

Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey

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Abstract

Theory suggests that variation in the attractive solvation force associated with cell-surface hydrophobicity can significantly affect contact rates among small cells in aqueous environments and consequently may influence rates and selective impacts of marine nanoflagellate grazers feeding on picoplankton assemblages. To investigate this hypothesis, we assayed the natural range in hydrophobic characteristics of subtropical picoplankton from the oligotrophic subtropical Pacific (Station Aloha, 22°45'N, 158°W) and mesotrophic Kaneohe Bay, Hawaii, using hydrophobic interaction chromatography (HIC) in conjunction with analytical flow cytometry. Variability in a relative index of cell-surface hydrophobicity (HIC index) for heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*, exhibited some consistent spatial patterns. The HIC index for *Prochlorococcus* at Station Aloha varied about threefold, being consistently more hydrophobic in the upper 80 m of the water column and dropping abruptly below this depth. Heterotrophic bacteria were more hydrophobic near the surface and decreased slightly, but steadily, with increasing depth. The hydrophobicity of heterotrophic bacteria steadily increased along a Kaneohe Bay transect extending from oligotrophic to mesotrophic conditions. In experiments involving nanoflagellates grazing on laboratory cultures of *Prochlorococcus*, cell cultures exhibiting the highest HIC indices were grazed upon at the highest rates. An additional experiment involving mixtures of *Prochlorococcus* cells exhibiting high and low hydrophobicities showed that the average hydrophobicity of the uningested prey mixture was driven progressively toward lower hydrophobicity as the more hydrophobic cells were selectively removed through time. If these laboratory grazing results hold in nature, the rate at which picoplankton cells are cleared from suspension by nanoflagellates could vary by as much as twofold due solely to natural variation in cell surface hydrophobicity.

Heterotrophic nanoflagellates are the principal consumers of picoplankton in many oceanic systems (Fenchel 1982a,b; Azam et al. 1983; Sherr and Sherr 1994). In the oligotrophic open ocean, where photosynthetic picoplankton can dominate total autotrophic carbon fixation (Li et al. 1983; Platt et al. 1983; Goericke and Welschmeyer 1993; Vaulot et al. 1995), heterotrophic nanoflagellates are typically the most important herbivores (Herbland et al. 1985; Campbell and Carpenter 1986; Murray et al. 1994; Landry et al. 1995, 1997; Monger et al. 1997). In coastal ecosystems where picophytoplankton are less important relative to larger forms, small flagellates are the main pathway through which the considerable secondary production of heterotrophic bacteria is transferred to higher trophic levels or remineralized back

to primary producers (Johannes 1965; Caron et al. 1985; Goldman and Caron 1985).

While heterotrophic nanoflagellates as a group include morphologically complex forms (Patterson and Larsen 1991), the dominant open-ocean flagellates often lack elaborate filtering structures and capture prey by a process of direct interception as described by Fenchel (1984). The physical basis for this suspension-feeding mode bears many similarities to particle-particle interactions in colloidal aggregation systems. As a consequence of their similarities, attractive molecular forces that enhance colloidal aggregation should also increase contact frequencies between nanoflagellates and picoplankton prey, while repulsive molecular forces that promote colloidal stability should result in a reduction in prey contact rate (Fig. 1).

As a first attempt at predicting patterns and rates of flagellate grazing interactions based on principles of colloid aggregation theory, Monger and Landry (1990) adapted a first-order numerical model of colloid aggregation (Fitzpatrick 1972) to predict contact rates between nanoflagellates and picoplankton prey. The size dependency of grazing interactions predicted by the numerical model was generally consistent with observations (Monger and Landry 1991, 1992). However, predicted grazing rate estimates were often substantially lower than measured estimates, suggesting that the first-order numerical model might have neglected im-

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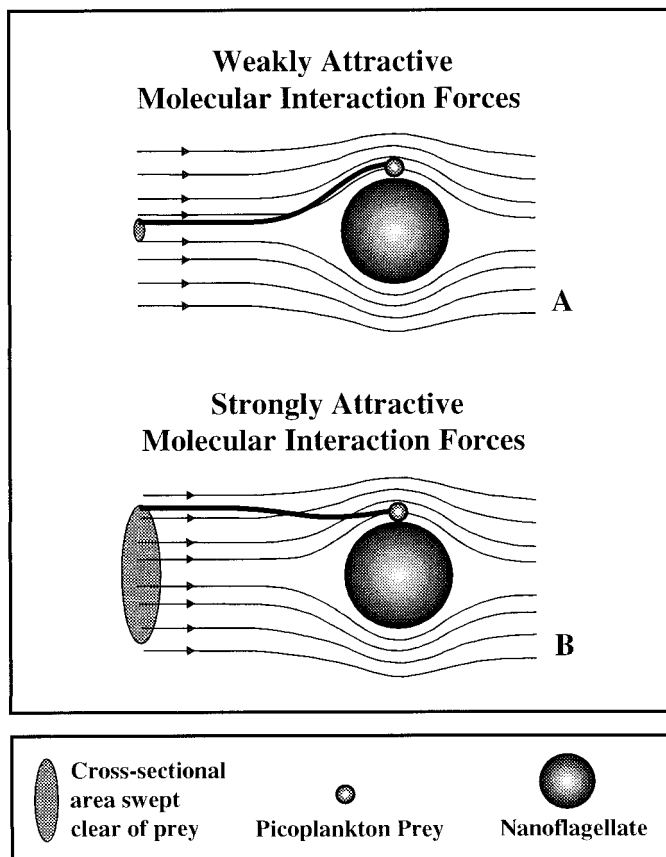


Fig. 1. Schematic representation of the effect that molecular interaction forces have on nanoflagellate clearance rates. As a prey approaches a nanoflagellate, attractive molecular interaction forces act to counterbalance other repulsive molecular interaction forces and a hydrodynamic resistance force. When attractive molecular forces are weak, the upstream prey must be relatively close to the axis defining the swimming path of the nanoflagellate in order to be captured and, consequently, the cross-sectional area swept clear of prey per unit time by the nanoflagellate (i.e., clearance rate) is relatively small (A). When the attractive molecular forces are strong (e.g., with the addition of a strong hydrophobic interaction force component), prey are pulled off their original streamline and, consequently, the nanoflagellate clearance rate is relatively high (B).

portant attractive molecular forces or behaviors that enhanced the frequency of encounters with prey. Based on arguments presented by Monger (1993), model underestimates may have been caused by the neglect of the hydrophobic interaction force.

