



Padilla Bay

National Estuarine Research Reserve

Reprint Series No. 24
Reprinted December 1995

**BACTERIAL PRODUCTION AND CONSUMPTION IN
MICROLAYER AND SUBSURFACE WATERS OF
PADILLA BAY, WASHINGTON**

Karen M. Thompson

August 1995

Publication No. SWR-95-76

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A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Karen M. Thompson

August 1995

Bibliographic citation: Thompson, Karen M. 1995. Bacterial production and consumption in microlayer and subsurface waters of Padilla Bay, Washington. Master's Thesis. Western Washington University, Bellingham, Washington. 82 pp. Padilla Bay National Estuarine Research Reserve Reprint No. 24, Reprinted December, 1995.

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BY

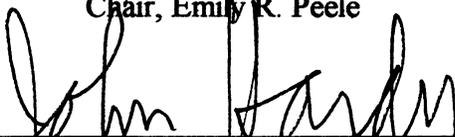
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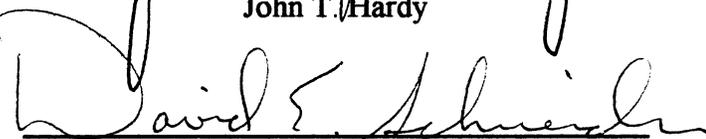
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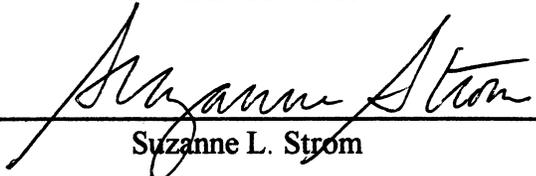

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BACTERIAL PRODUCTION AND CONSUMPTION IN MICROLAYER AND SUBSURFACE WATERS OF PADILLA BAY, WASHINGTON

by
Karen M. Thompson

ABSTRACT. Bacterial numbers, bacterial production and protistan bacterivory were measured in microlayer (~100 μm) and subsurface (~25 cm) water in Padilla Bay, Washington. Objectives were to compare bacterial numbers and heterotrophic activity in microlayer and subsurface waters, and to investigate the fate of bacterial production. Bacterial abundance was higher in microlayers than in subsurface waters. In contrast, subsurface bacterial communities were metabolically more active than microlayer communities. Bacterial production in the microlayer may have been limited by substrate composition or inhibited by pollutants concentrated in the surface film. Differences in grazing pressure may also explain lower metabolic activity in microlayers. Heterotrophic activity in microlayers can exceed subsurface activity if enrichment of bacteria in the surface film is significant. Small flagellates <5 μm were the most abundant members of the heterotrophic protistan community in both the microlayer and the subsurface. Other heterotrophic protists included flagellates 5 to 20 μm , dinoflagellates, and ciliates. Bacterivory occurred in both microlayer and subsurface waters and was attributed largely to flagellates. Grazing rates were not equivalent to rates of bacterial cell production, and usually were less than 1% of the estimated bacterial cell production. It appears that bacterivory by heterotrophic protists is not a major path of energy transfer in microlayer or subsurface waters of Padilla Bay. Other factors may be more important in balancing bacterial growth, such as physical advection and viral lysis.

ACKNOWLEDGMENTS

I thank my committee members, who have provided immeasurable guidance and support throughout the production of my Master's Thesis. My advisor, Emily Peele, spent many hours with me, providing encouragement when I needed it most. Her patience and enthusiasm, and sometimes candid reality checks during this process are greatly appreciated. Suzanne Strom provided me with equipment and supplies as well as work experience that were necessary for my research. She was always available when I had questions about my research, or many other things that seemed relevant at the time. I also thank Suzanne for my memories of the Point Sur and the SCM cruises which range from lurching decks in heavy seas to spectacular sunsets over the blue Pacific Ocean. Dave Schneider's accessibility and gentle kindness in a sometimes harsh academic environment will always be appreciated. When I go paddling, I will always consider the state of the sea-surface microlayer, and the organisms that depend on it because of the work of Jack Hardy.

Others who contributed to my research are to be thanked as well. From Shannon Point Marine Center, Gene McKeen was an absolute pleasure to work with. I am grateful for Gene's assistance while sampling in Padilla Bay, and helpful technical advice. I thank Brian Bingham for his generous advice on statistics and modeling. On campus, Geoff Landis made my rough idea of a microlayer sampler into reality.

I would also like to thank Doug Bulthuis and Sharon Riggs at Padilla Bay National Estuarine Research Reserve. Sharon is the best contract administrator I have ever worked with. I thank The Padilla Bay Foundation, and Shell Oil Company for the Assistantship in

Estuarine Science and Coastal Zone Management. The preparation of this thesis was financially aided through a grant to the Washington State Department of Ecology with funds obtained from NOAA/Office of Ocean and Coastal Resource Management, and appropriated for Section 306 of the Coastal Zone Management Act of 1972, as amended.

Finally, I can't begin to express thanks to my network of friends who are or have been MESP students for their support. I will always be grateful for the encouragement I received from Marlene Sanborn, and from my other friends at Shannon Point Marine Center. Mom and Dad were always there to listen to me as well, always offering loving words of support.

To remind myself of my reasons for pursuing this degree, I would often walk to the beach near the lab at Shannon Point, and watch the sunset over the islands. I frequently thought of a verse from a favorite song recorded by Bruce Cockburn:

“All the diamonds in this world that mean anything to me,
are conjured up by wind and sunlight sparkling on the sea.”

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INTRODUCTION

The sea-surface microlayer has unique chemical, physical and biological properties. Covering the surface of the world oceans, it is an important point of gaseous exchange between the atmosphere and water column (Hardy, 1982). The microlayer provides habitat for a variety of organisms known collectively as neuston. Neustonic organisms include bacterioneuston, zooneuston (including protists and invertebrate larvae), phytoneuston, and eggs and larvae of fish and shellfish (Tysban, 1971; Fuhs, 1982; Hardy, 1982; Hardy and Apts, 1984; 1989). Surface films can provide an enriched substrate for microbial growth. Enrichments of lipids, polysaccharides and proteins have been reported in the sea-surface microlayer, as well as enrichments of inorganic nutrients including phosphate, ammonium, nitrate and nitrite (see review by Maki, 1993). Hardy and Apts (1984, 1989) reported enrichments of bacterioneuston, phytoneuston, total chlorophyll and particulate carbon fixation in the sea-surface microlayer.

Norkrans (1980) described the sea-surface microlayer as consisting of three layers, including a surface hydrophobic lipid layer, a polysaccharide-protein film, and a lower layer of bacterioneuston extending to a depth of about 1.0 μm . Sieburth (1983) described the surface microlayer as consisting of proteins, carbohydrates, lipids and humic substances in a gel-like matrix, below which a layer of bacterioneuston extends down to about 10 μm . Both models differentiate between the sea-surface *microlayer* and the sea-surface *film*. The sea-surface film is defined as all other layers associated with the surface which may contain particulate organic matter and neustonic organisms. Hardy (1982) developed a model of the sea-surface microlayer that extends to about 50 μm , where

unique properties such as surface tension, reduced mixing, and particulate and organic accumulations occur (Fig. 1).

The term *sea-surface microlayer* is often used to describe both the microlayer and surface film. The depth of the sea-surface microlayer depends on the method used to collect samples, and ranges from 0.5 μm to 800 μm (Harvey and Burzell, 1972; Hatcher and Parker, 1974; Kjelleberg *et al.*, 1979; VanFleet and Williams, 1980; Hardy, 1982; Hardy *et al.* 1985; Hardy *et al.* 1988). Many particles and organisms associated with the sea-surface microlayer are larger than the depth of the microlayer as defined by Norkrans (1980), Sieburth (1983) and Hardy (1982). Additionally, some samplers are preferential in the collection of materials, depending on the chemical nature of the material. Therefore, the method chosen depends on the material being collected. In this study, the microlayer depth extends to $\sim 150 \mu\text{m}$, so organisms associated with the microlayer are included.

Mechanisms for the transport of substances to the sea surface include atmospheric deposition, rising air bubbles, upwelling (including Langmuir circulation), convection and diffusion from sediments and subsurface water (Hardy, 1982; Wangersky, 1976; Norkrans, 1980). Upwellings are sometimes responsible for the formation of surface slicks. Slicks are visible areas where surface films lower surface pressures, resulting in the dampening of capillary waves (Hardy, 1982). Two major sources of materials that can be transported to the microlayer via processes described above include runoff from terrestrial sources, and *in situ* primary and secondary production. Estuaries receive allochthonous inputs of dissolved and particulate organic matter from rivers and marshes, in addition to *in situ* inputs generated by phytoplankton-based food webs (Ducklow and Shiah, 1993).

