irradiance are related to cloud cover and weather conditions. Latitudinal variation in solar irradiance may influence bacterial growth and metabolism directly (Vincent & Neale 2000) or indirectly by affecting the production and availability of bacterial substrates derived from photochemical processes (Kieber 2000) and photosynthesis (Marañón et al. 2004). Viruses and heterotrophic nanoflagellates also contribute to mortality of bacteria, and are negatively impacted by solar radiation (Sommaruga et al. 1996, 1997, Wilhelm et al. 1998). Additionally, latitudinal and seasonal changes in irradiance influence surface water temperatures and water column stratification. Temperature may directly affect bacterial growth and metabolism (Pomeroy & Wiebe 2001), whereas stratification may influence the depth of the mixed layer and primary production (Sverdrup 1953); thus, the general availability and distribution of bacterial substrates derived from photosynthesis. Therefore, direct and indirect effects of solar radiation may influence growth, metabolism, and community structure of surface dwelling bacteria over large latitudinal gradients.

Responses of bacteria to large-scale geographical variability in solar radiation have not been investigated previously. Here, we report results from a ship-of-opportunity cruise: Translatitudinal Assessment of Bacterial Acclimation to Solar Conditions in the Ocean (TABASCO), where we examined the effect of ambient solar radiation on bacteria in the southeastern Pacific Ocean during the austral winter.

MATERIALS AND METHODS

Sample collection. Sample collections and experiments were conducted aboard the RV 'Nathaniel. B. Palmer' from 41.18° S, 78.07° W to 4.05° N, 81.93° W from 24 July to 4 August 2000 (Fig. 1). The vessel was transporting waste materials from Palmer Station, Antarctica to Louisiana and was authorized to stop for sample collections for a total of 1 h each day and obtain CTD profiles in the morning and afternoon.

Hydrography and light measurements. CTD profiles (<500 m) were obtained twice daily (N = 26), ~1 h before sunrise and 16:00 h local time at intervals of ~2° of latitude (Fig. 1). Continuous measurements of surface salinity and temperature along the entire south-to-north transect were collected by a Seabird SBE21 underway CTD. Surface water (3 m) for deck incubation experiments, bacterial abundance and community structure, chlorophyll, nutrients, and $\delta^{13}\text{C}_{\text{DIC}}$ analyses were collected in 10-l Go-Flo bottles. Surface irradiance was measured continuously by a radiometer (GUV 511C, Biospherical Instruments). Integrated exposure values for UVB (305 nm), UVA (320 nm), long-UVA (380 nm), and PAR (400–700 nm) were estimated by summation of 1-min measurements during deck experiments. Daily integrated irradiances along the cruise track, reported by Wilhelm et al. (2003), were used to examine statistical relationships among physical, chemical, and biological properties in this study.

Water chemistry. Samples for inorganic nutrients (N = 23) were collected in polyethylene bottles rinsed with 10% HCl, Milli-Q water, and sample water. Samples were frozen (–80°C) immediately after collection. Nutrient concentrations were measured after the cruise by automated methods (US Environmental Protection Agency 1983). Samples for $\delta^{13}\text{C}_{\text{DIC}}$ analyses (N = 24)



Fig. 1. TABASCO cruise track and location of the sampling stations

were poisoned with HgCl_2 and stored in airtight vials. Subsamples (5 ml) were later acidified *in vacuo* with ~0.5 ml of purified ortho-phosphoric acid. Carbon isotope values of the evolved CO_2 gas were measured with an isotope-ratio mass spectrometer (Micromass Optima). Data are presented in standard δ notation as the per mil (‰) difference from the Pee Dee Belemnite standard (Hut 1987). The analytical precision of the measurements was $\pm 0.08\text{‰}$ (2σ) determined by replicate analyses of a laboratory water standard.

Chlorophyll, bacterial abundances, and community structure. Chlorophyll samples ($N = 23$) were collected on GF/F filters, extracted in 90% acetone, and quantified fluorometrically (Welschmeyer 1994) against spinach chlorophyll *a* standards (Sigma) and acid-corrected for phaeopigments. Bacterial cell concentrations ($N = 23$) were measured by epifluorescence microscopy of formalin-preserved (2% final concentration, stored at 4°C until returned to home institution) DAPI-stained samples (Porter & Feig 1980). Samples for bacterial community structure (morning samples only, $N = 12$) were collected by filtration of ~0.5 to 1.0 l seawater through 47 mm, 0.2 μm nominal pore-size filters (Gelman Supor). The filters were frozen and stored at -80°C prior to phenol-chloroform extraction of bacterial DNA. Bacterial community structure was determined using Denaturing Gradient Gel Electrophoresis (DGGE; Murray et al. 1998) of PCR amplified bacterial 16S rDNA. Variable region 3 of the 16S rRNA gene (Neefs et al. 1990) was amplified by PCR with primer sequences complementary to positions 341 to 358 (primer 358f; eubacterial) and positions 517 to 534 (primer 517r; universal) corresponding to *Escherichia coli* 16S rRNA numbering (Muyzer et al. 1993). A 40-bp G-C clamp was added to the 358f primer. The primer sequences were 5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3' for GC358f and 5'-ATT ACC GCG GCT GCT GG-3' for UNIV517r. Primers were obtained from Operon Technologies. DGGE was performed using the Dcode™ Universal Mutation Detection System (Bio-Rad Laboratories). Gel electrophoresis banding patterns were used to generate dendrograms of bacterial community similarity, using an image analysis program (Gel Compar II Image Software, Applied Maths) using the UPGMA method and presence or absence, but not intensity, of bands.