The hydrophobic interaction force is a strongly attractive and long-range solvation force that arises when two surfaces that exhibit weak molecular interactions with adjacent water molecules are placed near each other in an aqueous environment (Israelachvili and Pashley 1982; Pashley et al. 1985; Israelachvili 1987). A familiar example of this force's action is the coalescence and separation of a nonpolar liquid phase from a polar liquid phase after vigorous agitation in a common organic chemistry separation procedure. In the medical literature, bacterial cell surface hydrophobicity is known to vary widely and is viewed as an important parameter regu-

lating contact probability and vulnerability to phagocytosis by mammalian leukocytes (van Oss 1978; Absolom 1988; Maródi et al. 1990; Yamada and Matsumoto 1990). By analogy, if picoplankton populations in natural aquatic ecosystems exhibit a range of cellular hydrophobicity, and particularly if the strength of this attractive force varies among different groups or as a function of physiological state, such variations could be important for understanding the rates and selectivities of the predators feeding upon them.

In the current article, we present observational and experimental results to test hypotheses that (1) there are quantifiable spatial variations in the cell-surface hydrophobicity of naturally occurring marine picoplankton and (2) the clearance rates of heterotrophic nanoflagellates vary significantly with the hydrophobicity of their picoplankton prey.

Materials and methods

Hydrophobic interaction chromatography (HIC)—HIC was used, in combination with analytical flow cytometry, to determine the relative extent of cell-surface hydrophobicity of naturally occurring marine picoplankton populations. Following, generally, the HIC method described previously (Smyth et al. 1978; Hermansson et al. 1982), replicate seawater subsamples were passed, respectively, through preconditioned high-performance liquid chromatography preparatory columns (Waters) containing 100 mg of hydrophobic (C18) or hydrophilic (Diol) resin. A relative index of cell-surface hydrophobicity (HIC index) was then computed using the expression (Clark et al. 1985)

$$\text{HIC index} = \frac{E(\text{Diol}) - E(\text{C18})}{E(\text{Diol})} \quad (1)$$

where $E(\text{Diol})$ and $E(\text{C18})$ represent the number of cells eluted through columns containing Diol and C18 resins, respectively. Separate HIC indices were determined for natural assemblages of heterotrophic bacteria, *Synechococcus* and *Prochlorococcus*, by measuring cell abundance of the respective taxa in column elutants using the dual-beam flow cytometry method described below. In addition to HIC indices, naturally occurring abundances of the respective taxa were also determined using dual-beam flow cytometry.

The actual mechanics of running the HIC assays involved placing up to 12 pairs of C18 and Diol preparatory columns, representing a complete hydrocast or grazing experiment, into a custom-made test-tube rack. The 1.0-ml preparatory columns were attached to the end of 5.0-ml plastic Eppendorf repeating syringes to accommodate larger seawater samples. The pairs of columns were held firmly upright in the test-tube rack. Small plastic test tubes were placed just under each column to collect the elutant. Seawater subsamples (5 ml) were dispensed into the top of the respective column pairs, syringe plungers were put in place, and a flat board was positioned into vertical guides above the extended syringe plungers and gently depressed by hand to force the liquid through all columns at a uniform rate of about 1.0 ml min^{-1} . Approximately 2 ml of sample was initially forced through the columns to equilibrate the resin with the sample. The initial 2 ml of elutant was then discarded, and the re-

maintaining 3.0 ml was collected in clean plastic tubes. A subsample of the elutant (1.0 ml) was placed in a cryotube, fixed with 100 μl of 10% paraformaldehyde, and stored in liquid nitrogen for later analysis.

The C18 resin was preconditioned by passing 5 ml of ethanol through each column followed by 5 ml of sterile-filtered (0.22- μm pore diameter) seawater. The Diol resin was preconditioned by passing 5 ml of sterile-filtered seawater through each column. To avoid drying the resins before samples were run, columns were preconditioned less than 10 min before HIC analysis.

HIC precision—Sources of variability in the HIC method are associated with (1) the precision of flow cytometric abundance estimates, (2) variability within a single rack of preparatory columns, and (3) variability between successive racks of preparatory columns. The accuracy and precision of the flow cytometric abundance estimates has been discussed previously and coefficients of variability (CVs) are usually not more than a few percent (Monger and Landry 1993). To examine the other two sources of variability, HIC indices were determined for 16 subsamples drawn from a single seawater sample. The 16 subsamples were processed in four separate test-tube racks containing four pairs of C18 and Diol preparatory columns. Analysis of variance was performed to determine the precision of computed HIC indices associated within and between successive racks of samples. The seawater used in the precision analysis was obtained from Kaneohe Bay, Hawaii.

Flow cytometric analyses—An EPICS 753 flow cytometer (Coulter) equipped with two 5-W argon ion lasers (Coherent), Cytomation[®] analog-to-digital-converters (ADCs), and a Biosense flow cell (Coulter) was used for all flow cytometric analyses. Signals from each photomultiplier tube (PMT) were collected in log-integral mode (three decades; 256 channels) without the use of a gated amplifier or fluorescence compensation. Listmode files were analyzed on an IBM-PC-compatible microcomputer using a custom-designed software program (CytoCI) created by D. Vaultot (Station Biologique, Roscoff, France).

To estimate the abundances of heterotrophic bacteria, *Synechococcus* spp. and *Prochlorococcus* spp. in natural seawater samples and in HIC assays, we used the flow cytometric method of Monger and Landry (1993). Briefly, paraformaldehyde-preserved (1% final volume) natural seawater samples or HIC column elutants were stained with a DNA-specific fluorochrome (Hoechst 33342) and subsequently interrogated using colinearly focused dual laser beams. The wavelength and power of light for each of the two lasers were, respectively, 315 nm (ultraviolet) at 200 mW and 488 nm at 1.0 W. Data acquisition was triggered on either blue, red, or orange fluorescence. Forward angle light scatter (FALS), right angle light scatter (RALS), red fluorescence, orange fluorescence, and blue fluorescence were collected for each sample. Heterotrophic bacteria, *Prochlorococcus* and *Synechococcus* populations were distinguished by their unique fluorescence and scattering signatures as described by Monger and Landry (1993).