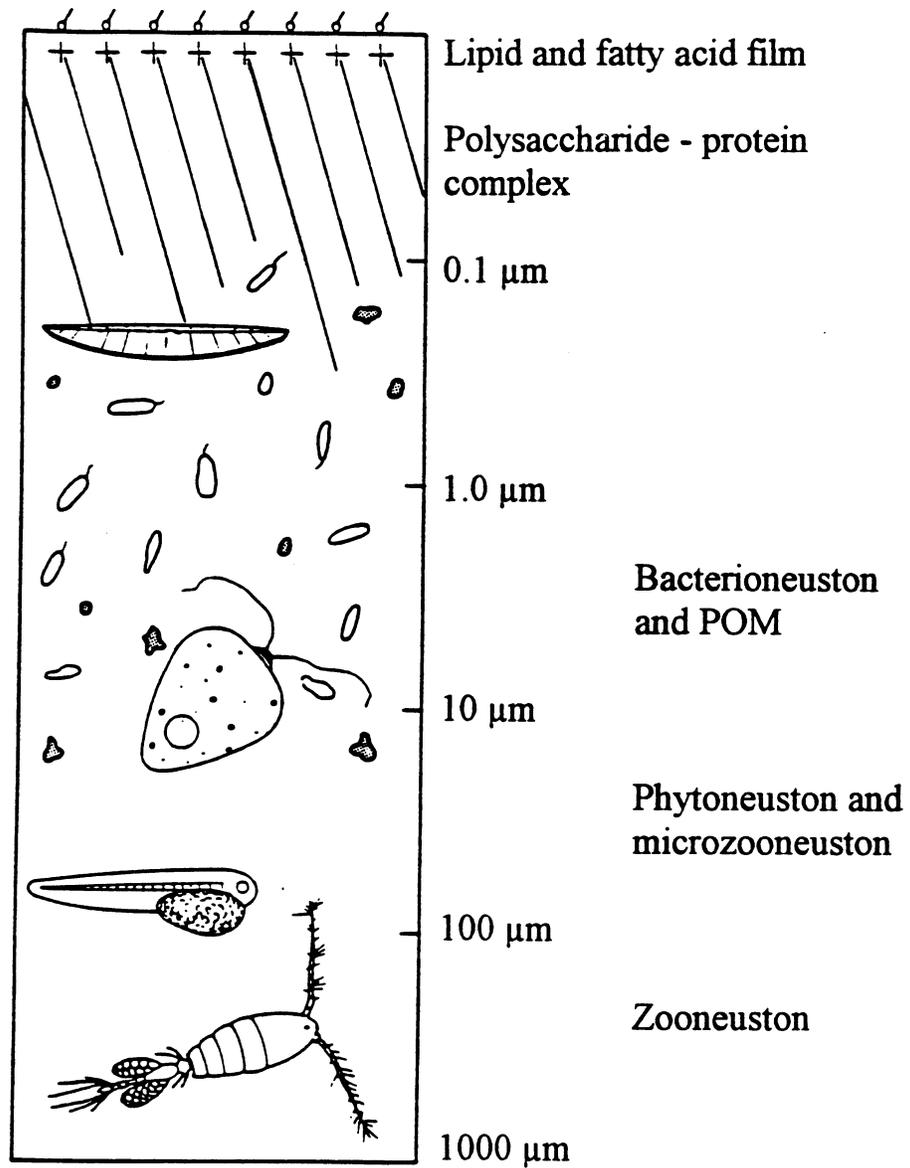


Fig. 1. Conceptual model of sea-surface microlayer (adapted from Hardy, 1982).

Mechanisms for dispersal from the surface film include bubbles from breaking waves moving down through the water, the production of atmospheric aerosols by breaking bubbles, evaporation of volatile fractions (Wangersky, 1976), and sedimentation of particles from the surface to the subsurface (Hardy, 1982). In estuaries and along shorelines surface films may deposit on beaches during tidal ebbs (Gardiner, 1992) and substances may be transported to the surface when tidal flooding occurs.

While the surface film is subjected to extremes in environmental conditions, strong surface tension creates physical stability, and a persistent substrate for the growth of microorganisms. Organic surface films are estimated to regenerate in approximately 0.2 seconds following disturbances, and are continuously renewed despite chemical or microbiological degradation (Dragčević and Pravdić, 1981).

Enrichments of organic matter and nutrients provide an advantage for neustonic organisms. Abundances of organisms can be high in surface microlayers compared with subsurface waters. Chlorophyll pigment concentrations in the microlayer of Sequim Bay, Washington were 18 times the concentrations found in subsurface waters (Hardy and Apts, 1989). Total phytoneuston abundance ranged from 37 (non-slick areas) to 154 (slicks) times phytoplankton abundance in subsurface waters. In 1984 Hardy and Apts reported enrichment ratios of phytoneuston to phytoplankton ranging from nearly 4 to 1500, and ratios of bacterioneuston to bacterioplankton ranging from 23 to more than 12,000 (microlayer depth $\sim 20\mu\text{m}$) in the same environment. Garabétian (1991) found concentrations of bacterioneuston five times the concentrations of bacterioplankton, and chlorophyll *a* concentrations in the microlayer more than 16 times concentrations in the

subsurface. Carlucci *et al.* (1986 and 1992) reported bacterioneuston to be slightly enriched in the microlayer (~150 μm).

In situ heterotrophic activities of bacterioneuston have been shown to be lower than those of the bacterioplankton, depending on the density of bacteria accumulated in the sea-surface microlayer (Dietz *et al.*, 1976). In contrast, Carlucci *et al.* (1986) concluded that surface films contain highly active microbial populations. However, diel differences in activity were detected and thymidine incorporation rates were lower in the microlayer than in the subsurface. This was attributed to differences in microbial population densities. In a subsequent study, Carlucci *et al.* (1991) found that bacterial production estimates were always lower in the subsurface than in the surface film, and per-cell thymidine incorporation rates were lower in surface films.

The same processes that create natural surface films and enrichments of organic matter and nutrients also attract pollutants from anthropogenic sources (Hardy, 1982). Pesticides and chlorinated hydrocarbons have been shown to inhibit neuston growth by affecting cell membranes, synthesis of nucleic acids, amino acids and proteins, and enzyme activity, in addition to increasing mutation frequency (see review by Maki, 1993). Natural toxins produced by marine plants, such as dissolved phenolic compounds, can be enriched in the microlayer and inhibit microbial growth (see review by Maki, 1993). Heavy metals are concentrated in surface microlayers (Hardy *et al.*, 1985); however, the toxicity of heavy metals toward organisms can be moderated when complexed with organic materials in the microlayer (Maki, 1993). Microlayer contamination and toxicity occurs inshore and offshore of the North Sea (Hardy and Cleary, 1992). Microlayers in Padilla Bay have

exhibited toxicity toward echinoderm larvae (Gardiner, 1992). Photoinhibition, which has been shown in phytoplankton (Hardy and Apts, 1984) and in subsurface heterotrophic communities (Sieracki and Sieburth, 1986) may also explain differences in heterotrophic activity. Differing sampling methodology and microlayer depths may also explain reported differences in heterotrophic activity.

Another process that can affect heterotrophic activity and specifically, bacterial production, includes bacterivory by protists. Azam *et al.* (1983) described the microbial loop as bacterial uptake of nonliving organic matter followed by the ingestion of bacteria by small protozoans, such as flagellates and ciliates. Recent research has implicated the importance of ciliate bacterivory, which would make bacterial production available to metazoan grazers (Sherr and Sherr, 1987; Gast, 1985; Turley *et al.*, 1986; Sherr *et al.*, 1987; Albright *et al.*, 1987). In 1988 Sherr and Sherr presented the microbial loop as an integral part of a larger food web, as opposed to a sink for fixed carbon due to respiratory losses. Small flagellates 2 to 20 μm , small ciliates $<30 \mu\text{m}$ and dinoflagellates are consumers of bacteria and cyanobacteria (Sherr *et al.* 1989; Bernard and Rassoulzadegan, 1990; Caron and Goldman, 1990; Lessard, 1991). Protists also play an important role as regenerators of nutrients, excreting a variety of phosphorus and nitrogen containing compounds, including phosphate and ammonium (Caron and Goldman, 1990).

Differences in microbial numbers, heterotrophic activity and growth rates between the sea-surface microlayer and subsurface waters may be due in part to the enrichment of organisms at the surface, utilization of an enriched surface substrate, extreme environmental conditions that may limit production, and possibly, differences in grazing

pressures. No known studies have attempted to measure rates of bacterivory by heterotrophic protists in the sea-surface film. The primary objectives of this research were to compare the numbers and heterotrophic activity of bacterioneuston and bacterioplankton in Padilla Bay, Washington, and to investigate factors that may affect bacterial productivity in microlayer and subsurface waters. A local estuarine embayment was selected to test the hypothesis that the microlayer contains bacterial populations that are higher in number, and metabolically more active than their counterparts below the surface. This research also looked at bacterivory and compared rates in microlayer and subsurface waters in Padilla Bay in an attempt to quantify its impact on bacterial production.

The following questions were asked concerning this thesis: 1) What is the composition of the heterotrophic microbial community in microlayer and subsurface waters in Padilla Bay? 2) What are the rates of bacterial production in the microlayer and subsurface? 3) What are the rates of bacterivory in microlayer and subsurface waters? 4) How do rates of bacterial consumption compare with rates of bacterial production?

METHODS

Study area

A 16-station square grid was located within Padilla Bay National Estuarine Research Reserve (Fig. 2). The southwest corner of the grid was located at 48° 30' N, 122° 32' W. Stations were located 371 m apart (equal to 12" of latitude). Padilla Bay, located northeast of the Skagit River delta in Skagit County, Washington, contains important estuarine habitats, including expansive seagrass beds (Bulthuis, 1991). Water flow in Padilla Bay is influenced by tidal currents flowing to and from Guemes Channel and adjacent deep water areas, and by flows to and from the Swinomish Channel. Freshwater inputs to the bay include the Skagit River via the Swinomish Channel, sloughs in the adjacent watershed, and large volume rivers located north of Padilla Bay, including the Fraser River, British Columbia (Bulthuis, 1991). The watershed east of Padilla Bay is primarily agricultural, with some forested land and a small residential community. West of the bay is March Point, the site of two large oil refineries. These features combined with heavy recreational boat use in nearby Fidalgo Bay and through the Swinomish Channel create the potential for anthropogenic enrichment in Padilla Bay waters, including both microlayer and subsurface waters.

Sample collection

Sampling took place April 11 and 12, June 6 and 7, and August 23 and 24, 1994. On the first day of each period, all sixteen stations were sampled for bacterial numbers. Two replicates from both microlayer and subsurface waters were collected at each station.