Deck incubation experiments. Water samples ($N = 12$) were collected at dawn and held in the dark at ambient seawater temperature for ~4 h prior to incubation on deck. Aliquots of sample water (75 ml) were amended with ^3H -leucine or ^3H -thymidine (final concentrations 10 nM). Subsamples consisting of 5-ml amended water were subsequently dispensed into UV-

transparent bags (Whirl-pak™, NASCO; Aas et al. 1996) to insure uniform labeling between treatments. Duplicate Leu and TdR amended Whirl-pak bags were placed under glass optical filters (Schott) with 50% cut-off wavelengths of 280, 320, 370, and 395 nm in an outdoor, flowing seawater bath. The filters allowed exposure to the following wavebands: UVB+UVA+PAR (280 nm filter), UVA+PAR (320 nm filter), long wavelength UVA+PAR (370 nm filter), and PAR (395 nm filter). Duplicate, dark and formalin-killed controls were incubated in the bath with the light-treatment samples. Samples were incubated for a total of 4 h: ~2 h before and after local solar noon while the ship was underway. Leu and TdR incorporation was measured by the micro-centrifuge method (Smith & Azam 1992). Dunnett's Test was used to determine statistically significant ($p < 0.05$) differences in rates between incorporation in the dark and light treatments. Where significant differences were observed between the light and dark treatments, the percent change in incorporation rates of Leu and TdR, relative to the dark controls, was determined as:

$$\text{Percent change from dark} = [1 - (\text{light rate} \div \text{dark rate})] \times 100$$

Negative values indicate inhibition, whereas positive values indicate stimulation. Where differences between light and dark treatments were not significant, the percent change from dark controls was assigned a value of zero.

RESULTS

Surface irradiance, temperature, salinity, and water chemistry

Integrated 305, 320, 380 nm irradiance, and PAR, measured during deck experiments, increased from 41° to 9° S and declined northward across the equator (Fig. 2A). Surface water temperatures and salinities increased between 41° and 5° S (Fig. 3A). From 5° S to north of the equator, temperatures increased and salinities decreased. Surface $\delta^{13}\text{C}_{\text{DIC}}$ remained relatively constant (~1.7‰) between 41° and 21° S, decreased to -0.32‰ at 4° S and then increased to 1.59‰ at 4° N (Fig. 2B). Between 33° and 15° S and north of 3° S, nitrate concentrations were below the limit of detection (Fig. 3C). High concentrations of nitrate at the surface between 13° and 3° S suggest that nutrient-rich water from the Peruvian Upwelling was present (Fig. 3C). At 4° intervals between 21° S and 3° N, correlation analyses (CORR procedure, SAS) indicated significant inverse relationships between $\delta^{13}\text{C}_{\text{DIC}}$ and daily integrated 305, 320, 380 nm and PAR irradiance,

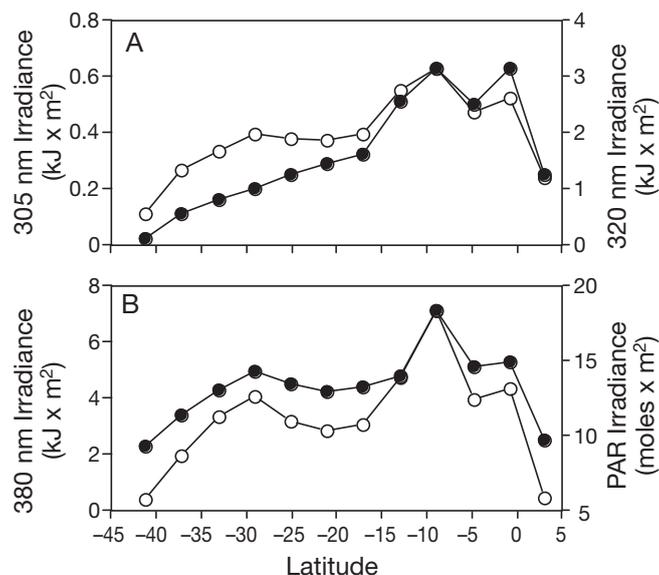


Fig. 2. Latitudinal trends in experimental light exposures (A) 305 nm irradiance (●), 320 nm irradiance (○), and (B) 380-nm irradiance (●), PAR (○)

