Particulate beam attenuation coefficient, bacteria abundance and production in marine nearshore waters

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ABSTRACT

Variability of particulate beam attenuation coefficient at 532 nm (c_p (532)) and microbial planktonic community (heterotrophic bacteria and phytoplankton) was analyzed in coastal waters of Southern California. The goal of this study was to explore heterotrophic bacteria (HB) response (cell abundance, BA, and carbon production, BCP) with respect to different particle characteristics (concentration, size distribution, and composition) related with c_p(532). We observed a fairly complex pattern of HB response and particle dynamics during seven experiments throughout the summer and winter, which reflected variations in c_p(532). The first experiment showed relatively high values of c_p(532), in conjunction with high chlorophyll *a* concentration (chl) of about 5.4 mg m^{-3}. For experiments 2 and 3, a sharp decrease of chl was accompanied by an increased role of detrital particles (non-living matter) as evidenced by increased detrital absorption (a_d). The highest values of particle-attached (>1 µm) and free living (<1 µm) BA and BCP were observed in experiment 3. These changes in particle assemblage including HB maintained c_p(532) at relatively high level, comparable to that observed when phytoplankton dominated. A significant decrease of c_p(532) was observed in experiment 4 and 5, which coincided with relatively low BA, BCP, and a_d values. In experiment 7, c_p(532) magnitude was comparable to the first experiment and was accompanied by high chl, BA and SPM (suspended particulate matter). Greatest changes in c_p(532) coincided with greatest variations in BA, even though our estimates of the direct contribution of heterotrophic bacteria to c_p(532) for all experiments remained quite low (<10%).

Keywords: heterotrophic marine bacteria, bacteria carbon production, marine optics, particle beam attenuation, particle size distribution, free-living bacteria, particle-attached bacteria, phytoplankton, detritus, coastal waters

1. INTRODUCTION

A better understanding of future climate change scenarios relies on how much we know about fundamental biological processes affecting the global carbon cycle. Key ecosystem functions (organic matter decomposition and respiration) affecting air-sea and sea-sediment exchange of greenhouse gases (carbon dioxide and methane) are mediated by heterotrophic marine bacteria or HB[1]. Large scale estimations (e.g., using satellite observations) of HB parameters such as bacteria abundance (BA) and bacteria carbon production (BCP) pose several limitations. First, most of the HB have no pigments[2] and cannot be detected based on light absorption signatures and using satellite sensors. Second, field measurements are expensive or time-consuming (e.g., flow cytometry). Lastly, indirect estimates using phytoplankton parameters are not always reliable since phytoplankton biomass and production may be uncoupled with HB variability[3, 4]. Despite these difficulties, the use of optical measurement of particle beam attenuation (c_p) may represent an alternative tool to derive the HB parameters directly (HB is an optical target[5]) or indirectly (HB bounded to optical targets such as dead particles[6]). Lab cultures[7] and *in situ* studies in oceanic waters[8] support this possibility. From the physics point of view, detritus (dead organic + inorganic particulate material) and HB contribute mainly to light scattering, thus affecting the scattering component of c_p ($c_p = a_p + b_p$, where a_p and b_p are the absorption and scattering coefficient of particles,

respectively). The objective of this preliminary work is to explore HB response with respect to different particle characteristics related to c_p variability in nearshore waters of the Southern California Bight.

2. METHODS

Parallel measurements of biological and optical data were conducted during seven experiments (Table 1) in nearshore waters at the pier of Scripps Institution of Oceanography in La Jolla, California. Samples for laboratory determinations of BA, BCP, concentration of chlorophyll *a* (chl), particulate organic carbon (POC), suspended particulate matter (SPM), particle size distribution (PSD), and particle absorption spectra were obtained from surface waters (0.5-2 m deep) around noon (10 am to 13:00 pm). Stormy or windy days were avoided to minimize resuspension of inorganic bottom sediments into the water column. The samples for chl, POC, SPM, PSD, and particle absorption were collected using buckets after disrupting the sea surface microlayer. *A posteriori*, water samples were dispensed in 20-L plastic carboys (Nalgene) previously rinsed (diluted HCl, microQ water, and seawater), transported in dark conditions, and analyzed in the laboratory. Water samples for biological determinations of bacteria parameters were obtained by diving and using pre-cleaned (diluted HCl, microQ water, and seawater) acrylic cylinders (0.3 to 0.6 L). Unlike carboys, the cylinder technique minimizes bacteria contamination (e.g., bubbling and aerosol sprays) and disruption of particle aggregates (e.g., marine snow). Cylinders were opened below the sea surface, filled with seawater, closed with silicon stoppers, and sealed with parafilm to avoid contamination. Biological samples were processed within two hours after sampling.