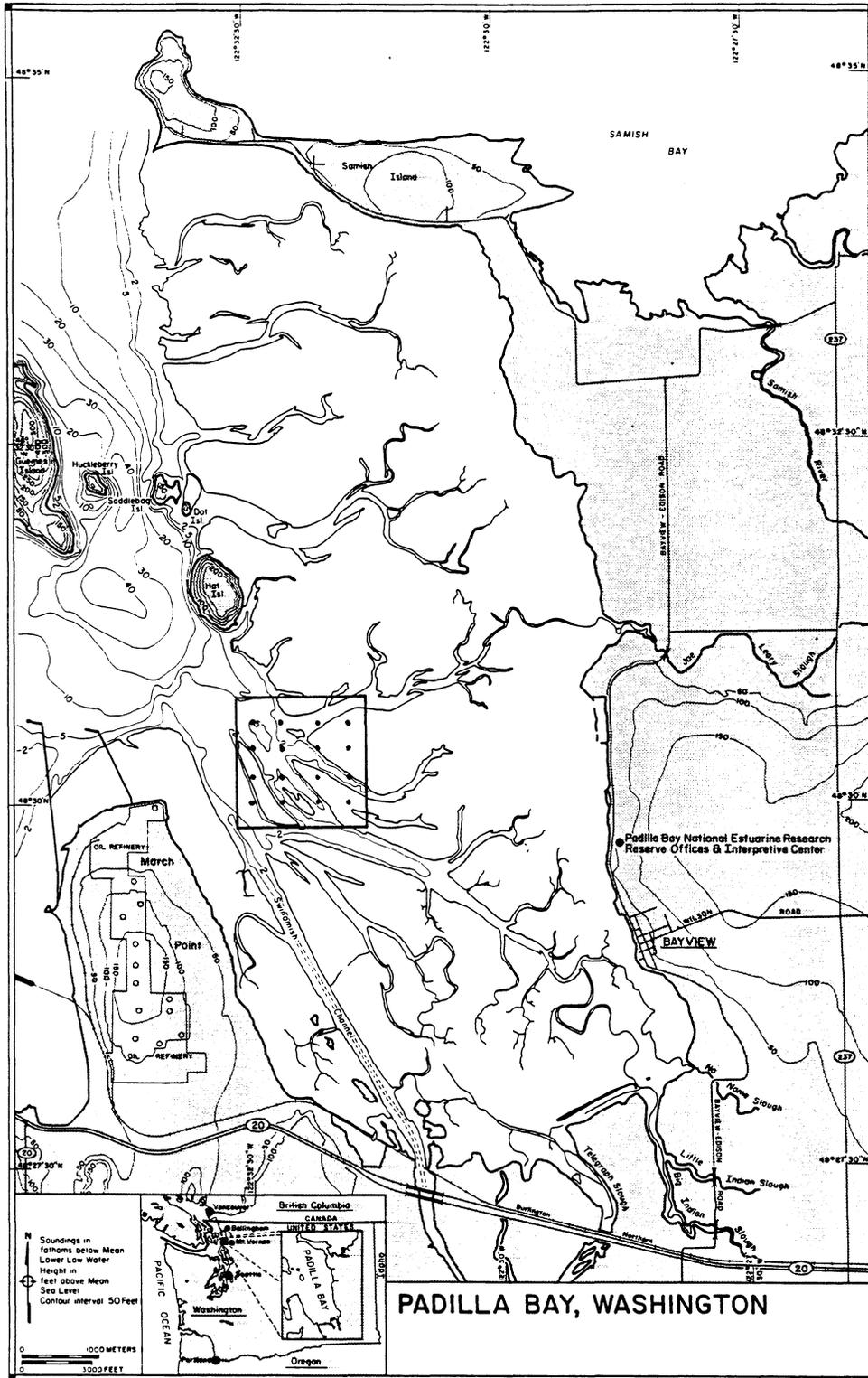


Fig. 2. Sixteen-station grid in Padilla Bay, Washington.

On day two, three replicates at each of four stations selected randomly from the 16-station grid were collected to determine bacterial numbers, rates of thymidine incorporation, and water quality parameters in microlayer and subsurface waters. Protist grazing experiments were conducted on two stations randomly selected from the four stations described above. Two replicates from each station from microlayer and subsurface waters were used to determine protist numbers and grazing rates.

The sea-surface microlayer was sampled by the glass plate method, modified from that described by Hardy *et al.* (1985). The microlayer sampler included a glass plate (33.0 cm x 25.4 cm) and a plexiglass frame, which supported a silicon rubber blade and a flask for water collection (Fig. 3). The glass plate was placed horizontally on the surface of the water. Each replicate was collected by quickly placing and withdrawing the glass plate from the surface of the water, draining for four to five seconds, and subsequently drawing the glass plate over the silicon rubber blade. The microlayer sample was collected into the polycarbonate flask.

On day one of each sampling period, each replicate from the microlayer represented a pooled sample of four or five dips. On the second day of each sampling period, approximately 300 ml were collected from the microlayer, requiring roughly 30 dips per replicate. Each dip of the glass plate collected nearly 10 ml. The depth of the microlayer was calculated as follows:

$$\frac{\text{sample volume (cm}^3\text{)} \times \text{number of dips}}{\text{area of glass plate (cm}^2\text{)}}$$

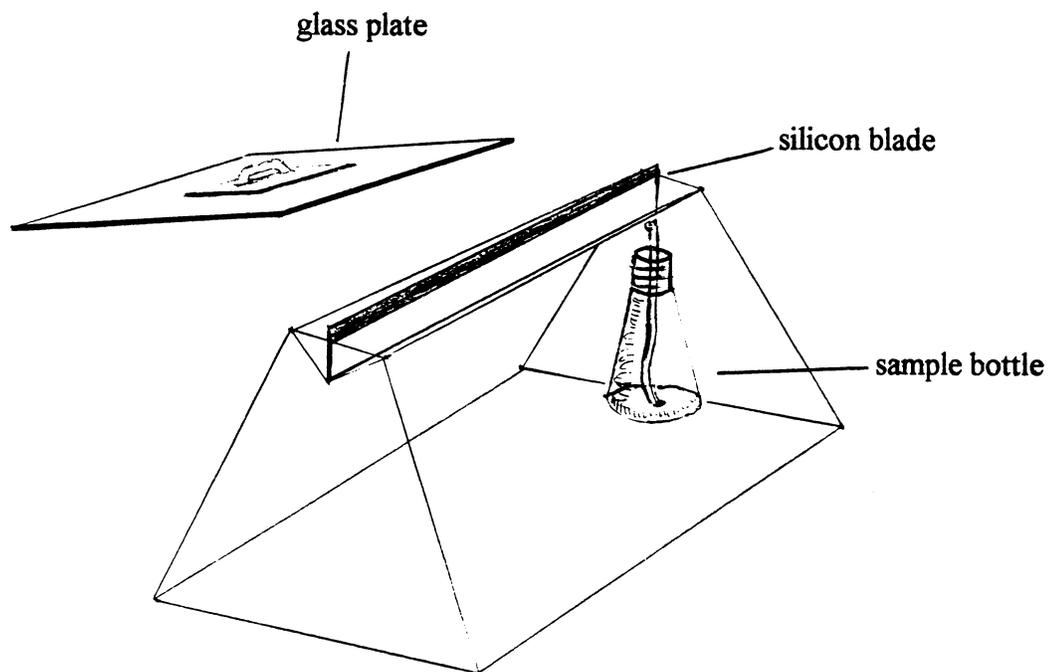


Fig. 3. Glass plate sampler used to collect microlayer water.

The mean microlayer depth in this study was 113.2 μm , but varied from station to station within each sampling period, ranging from 87.9 μm in August to 149.4 μm in April.

Microlayer samplers are generally not 100% efficient (Hardy, 1982). Sampling efficiency varies depending on the type of sampler used. The efficiency of the traditional vertical glass plate sampler is estimated to be 60-61% (Hardy *et al.*, 1985). Higher sampling efficiencies are reported for a Teflon-coated rotating drum (79-88%), Nitex screen ($72\pm 23\%$) and membrane filters ($>100\%$) (Hardy *et al.*, 1988; Van Fleet and Williams, 1980). The efficiency of the microlayer sampler developed for this study was tested in the laboratory by introducing a measured quantity of *Lycopodium* spores (Carolina Biological Supply) to a large container of seawater. The number of spores collected in the microlayer was determined microscopically. The change in spore density was used to measure collection efficiency, which was $>100\%$. Collection efficiency, however, can be affected by wind and wave action during sampling, and may not always approach 100%.

The subsurface was sampled by plunging a closed, double-ended polycarbonate bottle approximately 25 cm below the surface. The bottle was then opened by hand underwater. The turbulence created by opening a bottle underwater can destroy protists. For this reason, the double-ended bottle was allowed to remain open under water for 20 to 30 seconds to replace the disturbed water. The bottle was closed under water to minimize contamination from the surface microlayer, then brought to the surface. On day one of each sampling period, approximately 20 ml was collected from each replicate. Three 300-ml replicates of subsurface water were collected on day two of each sampling period.

Physical and water quality parameters

Tidal conditions varied during sampling periods, although an effort was made to sample near slack periods at low tide, and at similar times of day. In April, samples were collected during a tidal ebb, and ended at low tide. Sampling occurred during tidal flooding in June, starting at low tide. August samples were collected during a tidal ebb. Weather conditions, presence of slicks, time of sampling and tidal heights are provided in Appendix A.

Microlayer temperatures fluctuate seasonally and diurnally with changes in air temperature much more frequently and rapidly than subsurface temperatures (Hardy, 1982). In this study, air temperature was measured to indicate changes in microlayer temperature, as the measurement of microlayer temperature is difficult. Air temperature, subsurface temperature and salinity were measured *in situ* on day two of each sampling period. Temperatures were obtained using a mercury thermometer, and salinity was measured with a refractometer (Endeco, Type 102).

Microlayer and subsurface samples were filtered in the field (Whatman GF/F glass fiber filters, effective pore size 0.7 μm) for chlorophyll *a*, pheopigments and dissolved inorganic nutrients. Samples were transported in a cooler at *in situ* temperatures to the lab where they were stored frozen (-15°C) until analyzed. Filters were placed in 90% acetone to extract chlorophyll *a* and pheopigments. The extracts were analyzed the following day using a Turner Model 112 fluorometer as described by Parsons *et al.* (1984). Extracts were acidified for the analysis of pheopigments. Nitrate (nitrate + nitrite) concentrations were determined using an Alpkem Rapid Flow 300 Analyzer.