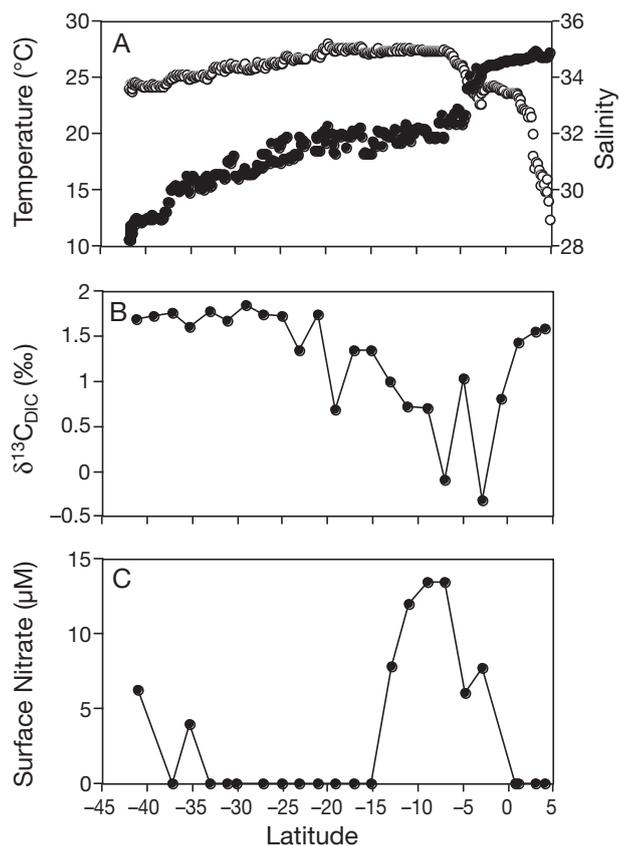


Fig. 3. Latitudinal trends in (A) surface temperature (●) and salinity (○), (B) dissolved inorganic $\delta^{13}\text{C}$, and (C) surface concentrations of nitrate

and dark Leu incorporation (Table 1). At 2° intervals in the high nitrate zone between 13° and 3°S , $\delta^{13}\text{C}_{\text{DIC}}$ values were negatively correlated with sea surface temperatures ($r = -0.84$, $p = 0.03$), but were not significantly correlated with nitrate, chlorophyll *a*, or abundance of bacteria.

Chlorophyll and bacterial production

Concentrations of chlorophyll *a* were elevated at the southern end of the survey and declined between 29° and 25°S (Fig. 4A). Chlorophyll concentrations were highest at 13°S , afterward decreasing steadily across the equator. Surface bacterial concentrations were elevated at the southern end of the survey and were highest at 13°S (Fig. 4A). Similar to the trend in chlorophyll, bacterial abundances declined from 9°S to 4°N . Bacterial production was low and relatively constant between 41° and 17°S (Fig. 4B) and increased ~5-fold by 13°S and, in general, declined northward across the equator.

Deck incubation experiments

We observed no effect of ambient sunlight on Leu incorporation in UVB+UVA+PAR treatments between 41° and 17°S and between 2°S and 3°N (Fig. 4C). However, Leu incorporation was inhibited in UVB+UVA+PAR treatments at 9° and 5°S . Stimulation of Leu incorporation in UVA+PAR treatments occurred at 29°S , 21°S , 13°S , and 3°N and inhibition of Leu incorporation occurred in UVA+PAR treatments only at 5°S . Stimulation of Leu incorporation in long-UVA+PAR occurred be-

Table 1. Correlations between $\delta^{13}\text{C}_{\text{DIC}}$ and physical, chemical, and biological variables between 21°S and 3°N ; 29 July to 4 August 2000. $N = 7$ stations. Daily-integrated radiation data are from Wilhelm et al. (2003). All other variables are from the present study

Comparison	$\delta^{13}\text{C}_{\text{DIC}}$	
	Pearson correlation coefficient	r
Daily integrated 305 nm	-0.97	0.0003
Daily integrated 320 nm	-0.90	0.005
Daily integrated 380 nm	-0.85	0.01
Daily integrated PAR	-0.82	0.02
Dark Leu incorporation	-0.75	0.049
Dark TdR incorporation	-0.63	0.12
Surface chlorophyll	-0.68	0.08
Bacterial cell abundance	-0.14	0.76
Surface temperature	0.05	0.91