A different flow cytometer configuration was used for

measuring cell abundances in laboratory cultures of *Prochlorococcus* and determining the grazing rates of heterotrophic nanoflagellates feeding on these cultures. In both cases a single laser beam was used to interrogate individual cells (wavelength = 488 nm; power = 500 mW). An ND1 neutral density filter was used in front of the FALS detector. A 488 nm dichroic rejection band filter (Coulter) was used to reflect 488 nm light to the RALS PMT while transmitting longer and shorter wavelengths. A 630-nm dichroic short-pass filter (Coulter) was used to reflect the red light caused by chlorophyll fluorescence to a second PMT. A 680-nm interference bandpass filter (Omega Optical) was placed in front of this second PMT. To determine cell abundance in the *Prochlorococcus* cultures both before and after passing through preparatory columns, 100- μl samples were run at a rate of 65 $\mu\text{l min}^{-1}$. Red fluorescence was used to trigger ADC data acquisition. Whenever cell abundance in the initial sample exceeded $\sim 1.0 \times 10^6$ cells ml^{-1} , they were diluted to give a final concentration of about 1.0×10^5 cells ml^{-1} and a count rate that did not exceed about 2,000 cells s^{-1} . Grazing rates were determined by running 100- μl samples at 65 $\mu\text{l min}^{-1}$ and using both FALS and red fluorescence to trigger ADC data acquisition. Count rates ranged between about 30 and 400 counts s^{-1} . More than a thousand nanoflagellates were analyzed for each time point. Particles forming a clearly defined population identified by large FALS and RALS signals, but lacking appreciable red fluorescence, were scored as flagellates without ingested prey and particles with a similar strong scattering signature and large red fluorescence were scored as flagellates with ingested prey. Uningested *Prochlorococcus* cells formed a uniquely defined population based on high red fluorescence, appreciable RALS, and very low FALS signals.

Kaneohe Bay—HIC measurements were made on picoplankton assemblages in various regions in Kaneohe Bay to examine possible changes in cell hydrophobicities with change in trophic status. HIC indices for *Synechococcus* and heterotrophic bacteria were determined for seawater samples collected at three locations in Kaneohe Bay. The three stations were representative of outer, central, and near-shore regions in the bay that fall along a gradient in trophic richness from semioligotrophic (outer) to mesotrophic conditions (near-shore, Coconut Island). Seawater samples were collected on 8, 12, and 13 September 1994. Samples were obtained from just below the sea surface in 50-ml sterile polypropylene centrifuge tubes and then placed in a cool dark ice chest until their return to the laboratory for subsequent determination of picoplankton abundances and hydrophobicities. Determination of extracted chlorophyll (Chl) *a* concentration (Strickland and Parsons 1972) was also made on separate seawater samples taken at each station. Chlorophyll samples of 225 ml were filtered onto 25-mm-diameter glass fiber filters (Whatman GF/F) and subsequently extracted in 90% acetone for 5 d in the dark at -20°C (Letelier et al. 1996). The fluorescence of extracted samples was determined with a Turner model 112 fluorometer. A Pearson correlation and a one-sample *t*-test for the significance of a correlation (Rosner 1982) were used to test statistically the

relationship between HIC indices of heterotrophic bacteria or *Synechococcus* and chlorophyll concentration.

Station Aloha—Seawater samples were collected from the subtropical north Pacific gyre (Station Aloha, 22°45'N, 158°W) at discrete depths to assess potential depth-dependent variations in the hydrophobicities of resident picoplankton assemblages under conditions more typical of the oligotrophic open ocean. Teflon-coated 10-liter GoFlo bottles were used to collect seawater between 20 and 21 August 1996 aboard the R/V *Moana Wave* during Hawaii Ocean Time Series cruise 75. Samples were drawn from GoFlo bottles into 50-ml polypropylene centrifuge tubes. HIC indices for picoplankton populations were determined as described above except that column elutants were stored in a -20°C freezer onboard the ship, and transferred to a -80°C laboratory freezer at the end of the cruise.

Laboratory cultures—Two types of controlled experiments were conducted with laboratory cultures of picoplankton and nanoflagellates to ascertain the extent to which the variations in picoplankton hydrophobicity that were observed in the field studies might translate into variations in nanoflagellate grazing rates. Laboratory cultures of *Prochlorococcus* and a heterotrophic chrysoomonad, *Paraphysomonas bandienseis*, were used, respectively, for the picoplankton prey and nanoflagellate grazer in all experiments. The original *Prochlorococcus* isolates were provided to us by L. Campbell (Texas A&M Univ.). *Prochlorococcus* cultures (~100 ml) were maintained in 250-ml polycarbonate plastic screw-cap flasks at several different light intensities in an environmental incubator held at 20°C on a 12:12 light:dark cycle. *P. bandienseis* was originally provided by D. Caron (Woods Hole Oceanographic Institution) and has been used by the present authors in previous grazing studies (Monger and Landry 1991, 1992). The nanoflagellate culture (~100 ml) was maintained in 125-ml polycarbonate plastic screw-cap bottles containing a medium of presterilized seawater supplemented with barley grains. Several days prior to each grazing experiment, the culture was transferred to a 500-ml polycarbonate bottle containing about 350 ml of medium to achieve a large volume of nanoflagellates growing in exponential phase.

Grazing on individual *Prochlorococcus* cultures—Laboratory experiments were conducted to determine the extent to which the hydrophobicity of a given *Prochlorococcus* culture affected nanoflagellate grazing rates. In each case, a series of *Prochlorococcus* cultures representing a wide range of HIC indices were systematically fed to a heterotrophic nanoflagellate culture to determine the hydrophobic dependency of grazing by the nanoflagellate. For each experiment, a set of approximately 20 *Prochlorococcus* cultures in various stages of growth was screened using the HIC technique described above. Between seven and nine cultures that spanned the full scale of HIC indices were selected from the initial set of cultures. Nanoflagellate grazing rates on each of the selected *Prochlorococcus* cultures were estimated from flow cytometric measurements of the time-course up-

take of prey by the nanoflagellate grazer population (Monger and Landry 1992).