Ammonium (alternative method) and phosphate were determined spectrophotometrically using methods described by Parsons *et al.* (1984).

Bacterial numbers

Microlayer and subsurface samples were fixed with glutaraldehyde (final concentration about 1%) in the field and stored in the dark at 4°C in 20-ml glass scintillation vials. Bacteria were counted using slides prepared with 4',6-diamidino-2-phenyl-indol (DAPI; final stain concentration, 0.01 µg ml⁻¹) for observation by epifluorescence microscopy (Leitz DMRB microscope with a UV filter set) (Porter and Feig, 1980; Parsons *et al.*, 1984). Slides were prepared within three days of sample collection. All reagents were filtered (0.2 µm) prior to use. Glassware was acid washed in 10% HCl and rinsed with filtered deionized water. Sample collection bottles were also autoclaved, and prerinsed with sample water in the field. Stained samples were filtered onto Poretics black polycarbonate filters (25 mm diameter, 0.2 µm pore size), with Poretics Membra-fil backing filters (25 mm diameter, 0.45 µm pore size). Slides were stored in the dark at -15°C until they were counted. At least 400 bacterial cells were counted on at least 10 fields per slide (Kirchman *et al.*, 1982).

[³H]Thymidine incorporation

Uptake of [³H]thymidine (TdR) by bacteria in microlayer and subsurface waters was measured using a modification of the tritiated thymidine incorporation technique described by Fuhrman and Azam (1980, 1982) and Bell (1994). Two 10-ml subsamples

from each replicate were measured into sterile plastic test tubes. One of the two subsamples was fixed with formalin, at a final concentration of 5%, and served as a killed control. Ten μl of a solution containing a final concentration of 10 nM [methyl- ^3H]thymidine (20 Ci mmol^{-1} ; New England Nuclear) was added to each subsample, and inverted to mix. Samples were incubated in the dark for three or four hours at *in situ* temperatures $\pm 2^\circ\text{C}$. Linearity of TdR uptake rates during the three to four-hour incubation period was established previously in time-series experiments on both microlayer and subsurface samples.

Bacteria were filtered onto a 0.22 μm pore-size 25 mm diameter Nuclepore Membra-Fil filter (vacuum pressure, 130 mm Hg), and rinsed with three 1-ml portions of ice-cold 5% trichloroacetic acid (TCA). TCA-insoluble material was then rinsed with three 1-ml portions of ice-cold 80% ethanol to remove the unincorporated thymidine. Each filter was placed into a scintillation vial. One ml of ethyl acetate was added and allowed to sit for approximately one hour to dissolve the filters. Nine ml of scintillation fluid (NEN-963) were then added. Samples were allowed to sit at least 12 hours in the dark. Radioactivity was then assayed using a Packard Tri-Carb liquid scintillation counter.

Thymidine incorporation rates were calculated as follows:

$$\text{mmoles } L^{-1} \text{ hr}^{-1} = \frac{\text{dpm}}{\text{SA}} \times \frac{4.5 \times 10^{-13}}{t} \times \frac{1}{v}$$

where 4.5×10^{-13} is the number of curies per dpm, SA is the specific activity in Ci mmole^{-1} , t is incubation time in hours, and v is the sample volume in liters.

Protistan grazing rates

Protist numbers

The density of heterotrophic protists in microlayer and subsurface samples was determined from slides made from grazing experiment samples. Bacterivorous protists, including flagellates <5 μm , flagellates 5 to 20 μm , heterotrophic dinoflagellates and ciliates (heterotrophic or mixotrophic) were counted. Samples were filtered onto 1.0 μm pore-size Poretics polycarbonate filters with 1.2 μm Poretics MCE backing filters. Slides were made using DAPI stain, as described by Sherr and Sherr (1993). Slides were made within three or four days of sampling, and stored at -15°C . They were scanned by epifluorescence microscopy as described in detail below.

Preparation of FLB

Consumption of bacteria by protists was estimated by measuring the uptake of fluorescently labeled bacteria (FLB), as described by Sherr *et al.* (1987) and Sherr and Sherr (1993). To prepare FLB, natural seawater samples were filtered through 0.8 μm pore-size 47 mm diameter polycarbonate filters. The bacterial assemblage in the filtrate was concentrated by centrifugation in a Sorvall Superspeed RC2-B centrifuge at 12,000 rpm (23,300 $\times g$). The cells were resuspended in 0.2 μm filtered seawater, and concentrated again. Two mg of 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) were added to the final concentrate. This solution was heated in a 60°C water bath for two hours. The concentrate was resuspended in sodium phosphate buffer (salinity 25‰, adjusted to pH 9.0), centrifuged, and the supernatant poured off. This washing procedure

was repeated three times. The FLB concentrate was then resuspended in a tetrasodium pyrophosphate solution (salinity 25‰, adjusted to pH 9.0), and concentrated again by centrifugation. The FLB concentrate was prepared and stored at -15°C before each experiment. The FLB were thawed at room temperature and sonicated in a water bath for 15 to 20 minutes to disperse clumps on the day of each experiment.

Grazing experiment

FLB concentrations ranging from 10 to 25% of the natural bacterial abundance were added to microlayer and subsurface samples to determine flagellate and ciliate grazing rates. Five ml of FLB were added to each sample. Twenty-ml subsamples were taken at time zero and at 10-minute intervals for up to 40 minutes following the addition of fluorescently labeled cells. Time series subsamples were immediately fixed with glutaraldehyde (final concentration about 1%).

Slides were scanned by epifluorescence microscopy (200x for ciliates and dinoflagellates, and 1000x for flagellates). When protists were located and tallied the filter set was switched from UV to blue light, and the objective lens was switched to 1000x to determine the number of FLB within each protist.

Eighty to 160 flagellates in microlayer and subsurface samples, and 60 to 100 ciliates and dinoflagellates (subsurface) were examined for each time point subsample. The average number of FLB particles per protist was determined for populations at each time point. FLB uptake was calculated for the following groups: heterotrophic flagellates $<20\ \mu\text{m}$, heterotrophic dinoflagellates and ciliates. The FLB ingestion rate, given in

particles ingested per protist per hour, was calculated using linear regression from the linear portion of the uptake curve (Fig. 4). Bacterivory was calculated by multiplying the FLB ingestion rate by the total number of protists per liter, then by the ratio of bacteria to FLB within each replicate.

Statistical Analysis

Physical parameters including temperature, salinity and microlayer depth were analyzed using a 2-way analysis of variance (ANOVA). Bacterial numbers on the first day of each sampling period were analyzed using a 3-way ANOVA (unnested). All other parameters, determined on day two of each sampling period were analyzed using a partially nested 3-way ANOVA. Statistical models for the three types of analysis are shown in Table 1. The level of significance for all statistical tests was set at $\alpha=0.05$. When interactions were significant, simple main effects were evaluated as described by Winer *et al.* (1991). Tukey's HSD (honestly significant difference) pairwise comparison was used when no interactions were present. The *t*-statistic was used to test the significance of simple main effects. Pearson *r* correlations were calculated to assess the degree of linear association between abundance, rate measurements and water quality parameters. Linear regression was used to determine FLB ingestion rates; the F-statistic was used to determine the significance of ingestion rates.