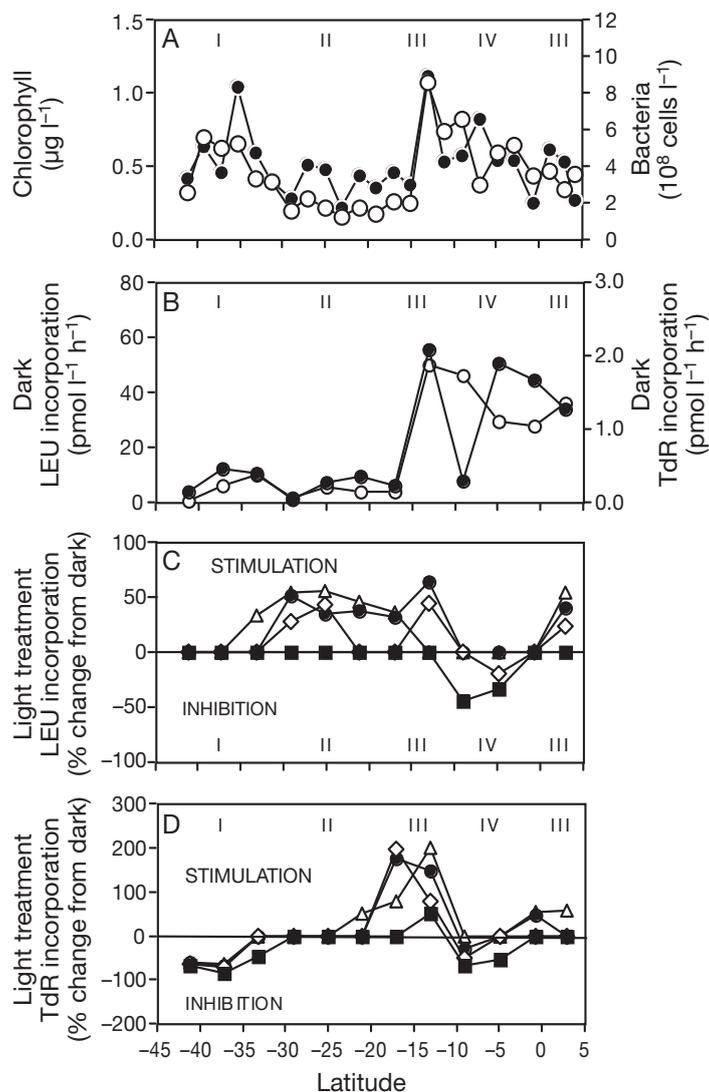


Fig. 4. Latitudinal trends in (A) concentrations of surface chlorophyll *a* (O) and bacterial cells (●), (B) dark treatment Leu (●) and TdR (O) incorporation, (C) light treatment for Leu incorporation during experimental exposure to UVB+UVA+PAR (■), UVA+PAR (◇), long wavelength UVA+PAR (●), and PAR (Δ), (D) light treatment TdR incorporation (symbols same as C). Roman numerals indicate approximate locations of bacterial communities identified by DGGE (see Fig. 7)

tween 29° and 13°S and at 3°N and PAR treatments between 33° and 17°S and 3°N. Inhibition of Leu incorporation was not measured in long-UVA+PAR and PAR-only treatments at any station.

TdR incorporation was inhibited in all light treatments between 41° and 37°S (Fig. 4D) and stimulated in all light treatments at 13°S (Fig. 5D). TdR incorporation in UVB+UVA+PAR treatments was inhibited between 41° and 33°S and was not significantly different from dark treatment rates between 29° and 17°S. North of 13°S, latitudinal trends of TdR incorporation

in UVB+UVA+PAR treatments were similar to Leu incorporation in UVB+UVA+PAR treatments. With the exception of stimulation at 1°S in the long-UVA+PAR treatment, latitudinal trends in TdR incorporation from UVA+PAR and long-UVA+PAR treatments were similar throughout the survey.

Between 41° and 13°S, there was a significant positive correlation between the PAR treatment for TdR incorporation and experimental PAR dose (Fig. 5A); however, there was a stronger correlation between the PAR treatment for TdR incorporation and daily, integrated PAR over this same interval (Fig. 5B). Between 25° and 13°S, a significant negative correlation ($r = 0.96$, $p = 0.02$) occurred between daily-integrated PAR and PAR treatment for Leu incorporation and a significant positive correlation ($r = 0.95$, $p = 0.03$) between daily-integrated PAR and PAR treatment for TdR incorporation (Fig. 6).