Bacteria abundance per unit volume of water was estimated by microscopic counting after staining with DAPI[9] whilst bacteria carbon production per unit volume of water was quantified using ^{3}H-leucine incubations[10, 11]. Free-living (<1 µm, FLB) and particle-attached (>1 µm, PAB) fractions of BA and BCP were obtained by filtration using GF/C (Whatman, 1.2 µm nominal pore size) glass fiber membranes after sonicating the original sample with a detergent[12,13]. Analysis of chl values was performed using fluorometry of samples extracted with 90% cold acetone[14]. To determine chl size fraction >20 µm, three sub-samples of seawater (0.2-0.5 L) were pre-filtered through nylon net (Millipore, 20 µm pore size) membranes before extracting the samples with acetone. The concentration of POC was measured on pre-combusted (5 hours, 450°C) GF/F filters using a Carlo Erba NCS 2500 elemental analyzer[15]. Measurements of SPM were obtained gravimetrically after collection and drying of particles on pre-weighed GF/F filters[16]. The absorption spectrum of particles retained on GF/F filter, $a_p(\lambda)$, was measured using the transmittance-reflectance (T-R) technique[17, 18]. The absorption coefficient of non-algal particles ($a_d(\lambda)$) was determined after pigment bleaching with sodium hypochlorite[19]. Note that inorganic particles rich in calcium carbonate or silica have a minor contribution to a_d with respect to organic particles[20].

Because c_p is an inherent optical property determined by several particle characteristics (abundance, size, and composition), a multiple instrument approach was applied to elucidate functionalities between c_p and bacteria parameters. Particle number concentration and particle size distribution (PSD) within the range 2-60 µm was measured in discrete samples with a Beckman-Coulter Multisizer III equipped with a 100-µm aperture (electrical sensing zone method)[21]. Estimations of PSD and particle volume concentration per unit volume of seawater (V*) over the size range from about 1 to 200 µm were also carried out using a LISST-100X (Laser In Situ Scattering and Transmissometry, Sequoia Scientific, Inc.), which measures the volume scattering function (at light wavelength λ = 532 nm) in forward directions (32 angles within the range 0.05 to 13.5°) and estimates the PSD from inversion of the scattering pattern[22].

Estimates of the particle beam attenuation coefficient were obtained *in situ* from the LISST instrument. In addition to the 32 detectors utilized in the forward scattering measurement, an additional detector is located at 0° (acceptance angle = 0.027°) allowing measurement of the total beam attenuation coefficient $c(532)$. A baseline measurement of 0.2 µm filtered seawater was used as a reference and subtracted from the measurement to estimate the attenuation coefficient due to particles, $c_p(532)$.

The contribution of HB to c_p was estimated at λ = 532 nm from bacteria abundance and attenuation cross-section values of bacteria cells ($\sigma_{HB}(532)$= 0.043727 µm^2 cell^{-1}) reported in the literature[2]. This bacteria contribution was likely underestimated since particle-attached bacteria are generally bigger (larger attenuation cross-section) than free living bacteria[23], for which the attenuation cross-section was determined.

General weather and hydrological parameters (sea surface temperature, wind, wave, and tidal data) during each experiment were obtained from the CDIP (The Coastal Data information Program, Scripps Institution of Oceanography) project (Ocean Engineering Research Group). Tidal height data was derived from predicted values (La Jolla water level station, NOAA).

3. RESULTS

Table 1 summarizes the hydrological and meteorological properties during the experiments.

Table 1. Hydrological and meteorological parameters at SIO pier during 2006-2007 experiments. ST: spring tide, NT: neap tide, ET: ebb tide, FT: flood tide, Z: bottom depth (m), H_S: wave height or 30-minute average of the 1/3 highest waves, W_S: wind speed (m s^{-1}), T: sea surface temperature (°C). Each measurement is the averaged value between 10 and 13 am.