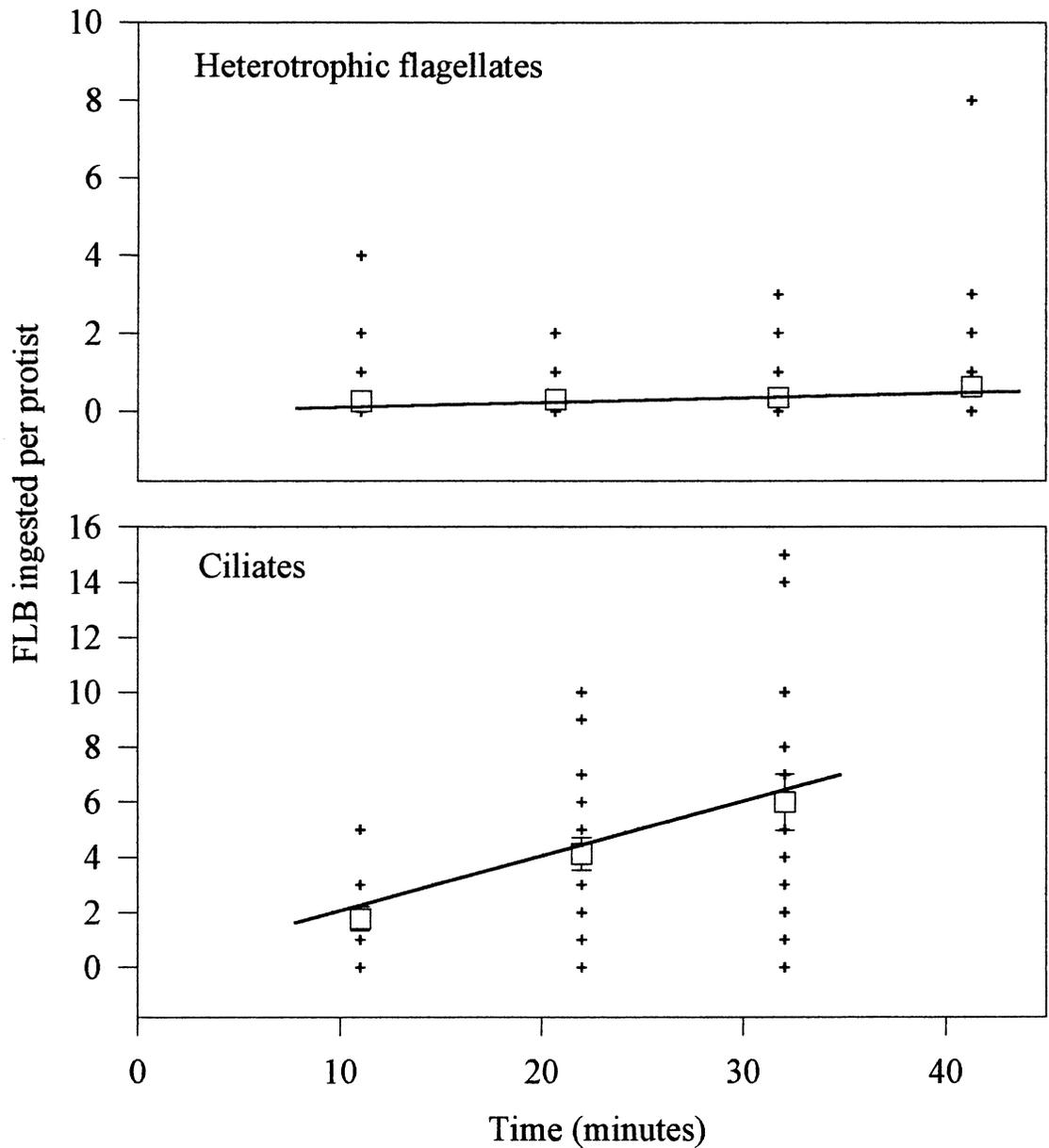


Fig. 4. Ingestion of fluorescently labelled bacteria by protists in microlayer and subsurface waters of Padilla Bay. Data points represent the number of ingested FLB by one or many protists. Squares represent means and error bars represent mean standard errors (n=60 to 160). Some squares obscure error bars. Examples of FLB ingestion by heterotrophic flagellates (A) and ciliates (B) are shown. Data for all heterotrophic flagellates (<5 μm and 5-20 μm) were pooled. FLB ingestion rates are represented by the slope of the solid lines, and were derived using linear regression.

Table 1. Experimental design models used to construct ANOVA tables, where M, P, S and ϵ represent month, position (microlayer or subsurface), station and replicate, respectively.

Parameters	Model
Temperature and salinity	$y = \mu + M_i + P_j + MP_{ij} + \epsilon_{(ij)k}$ <p>where $i=1..3$ (fixed) $j=1..2$ (fixed) $k=1..4$ (random)</p>
Microlayer depth	$y = \mu + M_i + S_{(ij)} + \epsilon_{(ijk)l}$ <p>where $i=1..3$ (fixed) $j=1..4$ (random) $k=1..3$ (random)</p>
Bacterial numbers, day 1	$y = \mu + M_i + S_j + MS_{ij} + P_k + SP_{jk} + MSP_{ijk} + \epsilon_{(ijk)l}$ <p>where $i=1..3$ (fixed) $j=1..4$ (fixed) $k=1..2$ (fixed) $l=1..2$ (random)</p>
All other parameters, day 2	$y = \mu + M_i + S_{(ij)} + P_k + MP_{ik} + SP_{(ijk)} + \epsilon_{(ijk)l}$ <p>where $i=1..3$ (fixed) $j=1..4$ (random) $k=1..2$ (fixed) $l=1..2$ (random)</p>

RESULTS

Physical and water quality parameters

Air temperature was higher in June and August than in April (Table 2). Air temperatures, and by inference, temperatures in the microlayer were significantly higher than subsurface temperatures in June and August. Subsurface temperatures were similar during all sampling periods. Microlayer and subsurface salinity was higher in June than in April, and higher in August than in June (Table 2). Salinity was highest in subsurface waters over all time periods. Temporal differences in salinity may have been related to weather conditions. Sampling periods in April and June were preceded by rain and wind. The August sampling period was preceded by a long dry spell with little wind. Weather conditions, presence of slicks, time of sampling and tidal heights are provided in Appendix A.

Mean chlorophyll *a* concentrations in the microlayer and subsurface were higher in June than in April, and were similar in June and August (Appendix C4). During April and June, chlorophyll *a* concentrations were not significantly different between microlayer and subsurface waters, but were higher in August in the microlayer (Table 3). Mean concentrations of pheopigment in the microlayer were similar in April and June and were more than 100% higher in August than in June (Appendix C4). Subsurface pheopigment concentrations were lower than concentrations in the microlayer during April and June. Mean microlayer pheopigment concentrations were nearly twice chlorophyll *a* concentrations measured in April and August.

Ammonium concentrations in microlayer and subsurface waters were significantly

Table 2. Ranges of physical parameters measured in microlayer and subsurface waters of Padilla Bay.

Date	Position	Temperature (air, °C)	Temperature (water, °C)	Salinity (‰)
Apr	microlayer	12.0-17.0		26.1-27.4
	subsurface		11.2-12.0	27.0-28.0
Jun	microlayer	12.0-23.0		28.0-28.9
	subsurface		12.0-13.3	28.1-29.7
Aug	microlayer	17.5-24.0		28.5-29.8
	subsurface		13.2-13.8	29.4-30.0

Table 3. Ranges of chlorophyll α , pheopigment and dissolved inorganic nutrient concentrations in microlayer and subsurface waters of Padilla Bay.

Date	Position	Chlorophyll α ($\mu\text{g L}^{-1}$)	Pheopigment ($\mu\text{g L}^{-1}$)	Ammonium ($\mu\text{g-at L}^{-1}$)	Nitrate ($\mu\text{g-at L}^{-1}$)	Phosphate ($\mu\text{g-at L}^{-1}$)
Apr	microlayer	1.15-2.49	1.97 - 5.54	0.10-0.89	9.1-13.8	1.08-2.68
	subsurface	0.19-2.97	0.06 - 2.86	0.02-1.03	8.6-15.2	1.04-2.44
Jun	microlayer	2.50-4.57	1.96 - 4.77	0.93-2.08	4.7-10.3	1.24-3.90
	subsurface	2.08-4.08	2.19 - 3.03	1.03-1.43	5.5 - 8.7	1.29-3.31
Aug	microlayer	0.65-7.96	2.30-11.47	0.76-1.56	11.9-14.7	2.06-3.46
	subsurface	1.25-3.07	2.21 -3.22	0.64-1.31	13.1-15.0	2.25-4.03

higher in June than in April, whereas concentrations were similar in June and August (Table 3). Ammonium concentrations were positively correlated with chlorophyll *a* concentrations in microlayer and subsurface waters. Nitrate concentrations were lower in June than in April or August in microlayer and subsurface waters (Table 3). Mean phosphate concentrations in the microlayer were higher in June than April, and subsequently higher in August (Table 3). In subsurface waters, phosphate concentrations were similar in April and June, but higher in August. Ammonium and phosphate concentrations were higher during summer than early spring in both microlayer and subsurface waters.

Ammonium concentrations were higher in the microlayer in June and August than in the subsurface. Microlayer nitrate concentrations were higher than concentrations in subsurface waters in April and June. Microlayer concentrations of phosphate were higher than subsurface concentrations in June, but similar in April and August. Dissolved inorganic nutrient concentrations in microlayer waters were usually at least as high as concentrations in subsurface waters. There were significant three-way interactions between month, station and position (microlayer or subsurface) for chlorophyll *a*, nitrate and phosphate (Appendix B and C).

Bacteria

Similar trends in bacterial numbers were observed in samples collected on day one and day two of each time period (Fig. 5). Both data sets showed enrichments of bacteria in microlayer water during August, and subsurface numbers that were similar during each

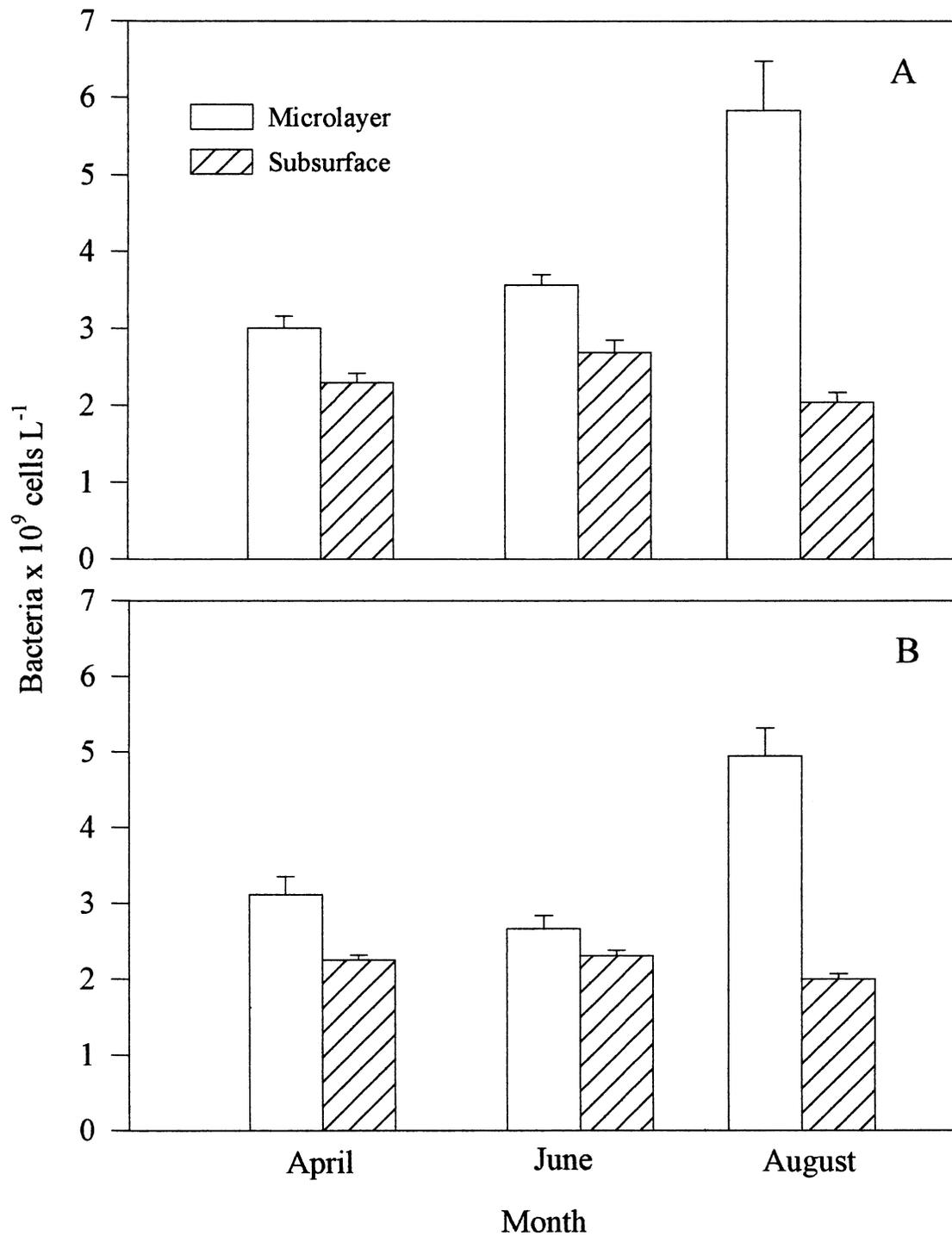


Fig. 5. Bacterial abundance in microlayer and subsurface waters of Padilla Bay on day one (A) and day two (B) of each sampling period. Samples were taken on 11-12 April, 6-7 June, and 23-24 August 1994. Error bars represent mean standard errors (n=32 on day one and n=12 on day two).