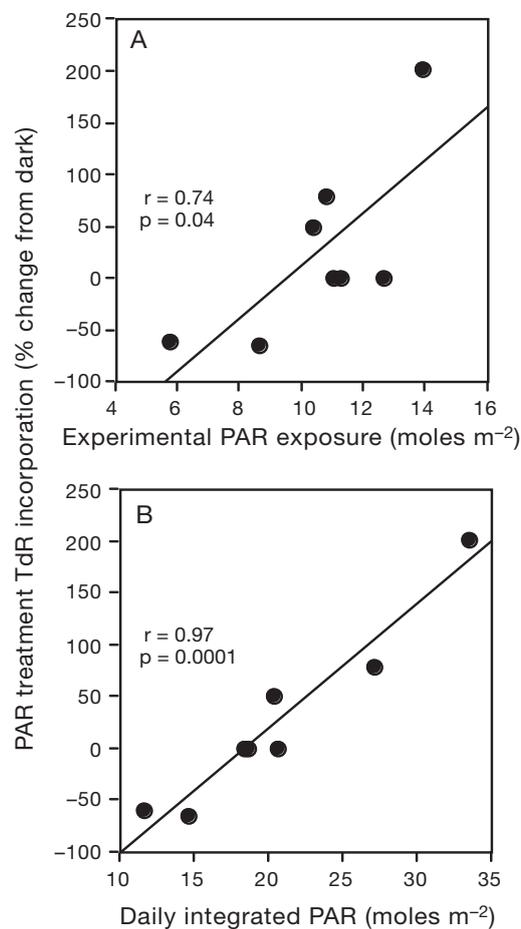


Fig. 5. (A) PAR treatment for TdR incorporation relative to dark rates and experimental PAR irradiances. (B) PAR treatment TdR incorporation relative to dark rates and daily-integrated PAR irradiances, 41° to 13°S. Positive values = stimulation relative to dark rates, negative values = inhibition relative to dark rates ($N = 8$)

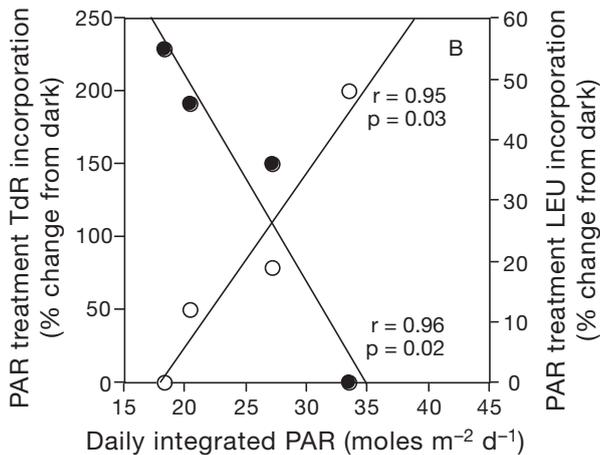


Fig. 6. Relationship between PAR treatment Leu (●) and TdR (○) incorporation and daily integrated PAR irradiance, 25° to 13° S (N = 4)

Bacterial community structure

DGGE analyses suggested that 4 broadly similar (>75% similarity) assemblages of bacteria were present along the survey route (Fig. 7). The southernmost communities (Group 1) were present from 41° to 34° S in cool (~10–17°C) surface water. Group 2 communities were present from 29° to 21° S in 18–20°C water. Group 3 communities were present from 17° to 13° S and at 1° S to 3° N, at the southern and northern boundaries of high nitrate waters located between 13° and 3° S. Group 4 communities were present in warm surface waters, 20–25°C, between 9° and 5° S. Each

community exhibited unique responses to ambient surface irradiance. Group 1 exhibited inhibition of TdR incorporation in all light treatments, whereas Leu incorporation was insensitive to exposure to ambient UVR and PAR. Incorporation of Leu was stimulated in Group 2 by PAR and Leu and TdR incorporation were not inhibited by ambient UVB, UVA, or PAR. Group 3 communities exhibited PAR stimulation of both Leu and TdR incorporation and were not inhibited by exposure to UVR. UVR inhibited Leu and TdR incorporation in Group 4 and Leu and TdR incorporation were not stimulated in any light treatment.

DISCUSSION

Heterotrophic bacteria exhibited a wide range of responses to ambient solar irradiance along the cruise track. At 41° S and 37° S, TdR incorporation was inhibited at all light treatments. The similarity of response among all light treatments indicated that PAR was primarily responsible for the inhibition of nucleic acid synthesis at these stations. Inhibition of TdR incorporation by UVR and PAR was previously reported in the Gulf of Mexico (Aas et al. 1996) and the Adriatic Sea (Sommaruga et al. 1997). Whereas UVR appears to inhibit bacterial growth by damage to DNA (Herndl 1997, Jeffrey et al. 2000, Moran & Zepp 2000), mechanisms responsible for PAR inhibition of bacterial production remain obscure. Morán et al. (2001) proposed that apparent PAR inhibition of Leu incorporation in Mediterranean surface waters was a result of artificial