Experiment #	Date	Tidal regime	Z	H_S	W_S	T
1	6/21/2006	NT, ET-FT	6.27	0.84	2.03	22.3
2	7/5/2006	ST, ET-FT	6.40	0.61	0.95	22.0
3	7/21/2006	ST,ET-FT	6.17	0.56	2.41	23.0
4	8/8/2006	ST, FT	5.67	0.71	0.66	21.0
5	8/17/2006	NT, ET-FT	6.44	0.48	1.52	21.0
6	1/19/2007	ST, ET	6.63	1.00	2.05	14.0
7	4/24/2007	NT, ET-FT	6.61	0.85	0.74	15.0

Maximum wind speed (experiment 3) was not related with maximum H_S (experiment 6) values. During experiment 4, a clear resuspension of bottom sediments was observed that coincided with lowest bottom depths. The highest and lowest sea surface water temperature values were observed during experiments 3 and 6, respectively.

Light attenuation due to particles in the green spectral range had multiple maxima throughout the period of the study (experiments 1, 3, and 7) and was always above 1 m^{-1} (Fig. 1). Greatest particle number concentration (particle diameter range = 2-60 µm, Beckman-Coulter Multisizer) did not necessarily coincide with greater $c_p(532)$ values (Fig. 1, Fig. 2A). In general, particle volume concentration per unit volume of seawater (V*) (particle diameter range = 1.05-198.6 µm, LISST) had a comparable experiment-to-experiment variability with respect to $c_p(532)$ values (Fig. 1, Fig. 2B).

The mass concentration of suspended particulate matter (SPM) did not necessarily covary with $c_p(532)$ (Fig. 1, Fig. 3A), and was always below 10 g m^{-3}. Particulate organic carbon (POC) was maximum in experiment 1 (> 0.5 g m^{-3}) and minimum in experiment 4 (0.28 g m^{-3}) (Fig. 3B, left y-axis). For the whole dataset, the organic carbon content of SPM was around 20% with minimum values calculated for experiment 2 (POC/SPM ~12%) (Fig. 3B, right y-axis). Phytoplankton biomass, as estimated from total concentration of chlorophyll *a*, chl_T, was high for the first (>5 mg m^{-3}) and the last (>3 mg m^{-3}) experiment (Fig. 3C, left y-axis). Contribution of microphytoplankton (cells >20 µm) to chl_T was highly variable during the period studied with greatest values measured in experiment 3 (>20%) and 7 (>55%) (Fig. 3C, right y-axis). In general, there was a good correspondence between BA and $chl_{(>20\ \mu m)}/chl_T$ values for the whole data set (Fig. 3C, Fig. 4A). The contribution of non-algal particles to total particulate absorption at λ = 412 nm, $a_d(412)/a_p(412)$, had the highest values during experiments 2 and 3 (>60%) (Fig. 3D).

Abundance of heterotrophic bacteria per unit volume of water had a drastic increase (3-fold) between experiment 2 and 3 (BA reached values >3 10^{12} cells m^{-3}) and a secondary maximum in the last experiment (Fig. 4A). Minimum bacteria abundance was observed in experiment 6 (0.67 10^{12} cells m^{-3}) when BCP was also minimum (Fig. 4A, B). As expected, the largest ^{3}H-leucine uptake was observed during experiment 3 (1.829 gC 10^{-6} m^{-3} h^{-1}) given the largest abundance of HB (Fig. 4A). However for experiment 7, there was not a clear correspondence between BCP and BA.

In general, there was a covariation between abundance of FLB and PAB during the experiments (data not shown). However, particle-attached HB parameters showed better relation to $c_p(532)$ variability and particle characteristics than free-living HB parameters. BA and BCP of PAB was related to V* (Fig. 2B, Fig. 4), while contribution of PAB to BA

was related to particle abundance N (Fig. 2A, Fig. 4A). Likewise, the relative contribution of PAB assemblage to total BCP was often positively related to carbon content of particles (POC/SPM) (Fig. 3B, Fig. 4B).

On average, FLB contributed ~50% to HB abundance during the period studied (Fig. 4A). Preliminary calculations using the bacterial attenuation cross-section and bacteria abundance suggest that HB had a small contribution to $c_p(532)$ magnitude (up to 10% in experiment 3). Unlike BA, BCP of PAB was, on average, one order of magnitude greater than BCP of FLB (Fig. 4B).