month (Table 4). Spatial and seasonal patchiness was observed in microlayer and subsurface waters when all 16 stations were sampled, although more variation was observed in the microlayer (Figs. 6 and 7). For example, a peak in bacterial numbers occurred in the microlayer at one station during the August time period (Fig. 6C). A significant three-way interaction between month, station and position (microlayer and subsurface) was present when all 16 stations were sampled for bacterial abundance (Appendix B9), suggesting seasonal and small-scale spatial patchiness between stations.

When four randomly selected stations were sampled on day two, bacterial densities in the microlayer were similar in April and June, and were 85% higher in August than in June (Fig. 5B). In the subsurface there was no difference in bacterial numbers between sampling periods. Bacterial abundance was generally higher and more variable in the microlayer than in subsurface waters, with significantly more bacteria in the microlayer during April and August (Table 5). Bacterial abundance was positively and significantly correlated with pheopigment concentration ($r=0.5994$).

Rates of [^3H]thymidine (TdR) incorporation were higher in June and August than in April in both microlayer and subsurface waters (Fig. 8). TdR incorporation rates were higher in the subsurface than in microlayer waters in April and June. However, rates in the microlayer were higher in August. Higher TdR incorporation rates corresponded with higher air temperature in June. Microlayer and subsurface TdR incorporation rates were positively and significantly correlated with ammonium ($r=0.6523$ and 0.6462 , respectively) and chlorophyll *a* concentrations ($r=0.4440$ and 0.6894). Cell-specific TdR incorporation rates were higher in the subsurface than in the microlayer over all time periods (Fig. 9).

Table 4. Ranges of bacterial numbers in microlayer and subsurface waters of Padilla Bay on day one and day two of each sampling period.

Date	Position	Day 1 (16 station) Bacterial numbers ($\times 10^9$ cells L ⁻¹)	Day 2 (4 station) Bacterial numbers ($\times 10^9$ cells L ⁻¹)
Apr	microlayer	1.84 - 6.41	2.18 - 4.99
	subsurface	0.80 - 4.38	1.90 - 2.94
Jun	microlayer	2.54 - 5.31	1.80 - 3.70
	subsurface	0.97 - 4.82	1.72 - 2.67
Aug	microlayer	2.01-20.95	3.11 - 7.99
	subsurface	0.58 - 3.29	1.47 - 2.38

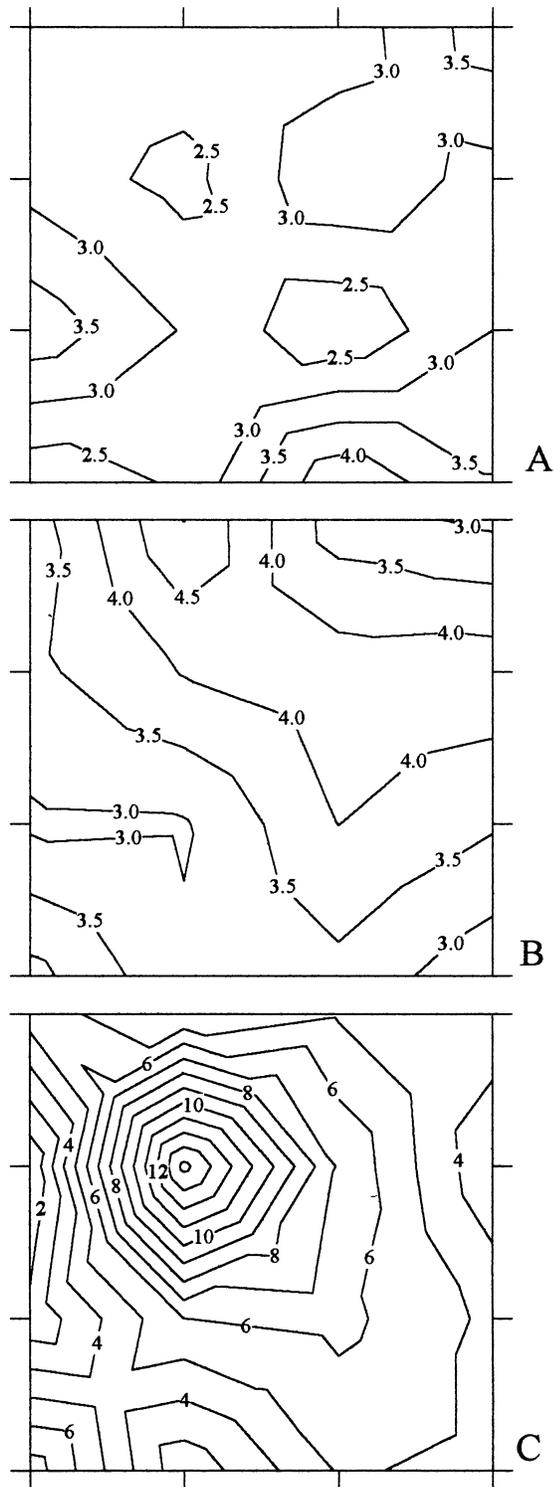


Fig. 6. Contour plots of bacterial abundance (10^9 cells L^{-1}) in the microlayer for 16 stations in Padilla Bay for 11 April (A), 6 June (B) and 23 August (C) 1994. Contours were plotted based on station means ($n=2$).

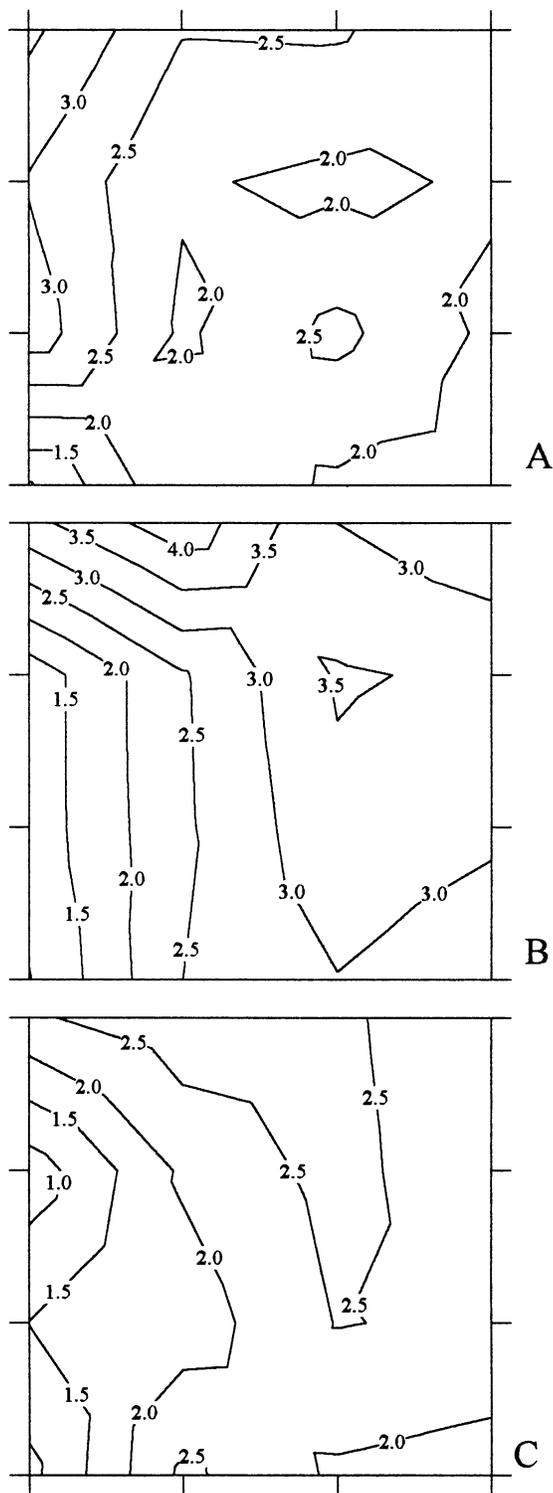


Fig. 7. Contour plots of bacterial abundance (10^9 cells L^{-1}) in subsurface waters for 16 stations in Padilla Bay for 11 April (A), 6 June (B) and 23 August (C) 1994. Contours were plotted based on station means ($n=2$).

Table 5. Ranges of bacterial numbers, [³H]thymidine incorporation rates, cell-specific TdR incorporation rates and total heterotrophic protist numbers measured in microlayer and subsurface waters of Padilla Bay.