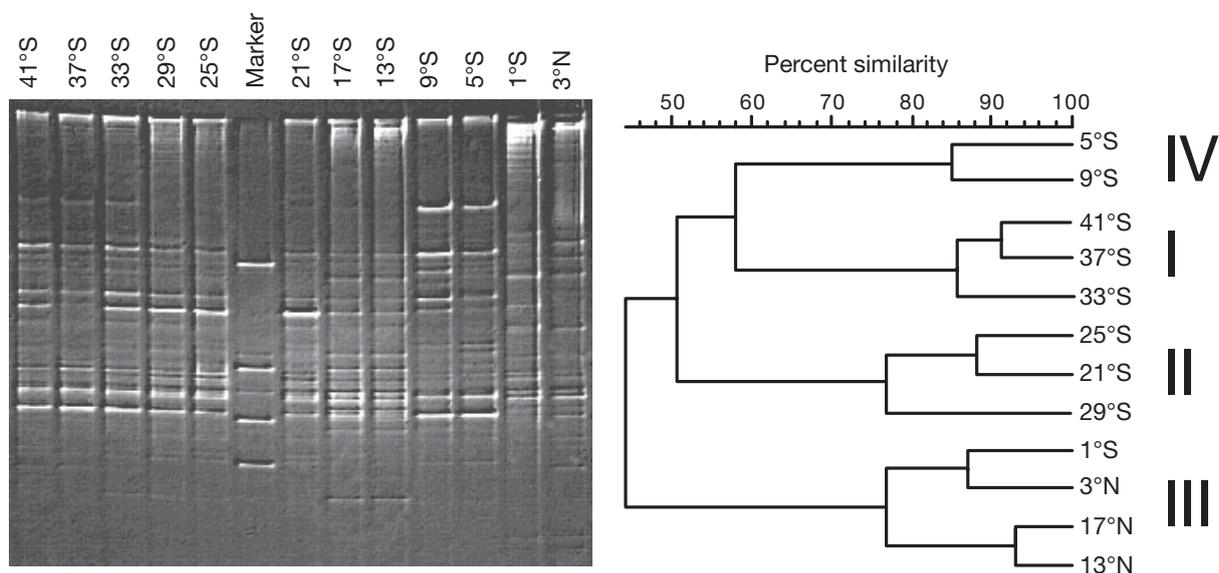


Fig. 7. Latitudinal trends in transect bacterial community structure visualized by banding patterns of bacterial 16S rDNA fragments after denaturing gradient gel electrophoresis and a similarity dendrogram of bacterial communities along survey route, based on DGGE analyses

stimulation of bacterial growth in the dark. However, mechanisms that could stimulate bacterial growth in the dark have not been identified. Marañón et al. (2004) reported that release of DOC by phytoplankton is a light-dependent process that ceases in the dark. Sommaruga et al. (1996) reported that exposure to UVR reduced bacterivory by nanoflagellates. Bacterivory in dark controls might be enhanced relative to light treatment rates and potentially reduce incorporation rates of Leu and TdR in the dark. We observed no effect of UVR or PAR on Leu incorporation at 41° and 37° S. It is unlikely that artificial stimulation of bacterial growth in our dark controls can account for PAR inhibition of TdR incorporation that we observed at 41° and 37° S or stimulation by sunlight of Leu and TdR incorporation that we observed elsewhere along the survey. We could not determine whether this inhibition was a direct or indirect effect of exposure to PAR.

In contrast to the 2 southernmost sample sites, we observed no significant effects of UVR on TdR incorporation between 29° and 21° S. This suggested that TdR incorporation at these locations was insensitive to ambient surface intensities of UVR at these latitudes and season. However, TdR incorporation was stimulated by exposure to UVA and PAR at 17° S and UVB, UVA, and PAR at 13° S. Stimulation of TdR incorporation in natural bacterial communities exposed to ambient sunlight has not been reported. A number of mechanisms may be responsible for stimulation of nucleic acid synthesis at these localities. Chlorophyll concentrations peaked at 13° S along the survey. PAR-stimulated release of DOC from phytoplankton (Zlotnik & Zobinsky 1989) may have enhanced substrate availability and growth-related nucleic acid synthesis in this region. Sommaruga et al. (1996) proposed that UVR-inhibition of nanoflagellate bacterivory resulted in a positive feedback between UVB exposure and bacterial production. Inhibition of protistan bacterivory in light treatments, relative to dark controls, may also have contributed to the apparent UVR stimulation of TdR incorporation in these 2 sampling areas. According to Ögrünç et al. (1998), bacterial and archeal nucleotide excision repair of UVR-induced CPD or 6–4 photoproducts results in the excision and subsequent polymerization of 10–13 nucleotide oligomers at each repair site. Exogenous TdR could be incorporated into UVR-damaged DNA and contribute to incorporation of TdR. Church et al. (2004) proposed that *Prochlorococcus* at station ALOHA were responsible for light-stimulated incorporation of leucine. Cyanobacteria can incorporate amino acids (Zubkov et al. 2003), but lack thymidine kinase (<http://cyano.genome.jp>), the enzyme essential for incorporation of exogenous TdR (Kornberg 1980). It is unlikely that cyanobacteria were responsible for the light-stimulated of TdR incorpora-

tion that we observed at 13° S. The greatest stimulation of TdR incorporation occurred in the PAR treatment, followed by the long wavelength-UVA+PAR, UVA+PAR, and UVB+UVA+ PAR treatments. This indicated that the net stimulatory effect of exposure to PAR diminished with increasing exposure to UVA and UVB. Photo-inhibition of DOC release from phytoplankton or an increase in the rate of DNA damage relative to the rate of DNA repair may account for reduced TdR incorporation with increasing exposure to the shorter wavelengths of UVR at 13° S.