4. DISCUSSION

In agreement with other studies[24,25], variability of c_p values in this study responded to diverse particle characteristics such as concentration, size distribution, and composition. Higher c_p values generally coincided with higher particle concentrations, including HB and particle volume concentration per unit volume of seawater V* (Fig. 1A, Fig. 2B). However, relatively high c_p values were not determined by one single type of particulate component, instead they were either associated with high values of chl-bearing particles (experiments 1 and 7) or non-algal particles (experiment 3), and also to some extent heterotrophic bacteria (experiments 3 and 7).

Heterotrophic bacteria in marine waters are mainly regulated by two principal interactive factors: substrate availability and temperature[26]. In general, both limiting variables have a positive influence on BA and BCP when one of them is held constant[26]. In this work, water temperature was probably not the unique factor affecting BA and BCP, since warmer waters were not necessarily related with greater bacteria abundance or production (Fig. 4, Table 1). Therefore, availability of organic substrate was likely another important limiting factor of bacteria parameters in our study area. Indeed, our preliminary $a_d(412)/a_p(412)$ and BCP data probably suggest a positive link between substrate availability and bacteria activities (Fig. 3D, Fig. 4B). A greater availability of organic dead and 'young' (formed within ~ 5 days) particles is expected to increase BCP since bacteria communities attached to dead organic-rich particles are more active (greater BCP) with respect to free living communities[27, 28].

In this study, organic content of suspended matter seemed to affect in a positive way the contribution of PAB to BCP. This finding agrees with previous studies where greater enzymatic and BCP values in organic aggregates (1-2 mm) were found to be a function of POC content per individual aggregate[6].

5. CONCLUSIONS

The variability of $c_p(532)$ in nearshore waters of the Southern California was often, but not always, associated with changes in bacteria parameters (BA and BCP) despite the low direct contribution of bacterial cells to $c_p(532)$ magnitude (up to 10%). These results suggest that a direct effect of bacteria on $c_p(532)$ (i.e., more bacteria greater particle beam attenuation) was not the main cause of c_p - bacteria (BA and BCP) relationships. Instead, other optically significant particle types covarying with BA or BCP (e.g., phytoplankton, non-living organic matter) were most likely indirectly responsible of those relationships. In this work, phytoplankton ('live photosynthetic particles') and organic detritus ('dead particles') were two optical components that were related to bacteria parameters and $c_p(532)$ values. Phytoplankton favors BCP by producing labile dissolved organic compounds (e.g., exudates)[29] whereas organic detritus represents a particulate food source for HB growth[30].