Date	Position	Bacteria (x10 ⁹ cells L ⁻¹)	TdR incorp. (pmol L ⁻¹ hr ⁻¹)	Specific TdR incorp. (x10 ⁻⁸ pmol cell ⁻¹ hr ⁻¹)	Total protists (x10 ⁵ cells L ⁻¹)
Apr	microlayer	2.18 - 4.99	33.02 - 56.49	0.66 - 2.19	-----
	subsurface	1.90 - 2.94	36.77 -104.26	1.77 - 5.09	-----
Jun	microlayer	1.80 - 3.70	80.86 -219.46	2.19 - 8.62	8.09 - 11.38
	subsurface	1.72 - 2.67	123.69 -207.14	4.70 -10.97	6.40 - 7.23
Aug	microlayer	3.11 - 7.99	105.64 -152.82	1.59 - 4.64	75.30 - 132.8
	subsurface	1.47 - 2.38	84.86 -131.67	4.33 - 6.76	17.31 - 83.66

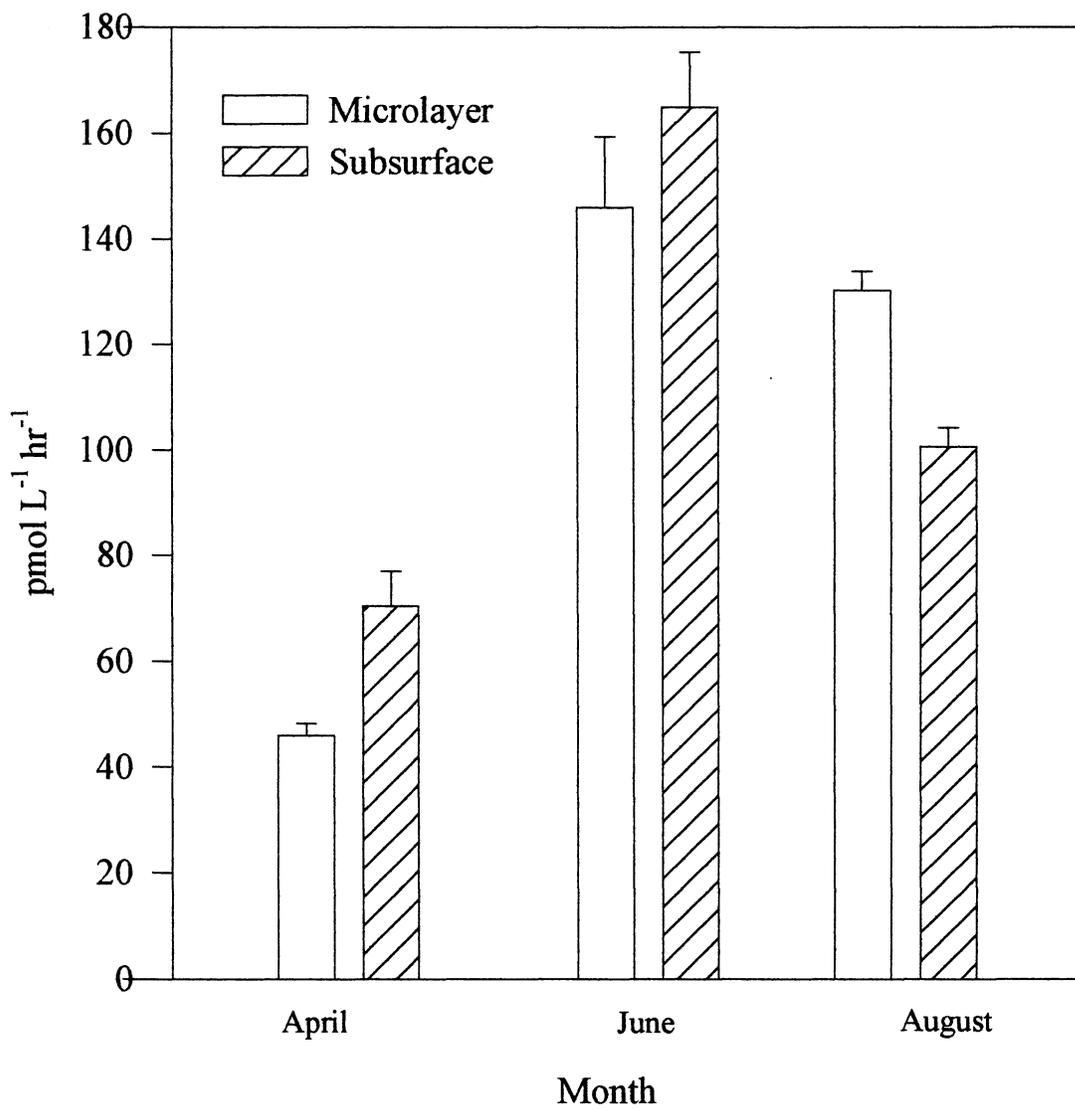


Fig. 8. [³H]Thymidine incorporation rates in microlayer and subsurface waters of Padilla Bay. Samples were taken on 12 April, 7 June, and 24 August 1994. Error bars represent mean standard errors (n=12).

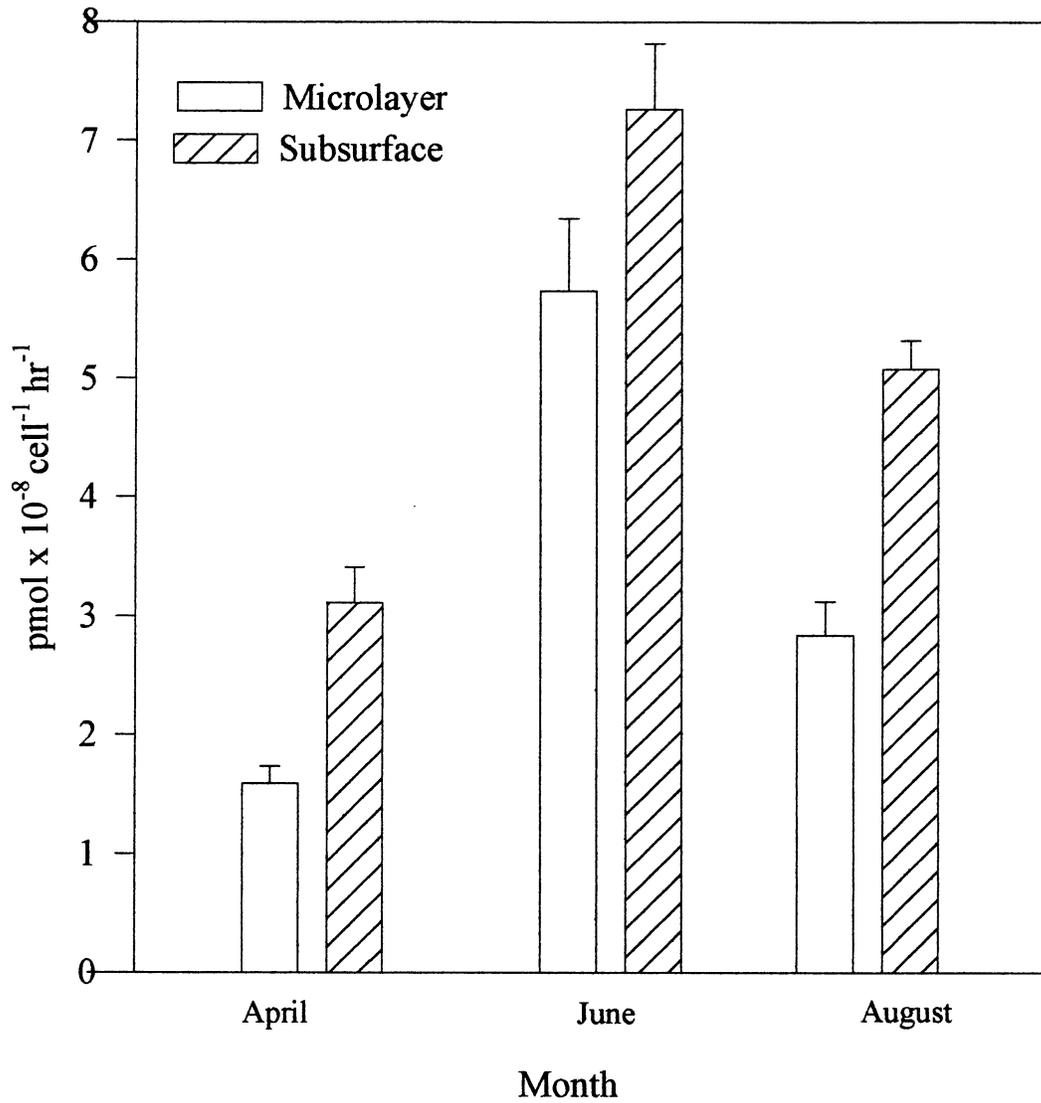


Fig. 9. [³H]Thymidine incorporation rates per bacterial cell in microlayer and subsurface waters of Padilla Bay. Samples were taken on 12 April, 7 June, and 24 August 1994. Error bars represent mean standard errors (n=12).

Heterotrophic protists

The total number of heterotrophic protists, including flagellates <5 μm , flagellates 5 to 20 μm , heterotrophic dinoflagellates and ciliates (heterotrophic and mixotrophic), was higher in August than in June in microlayer and subsurface waters (Table 5). Total protist numbers were generally two to three orders of magnitude lower than bacterial numbers (Table 5). There was a positive correlation between bacterial numbers and total heterotrophic protist numbers. The heterotrophic protist community was dominated by heterotrophic flagellates, and in particular, heterotrophic flagellates <5 μm in microlayer and subsurface waters (Fig. 10). Heterotrophic flagellates comprised more than 96% of the total protist population in June, and more than 99% of the total protist population in August. Heterotrophic flagellate abundance was generally two to three orders of magnitude higher than the number of heterotrophic dinoflagellates and ciliates numbers combined.