A nanoflagellate culture with an initial volume of about 300 ml was used for each full grazing experiment. A 25-ml aliquot was removed from the nanoflagellate culture and placed into a 50-ml polystyrene centrifuge tube and subsequently inoculated with a small volume (e.g., 50–200 μ l) of a selected high-density *Prochlorococcus* culture to achieve a final concentration between 5×10^4 and 5×10^5 *Prochlorococcus* cells ml⁻¹. A 1.0-ml sample was removed from the nanoflagellate-*Prochlorococcus* mixture every 3 min, over a 21-min period, and analyzed by flow cytometry immediately without fixation to determine the number of *Prochlorococcus* ingested by the flagellate population. Additional time-course uptake measurements were repeated with a new 25-ml aliquot of nanoflagellate culture for each of the selected *Prochlorococcus* cultures.

Ingestion rates were determined from the slopes of the linear portion of the prey uptake curves over time. Clearance rates were obtained by dividing ingestion rates by the densities of prey added to the culture at the beginning of the experiment. An entire grazing sequence involving seven to nine *Prochlorococcus* cultures was performed on three separate occasions over the course of a 2-week period. The results of all three experiments were pooled to determine the statistical significance of the correlation between nanoflagellate clearance rate and picoplankton hydrophobicity. A Pearson correlation and a one-sample *t*-test for the significance of a correlation were applied to the pooled data.

It was not possible to make direct measurements of cell size for the *Prochlorococcus* cultures used in the laboratory grazing experiments. However, the level of RALS is often used as an index of cell size (Campbell et al. 1994; Jacquet et al. 1998). Consequently, to examine the influence of prey size on grazing rate, a Pearson correlation and a one-sample *t*-test for the significance of a correlation were applied to RALS versus picoplankton hydrophobicity and to RALS versus nanoflagellate clearance rate to examine the influence of prey size on grazing rate. Multiple correlation analysis was also performed by treating clearance rate as the dependent variable and treating both RALS and picoplankton hydrophobicity as independent variables.

Selective grazing on a picoplankton assemblage of mixed hydrophobicities—A mixed-prey experiment was conducted to illustrate the principal that selective grazer removal of the most hydrophobic cells in a mixed picoplankton assemblage will drive the average hydrophobicity of the uningested cells toward more hydrophilic forms. Several *Prochlorococcus* cultures were screened using hydrophobic interaction chromatography to find two cultures that exhibited high and low levels of hydrophobicity, respectively. These endmember cultures were mixed in different proportions (75:25, 50:50) to obtain a mixed assemblage of cells with intermediate average hydrophobicity. Estimates of RALS for the two cultures were used to determine if there were obvious size differences between the two cultures.

Nanoflagellate culture aliquots of 100 ml were placed in separate 125-ml polycarbonate bottles and inoculated with a small volume of the endmember or mixed *Prochlorococcus*

Table 1. Results from an analysis of variance for repeated determination of HIC indices for a single seawater sample taken from Kaneohe Bay, Hawaii. Four separate test-tube racks, each containing four replicate preparatory column pairs, were processed as described in the Materials and methods section.

	<i>Synechococcus</i>	Pico-eukaryotes	Heterotrophic bacteria
Abundance (cells ml ⁻¹)	1.51×10^5	2.15×10^4	1.64×10^6
Average HIC index	0.264	0.705	0.335
SD* (between racks)	0.084	0.146	0.061
SD (within racks)	0.087	0.086	0.035
SD (pooled samples)	0.087	0.101	0.042

* SD = sample standard deviation.

populations (1×10^5 *Prochlorococcus* cells ml⁻¹, final concentration). At approximately 1–3-h intervals, 15-ml subsamples were removed for immediate HIC analysis of the uningested *Prochlorococcus* population. A 1.0-ml aliquot was also removed at the same time, fixed with 100 μ l paraformaldehyde, and stored in liquid nitrogen for later flow cytometric analysis of *Prochlorococcus* abundance. Grazing rates were determined from the decrease in the concentration of uningested prey over time assuming an exponential decline. Separate nongrazer controls were not used in the experiment because cell abundance estimates for the two *Prochlorococcus* cultures that had been monitored over the 2-week period leading up to the experiment, revealed that their growth rates would be an order of magnitude lower than the expected experimental grazing rates so that for all intents and purposes growth could be considered to be zero over the course of the experiment.

Results

HIC precision—Results from an analysis of variance on the 16 replicate HIC indices determined for a single Kaneohe Bay seawater sample are presented in Table 1. The standard deviations of the pooled HIC-index measurements for heterotrophic bacteria, *Synechococcus* and pico-eukaryotes were 0.042, 0.087, and 0.101 HIC-index units, respectively. An analysis of variance could not be performed on *Prochlorococcus* because they were absent in Kaneohe Bay at the time the sample was collected. HIC-index variability was significantly lower for heterotrophic bacteria than for either of the other two taxa ($P < 0.007$). There was no apparent difference in the magnitude of variability within and between processing of test-tube racks containing paired preparatory columns.

Kaneohe Bay field observations—Hydrophobicity of the heterotrophic bacterial assemblage in Kaneohe Bay (Fig. 2), as expressed by the HIC index, was positively correlated with Chl *a* concentration ($r^2 = 0.63$, $P = 0.01$). A similar correlation with regard to *Synechococcus* hydrophobicity and chlorophyll, however, was not evident ($r^2 = 0.10$, $P = 0.41$). It was not possible to determine an HIC index for *Prochlorococcus* at the three stations because *Prochlorococcus* cells were absent from the water column during the sampling period.