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REFERENCES

1. P.A. Del Giorgio, P.J. L.B. Williams, "Respiration in aquatic ecosystems", Oxford University Press, 325 p., 2005.
2. D. Stramski, D. A. Kiefer, "Can heterotrophic bacteria be important to marine light absorption?" Journal of Plankton Research, 20, 1489-1500, 1998.
3. C.M. Duarte, S. Agusti, D. Vaque, N.S.R. Agawin, J. Felipe, E.O. Casamayor, J.M. Gasol, "Experimental test of bacteria-phytoplankton coupling in the Southern Ocean", Limnology and Oceanography 50: 1844-1854, 2005.
4. S. Findlay, M.L. Pace, D. Lints, J.J. Cole, N.F. Caraco, B. Peierls, "Weak coupling of bacterial and algal production in a heterotrophic ecosystem: the Hudson River estuary", Limnology and Oceanography, 36, 268-278, 1991.
5. D. Stramski, D.A. Kiefer, "Light scattering by microorganisms in the open ocean", Progress in Oceanography, 2, 343-383, 1991.
6. H. Ploug, H.P. Grossart, F. Azam, B.B. Jørgensen, "Photosynthesis, respiration, and carbon turnover in sinking marine snow from surface waters of Southern California Bight: Implications for the carbon cycle in the ocean", Marine Ecology Progress Series, 179, 1-11, 1999.
7. T.D. Brock, D. Madigan, T. Michael, "Biology of Microorganisms", Prentice Hall Publications, Englewood Cliffs, New Jersey, 07632, 767-771, 1998.
8. R.W. Spinrad, H. Glover, B.B. Ward, L.A. Codispoti, G. Kullenberg, "Suspended particle and bacteria maxima in Peruvian coastal waters during a cold water anomaly", Deep-Sea Research, 36, 715-733, 1989.
9. K.G. Porter, Y.S. Feig, "The use of DAPI for identifying and counting aquatic microflora", Limnology and Oceanography, 25, 943-948, 1980.
10. D. Kirchman, E. K'Nees, R. Hodson, "Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems", Applied and Environmental Microbiology, 49, 599-607, 1985.
11. M. Simon, F. Azam, "Protein content and protein synthesis rates of planktonic marine bacteria", Marine Ecology Progress Series", 51, 201-213, 1989.
12. D.C. Smith, F. Azam, "A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine", Marine Microbial Food Webs, 6, 107-114, 1992.
13. W.B. Yoon, R.A. Rosson, "Improved method of enumeration of attached bacteria for study of fluctuation in the abundance of attached and free-living bacteria in response to diel variation in seawater turbidity", Appl. Environ. Microbiol., 56, 595-600, 1990.
14. O. Holm-Hansen, C.J. Lorenzen, R.W. Holmes, J.D. Strickland, "Fluorometric determination of chlorophyll", J. Cons. Cons. Int. Explor. Mer, 30, 3-15, 1965.
15. T.R. Parson, Y. Maita, C.M. Lalli, "A manual of chemical and biological methods for seawater analysis", 173 pp., Elsevier, NY, 1984.
16. D.W. Van der Linde, "Protocol for determination of total suspended matter in oceans and coastal zones, "Technical Note 1.98.182, Joint Res. Cent., Brussels, 1998.
17. S. Tassan, G.M. Ferrari, "An alternative approach to absorption measurements of aquatic particles retained in filters", Limnology and Oceanography, 40, 1358-1368, 1995.
18. M. Babin, D. Stramski, G. M. Ferrari, H. Claustre, A. Bricaud, G. Obolensky, N. Hoepffner, "Variations in the light absorption coefficients of phytoplankton, non-algal particles, and dissolved organic matter in coastal waters around Europe", Journal of Geophysical Research, 108(C7), 3211, doi:10.1029/2001JC000882, 2003.
19. G.M. Ferrari, S. Tassan, "A method using chemical oxidation to remove light absorption by phytoplankton pigments, Journal of Phycology, 35, 1090-1098, 1999.
20. M. Babin, D. Stramski, "Variations in the mass-specific absorption coefficient of mineral particles suspended in water", Limnology and Oceanography, 49, 756-767, 2004.
21. R.W. Sheldon, T.R. Parson, "Practical manual on the use of the Coulter Counter in marine sciences", Coulter electronics.
22. Y.C. Agrawal, H.C. Pottsmith, "Instruments for particle size and settling velocity observations in sediment transport", Marine Geology, 168, 89-114, 2000.
23. J. Iriberri, M. Unanue, I. Barcina, L. Egea, "Seasonal variation in population density and heterotrophic activity of attached and free-living bacteria in coastal waters", Applied Environmental and Microbiology, 53, 2308-2314, 1987.
24. J.K.B. Bishop, "Spatial and temporal variability of POC in the northeast Subarctic Pacific", Deep Sea Research II, 2699-2733, 1999.

25. R.W. Spinrad, C.M. Yentsch, J. Brown, Q. Dortch, E. Haugen, N. Relevante, L. Shapiro, “The response of beam attenuation to heterotrophic growth in a natural population of plankton”, Limnology and Oceanography, 34, 1601-1605, 1989.
26. L.R. Pomeroy, W.J. Wiebe, “Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria”, Aquatic Microbial Ecology, 23, 187-204, 2001.
27. C.M. Turley, E.D. Stutt, “Depth related cell specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the NW Mediterranean”, Limnology and Oceanography, 45, 4129-425, 2000.
28. H. Plough, H.P. Grossart, “Bacterial growth and grazing on diatom aggregates: Respiratory carbon turnover as a function of aggregate size and sinking velocity”, Limnology and Oceanography, 45, 1467-1475, 2000.
29. X.A.G. Morán, M. Estrada, C. Pedrós-Alió, “Dissolved primary production and the strength of phytoplankton-bacterioplankton coupling in contrasting marine regions”, Microbial Ecology, 44, 217-223, 2002.
30. K.D. Bidle, F. Azam, “Bacterial control of silicon regeneration from diatom detritus” significance of bacterial ectohydrolases and species identity”, Limnology and Oceanography, 46, 1606-1623, 2001.