Light stimulation of Leu incorporation was observed through most of the survey. PAR and UVA+PAR were responsible for most of this stimulatory effect. PAR stimulation of prokaryotic Leu incorporation has been reported from the Mediterranean and North Pacific Oceans (Morán et al. 2001, Church et al. 2004, Alonso-Sáez et al. 2006). Recent research suggested that photoheterotrophy is widespread in temperate and tropical surface waters and contributes to bacterial production in marine surface water (Béjà et al. 2000, Kolber et al. 2000, 2001, Zubkov et al. 2003). In our study, we could not determine whether photo-stimulated Leu incorporation was solely the result of bacterial photoheterotrophy. Other factors, such as light-enhanced release of DOC from phytoplankton and photolysis of DOC, might contribute to light-stimulated incorporation of Leu. As photo-stimulation of Leu incorporation was greatest in the long wavelength UVA+ PAR and PAR treatments, PAR-mediated biological, rather than UVR-mediated photochemical processes might have played a greater role in photo-stimulation of Leu and TdR incorporation by bacteria in our deck experiments. Many of our results were unexpected. The majority of studies reported that exposure to full UVR inhibits bacterial production (Herndl et al. 1993, Aas et al. 1996, Sommaruga et al. 1997, Pakulski et al. 1998). At several of the sampling locations, apparent photo-stimulation by longer wavelengths was great enough to offset the inhibitory effects of UVR on bacterial production.

Community structure may play a critical role in determining bacterioplankton response to solar radiation. Van Mooy et al. (2004) reported that bacterial community structure in the eastern Subarctic Pacific was strongly and independently related to geographical trends in PAR and primary production. Alonso-Sáez et al. (2006) recently reported differences in photo-sensitivity of different bacterioplankton groups in the Mediterranean Sea. In our study, latitudinal trends in light treatment of Leu and TdR incorporation also corresponded to shifts in community structure.

Photo-responses of Group 3 communities were similar despite geographical separation. These communities were located at the northern and southern bound-

aries of high-nitrate water associated with the Peruvian Upwelling. Baldwin et al. (2005) reported the development of genetically similar bacterial communities to the north and south of the Equatorial Upwelling in the central Pacific. Exposure experiments indicated that those communities exhibited similar responses to ambient solar irradiance (J. D. Pakulski et al. unpubl.), and that bacterial communities developing under similar environmental conditions, but separated geographically, exhibit similar responses to solar irradiance.

Light treatment for Leu and TdR incorporation varied with latitudinal trends in insolation. From 41° to 13° S, the PAR treatment for TdR incorporation transitioned from PAR inhibition at the southernmost sampling areas to PAR stimulation at 13° S. These data suggested that bacterial communities exposed to low intensities of solar irradiance were more susceptible to deleterious effects of sunlight than communities routinely exposed to higher intensities of UVR and PAR. The higher correlation between PAR treatment for TdR incorporation and daily-integrated PAR also suggested that photo-responses of bacterial communities during short-term experimental exposures were influenced by previous long-term exposure to ambient solar irradiance. Between 25° and 13° S, PAR-stimulated Leu incorporation declined, whereas PAR-stimulated TdR incorporation increased with increasing PAR exposure. These data are enigmatic, but suggest a shift in the metabolism of the bacterial community associated with latitudinal variation in solar irradiance.

Upwelling (Peeters et al. 2002) and the ^{13}C Suess Effect (Keeling 1979, Keeling et al. 2004) influence the distribution of $\delta^{13}\text{C}_{\text{DIC}}$ in marine surface water. However, the robust correlation we observed between daily-integrated UVB and $\delta^{13}\text{C}_{\text{DIC}}$ was unexpected. Our initial hypothesis was that $\delta^{13}\text{C}_{\text{DIC}}$ would reflect the distribution of upwelling waters off the coast of Peru and at the equator. Our data suggest additional factors contribute to the decrease in $\delta^{13}\text{C}_{\text{DIC}}$ in these waters. Baldwin et al. (2005) reported the distribution of sea surface temperatures, nitrate, chlorophyll, and bacteria across the Equatorial Upwelling zone at ~165° W. Sea surface temperatures were depressed and concentrations of nitrate, chlorophyll, and bacteria were elevated at the equator. Along the TABASCO cruise transect (~80° W), we did not observe these same trends across the equator. Fiedler et al. (1991) reported that surface currents in the far eastern Pacific were not divergent at the equator during August to November 1990, which suggested that upwelling was not well developed in the eastern Equatorial Pacific during this period. Equatorial surface water was also influenced by the ENSO cycle. TOPEX/Poseidon data collected during August 2000, however, indicated that

the ENSO cycle had entered a neutral phase and did not show sea surface temperature anomalies suggestive of equatorial upwelling along the survey route (http://topex-www.jpl.nasa.gov/science/timeseries/200008_P.html). In the high nitrate water off the coast of Peru, there was no significant correlation between concentrations of nitrate and $\delta^{13}\text{C}_{\text{DIC}}$ values at the surface. Furthermore, the negative correlation between $\delta^{13}\text{C}_{\text{DIC}}$ and sea surface temperatures suggested that isotopically-light DIC was associated with warm water, rather than cooler waters, as might be expected if upwelling accounted for the distribution of $\delta^{13}\text{C}_{\text{DIC}}$ along this section of the survey.