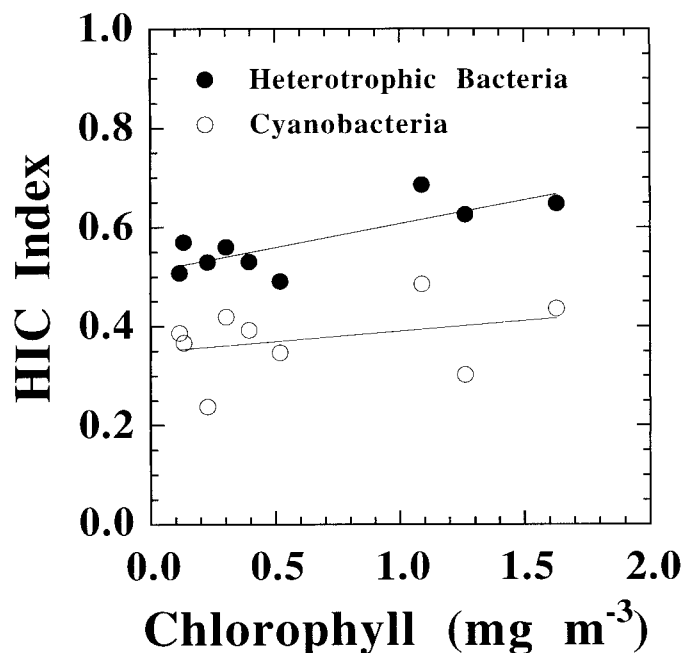


Fig. 2. Hydrophobic interaction chromatography (HIC) index of heterotrophic bacteria and cyanobacteria (*Synechococcus*) as a function of Chl *a* concentration. Samples were collected at three stations in Kaneohe Bay, Hawaii representing outer bay, central bay, and near-shore conditions. The HIC index is significantly correlated with Chl *a* concentration for heterotrophic bacteria ($r^2 = 0.63$, $P = 0.01$), but not for cyanobacteria ($r^2 = 0.10$, $P = 0.41$).

Station Aloha field observations—*Prochlorococcus* exhibited significant variations in hydrophobicity, as expressed by the HIC index, over the vertical extent of the water column at Station Aloha (Fig. 3A). A *t*-test for the difference between two means revealed that cells in the upper 100 m of the water column were significantly more hydrophobic than cells below this depth ($P \leq 0.001$). Heterotrophic bacteria hydrophobicity exhibited a negative correlation with increasing depth that was statistically significant ($P \leq 0.001$) when tested using a one-sample *t*-test for a correlation coefficient.

The vertical profiles of *Prochlorococcus* and heterotrophic bacterial abundances (Fig. 3C,D) were typical of prior observations at Station Aloha (Campbell et al. 1994). Both assemblages exhibited subsurface maxima, with those for the heterotrophic bacteria being somewhat less pronounced.

There was considerable temporal variability over the 24-h sampling period for both taxa, but no consistent temporal pattern of change in hydrophobicity could be discerned. Application of *F*-tests for the difference of two variances indicated that the levels of variability in HIC-index estimates for heterotrophic bacteria and *Prochlorococcus* were essentially the same at any given depth ($P \geq 0.27$). Additional *F*-tests revealed that the levels of temporal variability in the HIC-index estimates for heterotrophic bacteria of any given depth at Station Aloha were not significantly different from the basic level of measurement variability determined in the precision experiments described previously ($P \geq 0.15$).

Laboratory grazing experiments—Experiments involving nanoflagellates grazing on individual cultures of *Prochlo-*

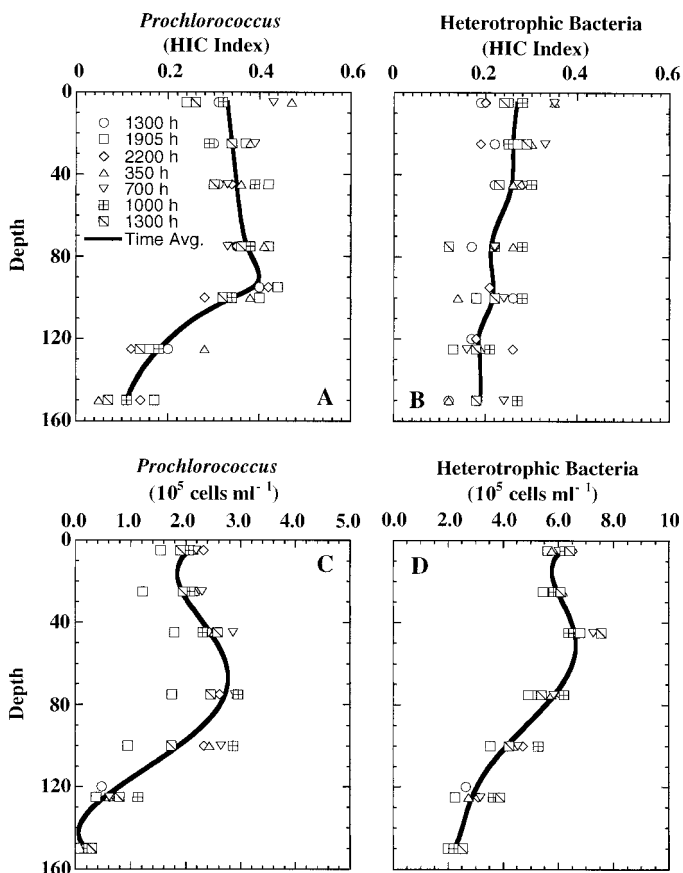


Fig. 3. Hydrophobic interaction chromatography index (HIC) and cell abundance of *Prochlorococcus* and heterotrophic bacteria at Station Aloha (22°45'N, 158°W). Samples were collected between 20 and 21 August 1996 during Hawaii Ocean Time-series cruise 75. Symbol legend given in A applies to all figures.

roccoccus demonstrated that clearance rates were higher on cultures with higher levels of hydrophobicity. Over the full range of HIC indices tested, the rate at which cells were cleared from suspension by the nanoflagellate varied by about twofold (Fig. 4). There was considerable variability about the generally increasing trend in the combined experiments ($r^2 = 0.37$), but the overall correlation was statistically significant ($P = 0.003$). The correlation between *Prochlorococcus* hydrophobicity and RALS was only slightly significant ($P = 0.08$) when analyzed using a one-sample t -test for a correlation coefficient. The correlation between nanoflagellate clearance rate and *Prochlorococcus* RALS was also only slightly significant ($P = 0.07$). Multiple correlation analysis using both RALS and hydrophobicity of the *Prochlorococcus* cultures as independent variables yielded a slightly better correlation with nanoflagellate clearance rate ($r^2 = 0.49$) than when hydrophobicity alone was considered.