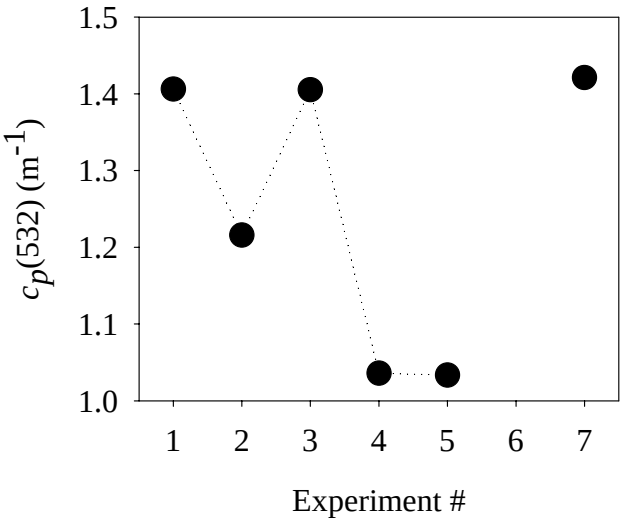

Fig. 1. Variability of particle beam attenuation coefficient, $c_p(532)$, in nearshore waters at Scripps pier in La Jolla, California, during 2006-2007 experiments. Values of $c_p(532)$ were derived from LISST measurements. No LISST data were collected in experiment 6.

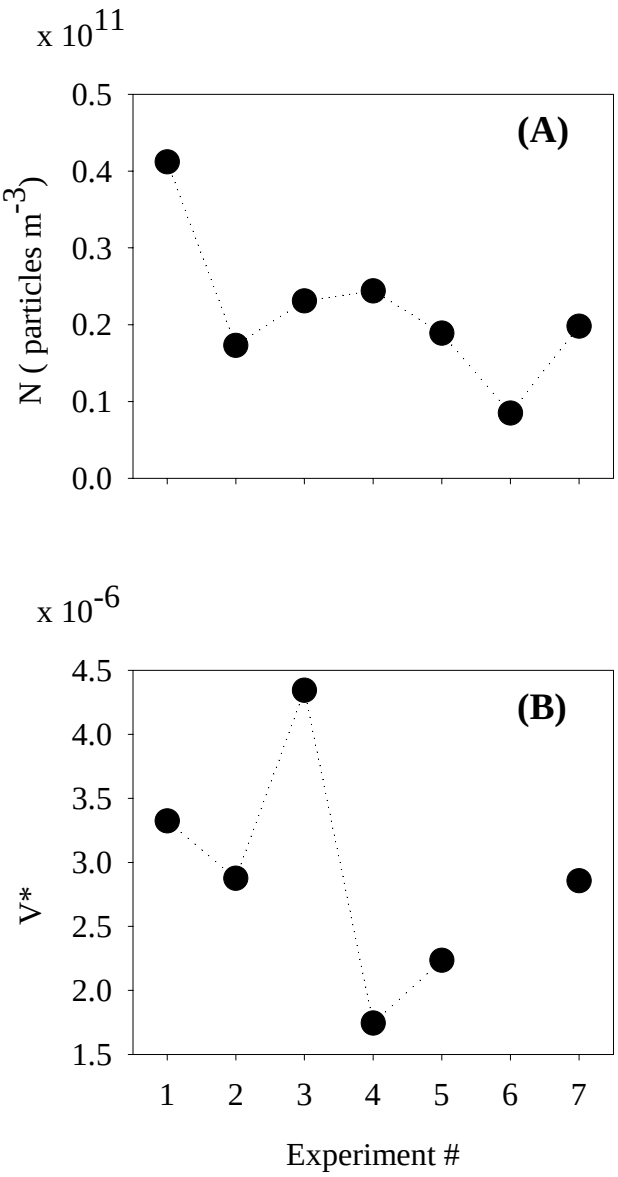

Fig. 2. Variability of (A) particle number concentration (N) for particles in the size range 2-60 µm estimated using Beckman-Coulter Multisizer, and (B) particle volume concentration per unit volume of seawater (V*) for particles between 1.05 and 198.6 µm estimated from LISST measurements in nearshore waters at Scripps pier in La Jolla, California, during 2006-2007 experiments. No LISST data were collected in experiment 6.