Correlations between $\delta^{13}\text{C}_{\text{DIC}}$, solar irradiance, and Leu incorporation between 21° S and 3° N suggest that photolysis and photo-oxidation of DOC might influence the distribution of $\delta^{13}\text{C}_{\text{DIC}}$ in surface water. Bulk marine $\delta^{13}\text{C}_{\text{DOC}}$ range from -24 to -22‰ and do not appear to vary appreciably with depth or season (Bauer et al. 2002). Photo-oxidation of DOC would deplete DIC in ^{13}C . In Antarctic waters, UVB influences $\delta^{13}\text{C}_{\text{DIC}}$ during the austral spring. Karentz & Spero (1995) reported that $\delta^{13}\text{C}_{\text{DIC}}$ in the Bellinghousen Sea, Antarctica, was positively correlated with total column ozone (TCO). Over 6 d, total column ozone declined from >350 to <200 Dobson Units (DU) and surface $\delta^{13}\text{C}_{\text{DIC}}$ declined from ~1 to -2.3‰. The decline in $\delta^{13}\text{C}_{\text{DIC}}$ was attributed to the rapid remineralization of organic matter during periods of low TCO and elevated UVB. In our study, the lowest surface water $\delta^{13}\text{C}_{\text{DIC}}$ occurred in the zone of elevated UVB near the equator. Direct UVR-oxidation and photolysis and subsequent bacterial oxidation of recently upwelled DOC (Kieber et al. 1989, Benner & Biddanda 1998, Miller & Zepp 1995, Miller & Moran 1997) might, in part, account for the spatial patterns we observed among $\delta^{13}\text{C}_{\text{DIC}}$, daily-integrated UVB, and dark incorporation of Leu between 13° and 3° S.

A significant quantity of organic carbon would have to be oxidized to account for the $\delta^{13}\text{C}_{\text{DIC}}$ distribution we observed along the survey. The difference in $\delta^{13}\text{C}_{\text{DIC}}$ measured between 21° and 3° S was -2.07‰. To produce a 1‰-decrease in a 2 mM, DIC pool with an initial $\delta^{13}\text{C}$ value of 0 ‰ would require the oxidation of 100 μM of organic carbon with a $\delta^{13}\text{C}$ value of -22‰. DOC concentrations in Equatorial Pacific surface waters are ~60 to 70 μM (Sharp et al. 1995) and ~65 μM in the Peruvian Upwelling (Archer et al. 1997). If photolysis and photo-oxidation of DOC were solely responsible for the changes in $\delta^{13}\text{C}_{\text{DIC}}$ that we observed, it would require the complete oxidation of the surface DOC pool over some unknown interval of time. Whereas rates of DOC photo-mineralization have been measured in temperate coastal waters (0.1 to 0.4 $\mu\text{mol C m}^{-1} \text{ h}^{-1}$, Miller & Zepp 1995), photo-mineralization

rates of DOC in tropical and subtropical pelagic waters are not known. Whether photo-oxidation of DOC is a significant component of the carbon cycle of the eastern tropical Pacific will require further investigation.

Latitudinal trends in dark treatment Leu and TdR incorporation in our deck experiments were quite different from trends in light treatment protein and nucleic acid synthesis and, unlike light treatment responses, did not appear to be closely related to shifts in community structure. Several other studies have observed differences in the effects of solar radiation on leucine and thymidine incorporation (Aas et al. 1996, Sommaruga et al. 1997, Pakulski et al. 1998). Much of our current understanding of bacterial production in the ocean is derived from Leu and TdR incorporation measurements performed in the dark. However, recent work (Aas et al. 1996, Pakulski et al. 1998, Morán et al. 2001, Church et al. 2004, Alonso-Sáez et al. 2006) and this study suggest that dark incubation measurements may seriously under or over-estimate *in situ* bacterial production in aquatic environments. Estimates of *in situ* bacterial production should therefore be conducted under ambient light conditions, including diurnal light treatment and nocturnal dark treatment measurements of Leu and TdR incorporation. It is apparent that light may have inhibitory or stimulatory effects on bacterial production. While very short incubations may reduce these effects on measurements, the kinetics of these responses remains largely unknown and warrant further investigation.

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