In the experiment with mixed *Prochlorococcus* cultures, exponential loss rates ranged from 0.01 and 0.015 h^{-1} that were about an order of magnitude greater than the growth rates of the *Prochlorococcus* cultures that were monitored over a 2-week period leading up to the grazing experiment (growth rate = -0.00084 h^{-1} and 0.0014 h^{-1} for Pro 1 and

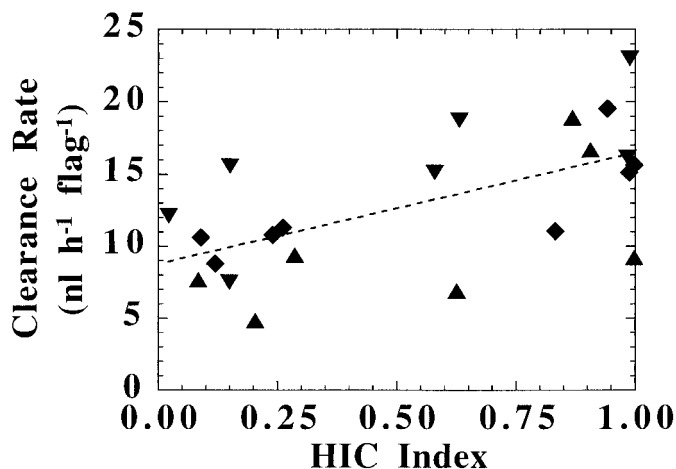


Fig. 4. Clearance rate of a *P. bandienseis* grazing on individual *Prochlorococcus* cultures with differing hydrophobic indices. *Prochlorococcus* hydrophobicity and nanoflagellate clearance rate are significantly correlated for the pooled experiments ($r^2 = 0.32$, $P = 0.002$). The dashed line represents a least-squares fit line through the data. Individual symbols represent measurements of the three respective grazing experiments that were pooled together.

Pro 2, respectively). Consequently, exponential loss rates measured in this experiment were essentially equivalent to grazing mortality. With this assumption, grazing rates showed a significant positive correlation ($P = 0.02$) between nanoflagellate grazing rate and overall hydrophobicity of the mixed *Prochlorococcus* assemblage (Fig. 5A). The scatter about this trend was considerably less than the scatter observed in the trends derived in the previous experiments (Fig. 4). The average hydrophobicity of uningested prey were driven progressively toward lower hydrophobicity as the more hydrophobic cells were removed through time (Fig. 5B). The high hydrophobic endmember culture was unchanged through time while the low hydrophobicity endmember culture became even more hydrophilic. The magnitude of the RALS for the two respective endmember *Prochlorococcus* cultures were not significantly different ($P = 0.14$) when analyzed with a two-sample t -test. This suggests that there was no appreciable size difference in the two cultures.

Discussion

Natural variability in the hydrophobicity of marine picoplankton—The present results reveal that naturally occurring assemblages of *Prochlorococcus* and heterotrophic bacteria exhibit measurable variations in cell-surface hydrophobicity. Rather than being completely random, these variations seem to exhibit some consistent patterns with respect to changes in environmental conditions (Figs. 2, 3). The fact that there was measurable variation in the hydrophobicity of marine picoplankton is not completely surprising because past studies have shown that the hydrophobicity of laboratory cultures of bacteria isolated from a variety of marine habitats can vary widely (Dahlbäck et al. 1981; Dawson et al. 1981; Kjelleberg and Hermansson 1984; Taylor et al. 1997). The

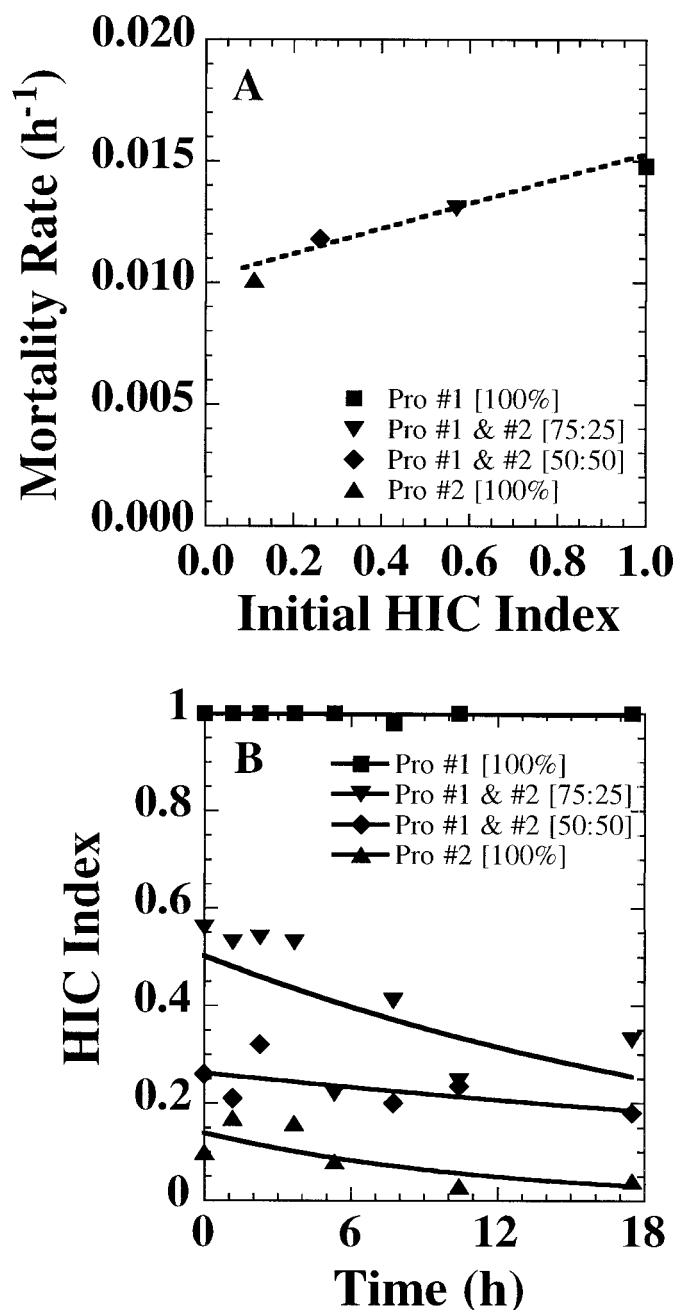


Fig. 5. (A) Mortality rates due to nanoflagellate grazing on variable mixtures of highly hydrophobic (Pro 1) and weakly hydrophobic (Pro 2) *Prochlorococcus* cultures, and (B) the average surface hydrophobicity of the uningested cells in the same *Prochlorococcus* mixtures resulting from the steady removal of the most hydrophobic cells by the nanoflagellate grazers. The correlation between the average hydrophobicity of the mixed *Prochlorococcus* culture at the beginning of the experiment and its subsequent mortality due to nanoflagellate grazing (A) was statistically significant ($r^2 = 0.96$, $P = 0.02$). The dashed line in panel A represents a least-squares fit through the data.