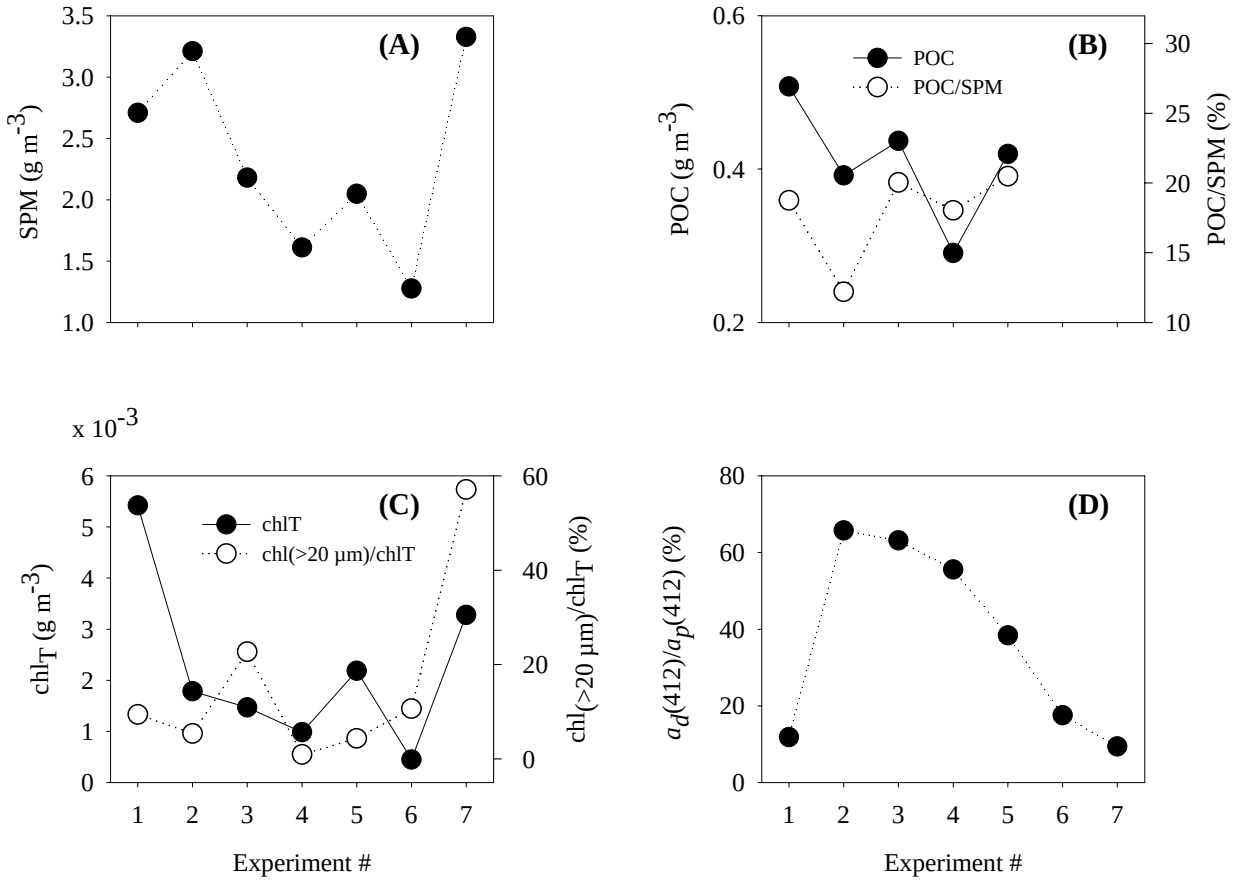

Fig. 3. Variability of properties of particulate assemblages in nearshore waters at Scripps pier in La Jolla, California, during 2006-2007 experiments. (A) mass concentration of suspended particulate matter (SPM), (B) concentration of particulate organic carbon (POC) (left y-axis) and contribution of POC to SPM (right y-axis), (C) total concentration of chlorophyll *a* (chl_T) (left y-axis) and microphytoplankton contribution (cells >20 µm) to chl_T (right y-axis), (D) contribution of non-algal absorption to total particulate absorption at λ = 412 nm, $a_d(412)/a_p(412)$. No POC data available for experiments 6 and 7.