present study, however, represents the first time that variability in cell hydrophobicities has been determined directly for naturally occurring assemblages.

It is not clear from the present results whether the observed spatial variation in picoplankton hydrophobicity resulted from a uniform phenotypic response by cells to changes in growth conditions, a genotypic shift in a mixed assemblage due to differential growth and grazing mortality of individual genotypes, or a combination of these two processes. Past studies have shown that changes in growth conditions can strongly influence surface hydrophobicity of a given bacterial strain (McEldowney and Fletcher 1986; van Loosdrecht et al. 1987; Eisen and Reid 1989). It is reasonable to assume by analogy that environmental variability might also directly influence the hydrophobicity of *Prochlorococcus* and *Synechococcus* and contribute to the observed patterns. However, large strain-dependent differences in cell-surface properties of heterotrophic bacteria have also been reported (van Loosdrecht et al. 1987; Eisen and Reid 1989; Yaskovich and Yakovleva 1996), and the existence of a significant depth-dependent genetic variability in the resident *Prochlorococcus* assemblage is now well established (Partensky et al. 1999 and references therein). It then seems possible that genotypic differences could make a measurable contribution to observed vertical variations in the hydrophobicity of *Prochlorococcus*.

As a final remark on the observed variations in the HIC index of marine picoplankton, it should be noted that changes in ionic strength, and possibly other dissolved constituents in seawater, will alter the strength of the hydrophobic interaction force irrespective of any variation in the surface chemistry of picoplankton cells. This effect is folded implicitly into the HIC method because the cells interact with the hydrophobic column resin while suspended in the original seawater medium. It is important to note that the HIC index is not an absolute measure of cell surface hydrophobicity but rather a measure of a hydrophobic surface's reactivity under the particular chemical makeup of the suspending medium.

Grazing on Prochlorococcus cultures exhibiting different hydrophobicities—Higher clearance rates on more hydrophobic prey (Fig. 4) implies that increased hydrophobicity enhances contact rates between nanoflagellates and picoplankton prey, and/or successful phagocytosis of prey after the initial contact. Enhanced contact rate is consistent with modern colloid theory that predicts a stronger attractive solvation force between small particles exhibiting higher levels of hydrophobicity (Ninham 1980; Israelachvili 1985; Pashley et al. 1985; Israelachvili and McGuiggan 1988; Christenson 1992). Present observations are also consistent with prior studies of bacterial adhesion to immersed surfaces of different hydrophobic characteristics (Dahlbäck et al. 1981; Dawson et al. 1981; Absolom et al. 1983; Stenström 1989; Taylor et al. 1997). Moreover, the positive correlation between picoplankton prey hydrophobicity and nanoflagellate grazing rate is consistent with a large body of literature dealing with empirical and theoretical studies of phagocytic processes in mammalian immune systems (van Oss 1978; Absolom et al. 1982; Absolom 1988; Maródi 1990; Yamada and Matsumoto

1990; Yamada et al. 1993). Finally, the positive correlation between grazing rates and cell hydrophobicities for *Prochlorococcus* cultures (Fig. 4) is consistent with prior experiments involving the same chryomonad population that was fed plastic microspheres (0.5 μm diameter) that were coated with various proteins and block copolymers. The microspheres were coated to elicit different levels of surface hydrophobicity while keeping size constant (Monger 1993: figs. 6.1, 6.2).

The level of correlation between nanoflagellate clearance rate and *Prochlorococcus* hydrophobicity was reduced by variation in *Prochlorococcus* size as expressed by the magnitude of *Prochlorococcus* RALS. The two variables taken together explained about half of the total variability in the nanoflagellate clearance rates. It is important to point out that there are many factors other than picoplankton hydrophobicity and size that can influence picoplankton grazing mortality. Jürgens and Güde (1994) give a good review of this topic. Specific examples include picoplankton cell shape (Parnthaler et al. 1997; Jürgens et al. 1999; van Hannen et al. 1999), nanoflagellate chemotaxis (Sibbald et al. 1987; Bennett et al. 1988), and fluid turbulent motions (Shimeta et al. 1995).

It is also important to note that variation in the surface hydrophobicity of the nanoflagellate grazer can influence the clearance rate. Absolom (1988) offers a good theoretical discussion of this problem and presents results of an elegant experiment that demonstrates basic principles (Absolom 1988: fig. 9). Briefly, if a phagocytic organism's surface tension—which is inversely related to the level of surface hydrophobicity—is lower than the surface tension of the suspending liquid, the organism will ingest bacteria that have the highest hydrophobicity at the highest rate. A reverse selective grazing relationship results if the surface tension of a phagocytic organism is higher than the surface tension of the suspending liquid. If a phagocytic organism's surface tension is the same as the suspending liquid, there is no hydrophobic prey dependency. Presumably, in the current experiments the marine nanoflagellate had a surface tension that was lower than that of the suspending seawater. The extent of inter- and intraspecies variability in the surface hydrophobicity of marine nanoflagellates is currently not known. It is assumed to be constant in the laboratory experiments presented in this article.

Grazing on mixed picoplankton assemblages—Further confirmation of a nanoflagellate grazing preference for picoplankton cells that are more hydrophobic comes from the results of the experiment involving nanoflagellate grazing on mixtures of two *Prochlorococcus* cultures representing high and low hydrophobic endmember populations (Fig. 5). The average grazing rate on each of the culture mixtures was highly correlated with the initial hydrophobicity (Fig. 5A). The goodness of the fit of these data was considerably greater than in the previous experiments involving grazing on a series of seven to nine different *Prochlorococcus* cultures of different hydrophobicities (Fig. 4). The higher degree of variability in the previous experiments may have been due to size differences in the various cultures. The variability in the experiment involving the mixture of prey was probably min-

imized because only two different cultures were used and any bias in grazing rate caused by size difference would not be random.

The shift through time in the makeup of the assemblage of uningested *Prochlorococcus* cells toward a composition of cells with relatively lower levels of hydrophobicity is consistent with higher grazer removal rates of more hydrophobic cells. The hydrophobicity of the uningested cells in the high hydrophobicity member culture did not change through time. The best explanation for this result is that the endmember culture was composed of cells that were all very similar in their level of hydrophobicity so that grazer selection had no measurable effect on the *Prochlorococcus* population.

Variability in hydrophobicity of the different *Prochlorococcus* cultures resulted in a nearly twofold variation in grazing mortality (Fig. 4). The variability in hydrophobicity of natural *Prochlorococcus* populations (Fig. 3A) was less than that of the laboratory *Prochlorococcus* cultures, and so it might be presumed that grazing variability in natural systems may be proportionally less. It is probable, however, that the most hydrophobic cells are growing at a relatively higher rate than the average population and are consumed at a higher rate by the resident nanoflagellate assemblage. Under these circumstances, the range of picoplankton hydrophobicity would appear to be less dramatic than the measured level and so it leaves open the possibility of significant hydrophobic-dependent grazing variability in spite of the lower levels of variability in picoplankton hydrophobicity observed in the field.

For completeness it should be stated that it is feasible to think that the observed positive correlation between picoplankton hydrophobicity and nanoflagellate grazing rate may have been an indirect result caused by the aggregation of hydrophobic picoplankton cells and subsequent size-selective grazing of the cell aggregates by nanoflagellate grazers. In the end, however, the result is still the same in that picoplankton cells with greater hydrophobicity are more vulnerable to grazing mortality.

Implications for food web dynamics—Despite the obvious advantage of reduced grazing mortality for picoplankton cells that exhibit lower hydrophobicity, the present field studies reveal that there are certain environments where picoplankton hydrophobicity is relatively high (Figs. 2A, 3A). We speculate that there must be an enhanced growth advantage for cells that exhibit relatively higher hydrophobicity in these regions.

The hydrophobicity of *Prochlorococcus* at Station Aloha was highest in the upper region of the euphotic zone where growth-limiting nutrients are in low concentration. *Prochlorococcus* hydrophobicity decreased abruptly at approximately the thermocline depth where mineral nutrient concentrations increase abruptly and light begins to limit growth. These observations are consistent with past studies involving the effects of nutrient starvation in heterotrophic bacteria where the elimination of an essential nutrient often leads to increased cell-surface hydrophobicity (Dawson et al. 1981; Kjelleberg and Hermansson 1984). It has been suggested in past reports that an increase in bacterial hydrophobicity at extremely low nutrient conditions may be a survival

mechanism that causes an increase in bacterial attachment to particles leading either to enhanced vertical transport with the settling particles or to enhanced nutrient conditions within particle microzones (Dawson et al. 1981; Kjelleberg and Hermansson 1984). Alternatively, we believe it is possible that increased cell-surface hydrophobicity may be a survival response that facilitates the uptake of growth-limiting nutrients at low concentration. Results from the low-nutrient chemostat studies of van Loosdrecht et al. (1987: fig. 3) provide good evidence supporting this idea. They showed that as the dilution rate of the chemostat increased, the bacteria with lower hydrophobicity were washed out of the system leaving behind faster-growing bacterial isolates that had a higher level of hydrophobicity. Gurijala and Alexander (1990) examined the influence of bacterial hydrophobicity on the combined effects of growth rate and grazing mortality by *Tetrahymena thermophila*. They found that the advantage of more efficient growth was greater than the disadvantage of greater grazing mortality for the most hydrophobic bacterial strains.

A similar inverse relationship between picoplankton hydrophobicity and nutrient concentration did not occur along the transect in Kaneohe Bay (Fig. 2). One explanation for this observation is that the dominant bacterial strains in Kaneohe Bay naturally decrease in hydrophobicity in response to the decreasing nutrient concentrations. This somewhat unusual type of hydrophobic response to starvation has, in fact, been observed by others for a few bacterial strains (Kjelleberg and Hermansson 1984; Galdiero et al. 1993). Alternatively, a switch in the growth-limiting nutrient from one that is hydrophobic in nature to one that is hydrophilic could explain the trend in picoplankton hydrophobicity along the transect. Lemke et al. (1995) observed that under low nutrient conditions, hydrophobic bacterial strains had a growth advantage over hydrophilic strains only when the growth-limiting nutrient was hydrophobic in nature (e.g., organic-versus mineral-phosphate limitation). Observations of Zang and Miller (1994) generally support the conclusions of Lemke et al. (1995). The trend in hydrophobicity along the Kaneohe Bay transect could then be explainable if the limiting nutrient in the outer region of the bay was hydrophilic in nature while the limiting nutrient further in the bay was hydrophobic. Distinguishing which, if any, of these two hypotheses correctly explains the trend in picoplankton hydrophobicity along the transect is outside the scope of this article. The hypotheses are presented here only to stimulate thoughtful discussion.

In summary, it may be expected that whenever a picoplankter's growth is limited by the availability of a nutrient that is favorably taken up by cells that exhibit a high level of hydrophobicity, a balance must be struck between the picoplankter's need to compete efficiently for the limiting nutrient and its need to minimize cell hydrophobicity to reduce predation losses. On the other hand, when the uptake of a limiting nutrient is not dependent on a picoplankter's hydrophobicity, it should always be to a picoplankter's advantage to be as hydrophilic as possible to reduce predation losses. More generally, any shift in the type of limiting nutrient, or its concentration, should give rise to a shift in the level of picoplankton hydrophobicity needed to balance

growth and grazing constraints. Understanding the controls on this balance is an important area of continued research.

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