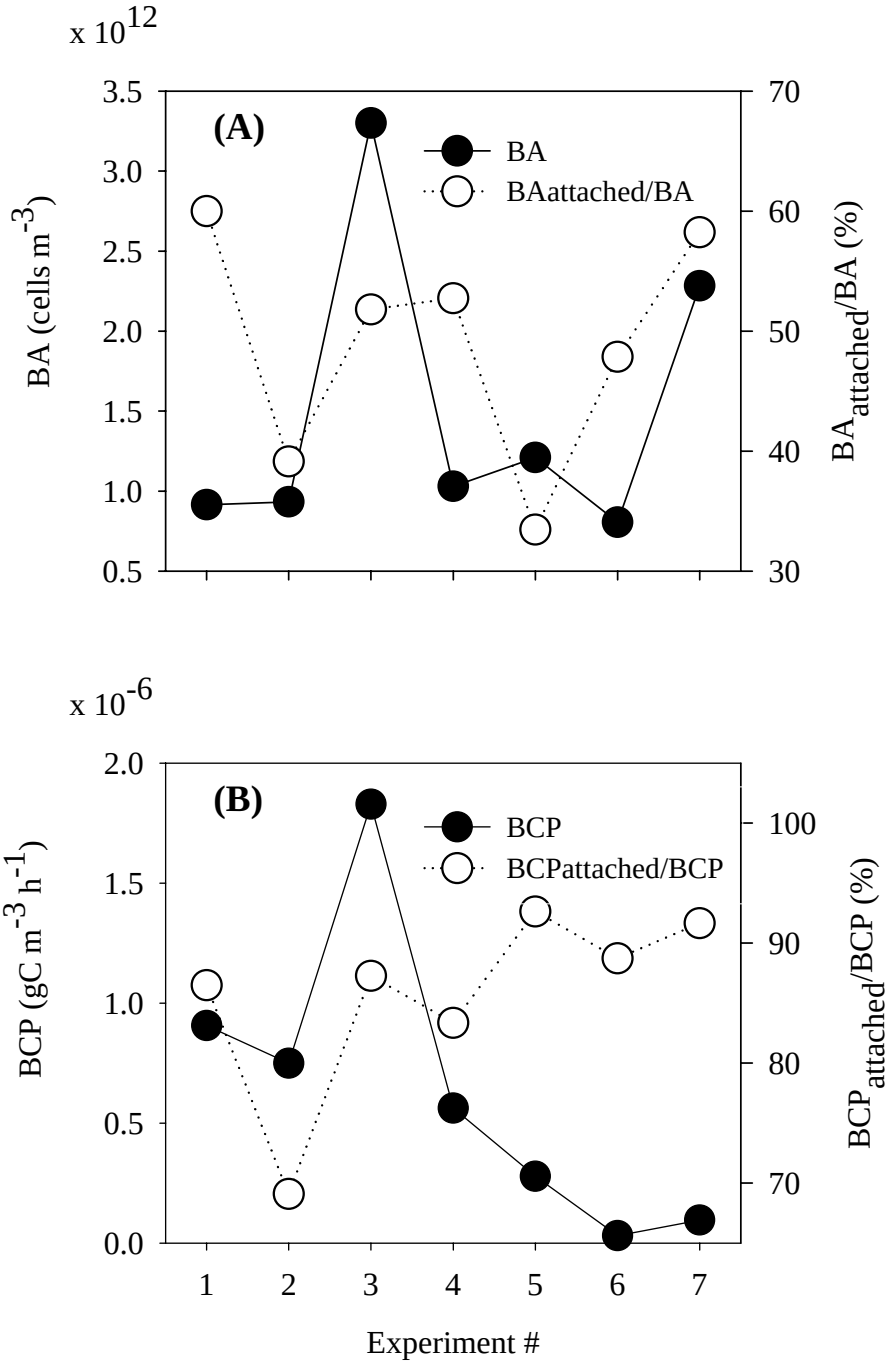

Fig. 4. Variability of marine heterotrophic bacteria in nearshore waters at Scripps pier in La Jolla, California, during 2006-2007 experiments. (A) total number of bacteria cells per unit volume of water (BA) (left y-axis) and contribution of particle-attached bacteria to BA (right y-axis), (B) total bacteria carbon production per unit volume of water (BCP) (left y-axis) and contribution of particle-attached bacteria to BCP (right y-axis).