There was no significant difference in the number of small flagellates, relative to sampling period in the microlayer or subsurface (Fig. 11A). Heterotrophic flagellates in the 5 to 20 μm size class were more abundant in August than in June in the microlayer. Total heterotrophic flagellate numbers were higher in August than in June in the microlayer. The abundance of heterotrophic flagellates <5 μm in the microlayer was not significantly different than abundances in the subsurface in August or June (Fig. 11A). Flagellates in the 5 to 20 μm size class were significantly more abundant in the microlayer than in the subsurface in August (Fig 11A). There was no difference in total heterotrophic flagellate numbers between the microlayer and subsurface in June, while there were higher

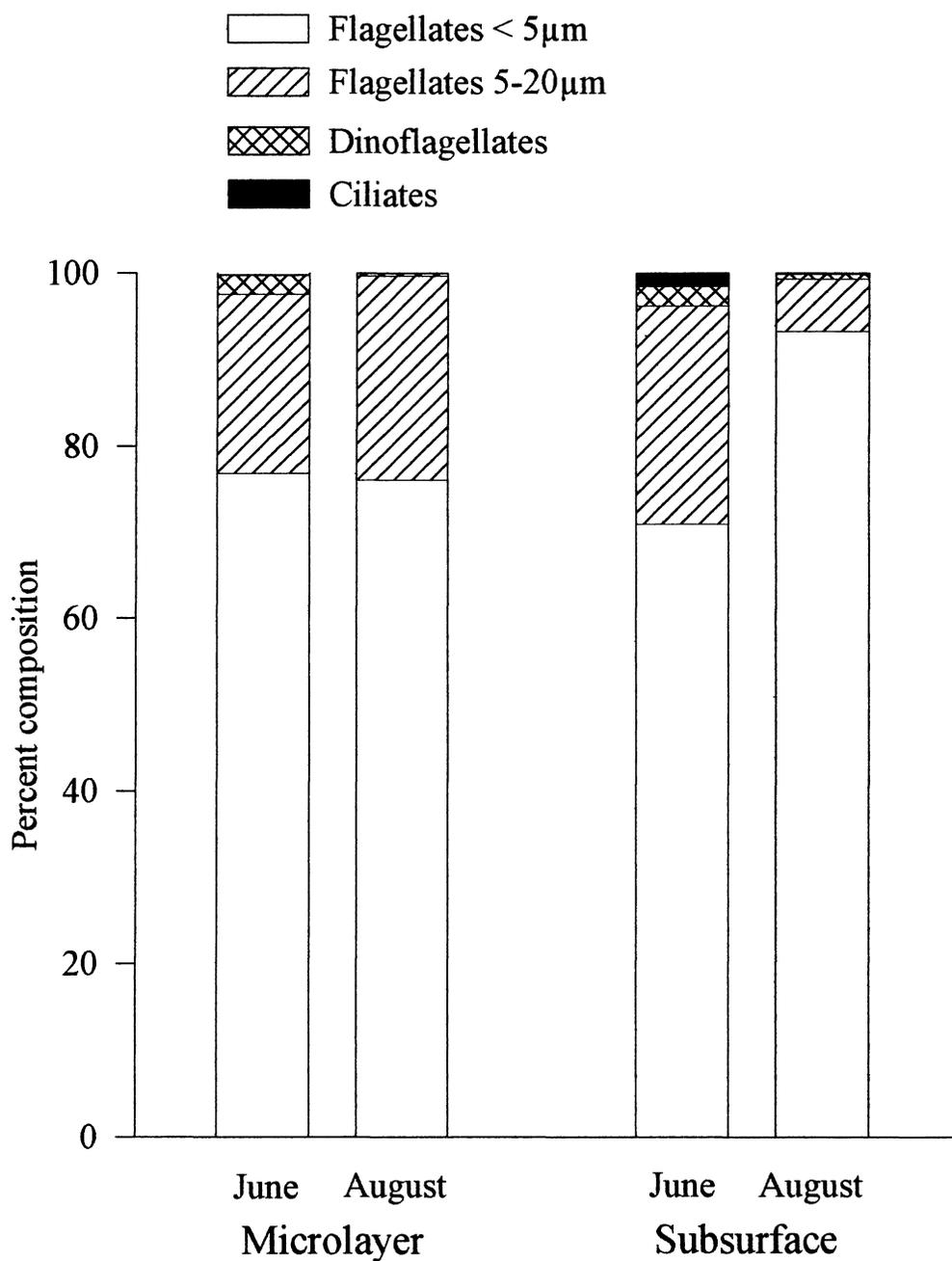


Fig. 10. Community composition of bacterivorous protists in microlayer and subsurface waters of Padilla Bay. Samples were taken on 7 June and 24 August 1994.

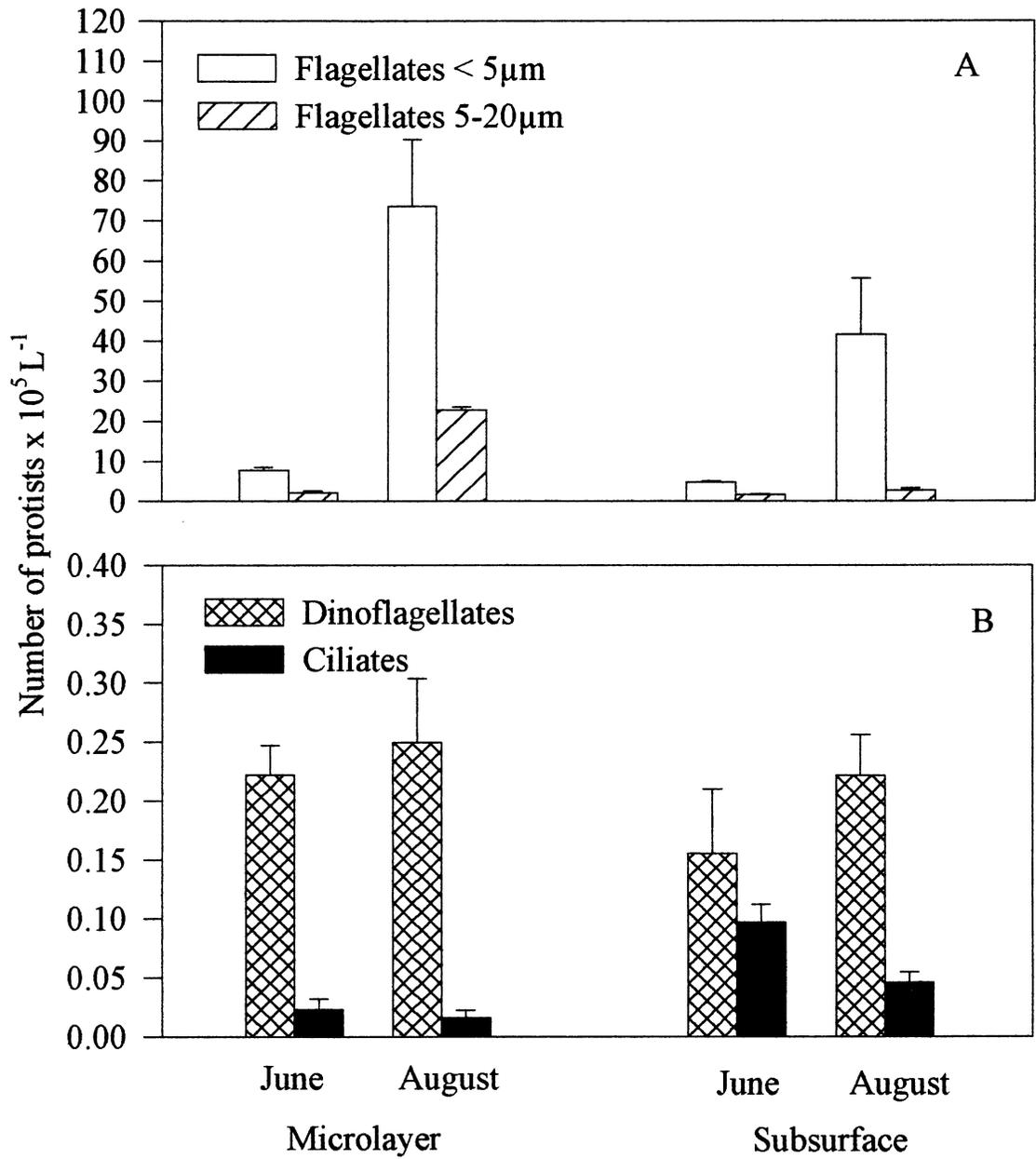


Fig. 11. Abundance of heterotrophic flagellates (A), and heterotrophic dinoflagellates and ciliates (B) in microlayer and subsurface waters of Padilla Bay. Samples were taken on 7 June and 24 August 1994. Error bars represent mean standard errors (n=4).

numbers in the microlayer in August. There was a significant three-way interaction between month, station and position for flagellates 5 to 20 μm . Numbers of heterotrophic flagellates in the microlayer and ciliate abundance in subsurface waters were positively and significantly correlated with bacterial numbers ($r=0.9067$ and 0.8819 , respectively).

Heterotrophic dinoflagellate numbers were similar in June and August, and there was no difference in heterotrophic dinoflagellate numbers between the microlayer and subsurface (Fig. 11B). However, dinoflagellate abundance as a percentage of total protist abundance in both microlayer and subsurface waters was higher in June (approximately 2%) than in August (<1%) (Fig. 10). Ciliate numbers were not significantly different between the microlayer and subsurface, nor from month to month (Fig. 11B). However, the highest number of ciliates were observed in the subsurface, and were found in June.

Grazing by heterotrophic protists on bacteria

Grazing by heterotrophic protists on FLB was examined in 16 samples. Grazing rates of bacterivorous protists, as determined by linear regression, were significant in eight out of the 16 samples. The ingestion of FLB can be attributed to all groups of protists; however, the contribution of each group to total grazing pressure varied depending on sampling month. In the microlayer, heterotrophic flagellates were the only group of bacterivorous grazers as defined by significant grazing rates (Fig. 12). Ciliates and dinoflagellates were the primary grazers isolated from subsurface in June, whereas in August, ingestion of FLB was due solely to heterotrophic flagellates in microlayer and subsurface waters (Fig. 12).

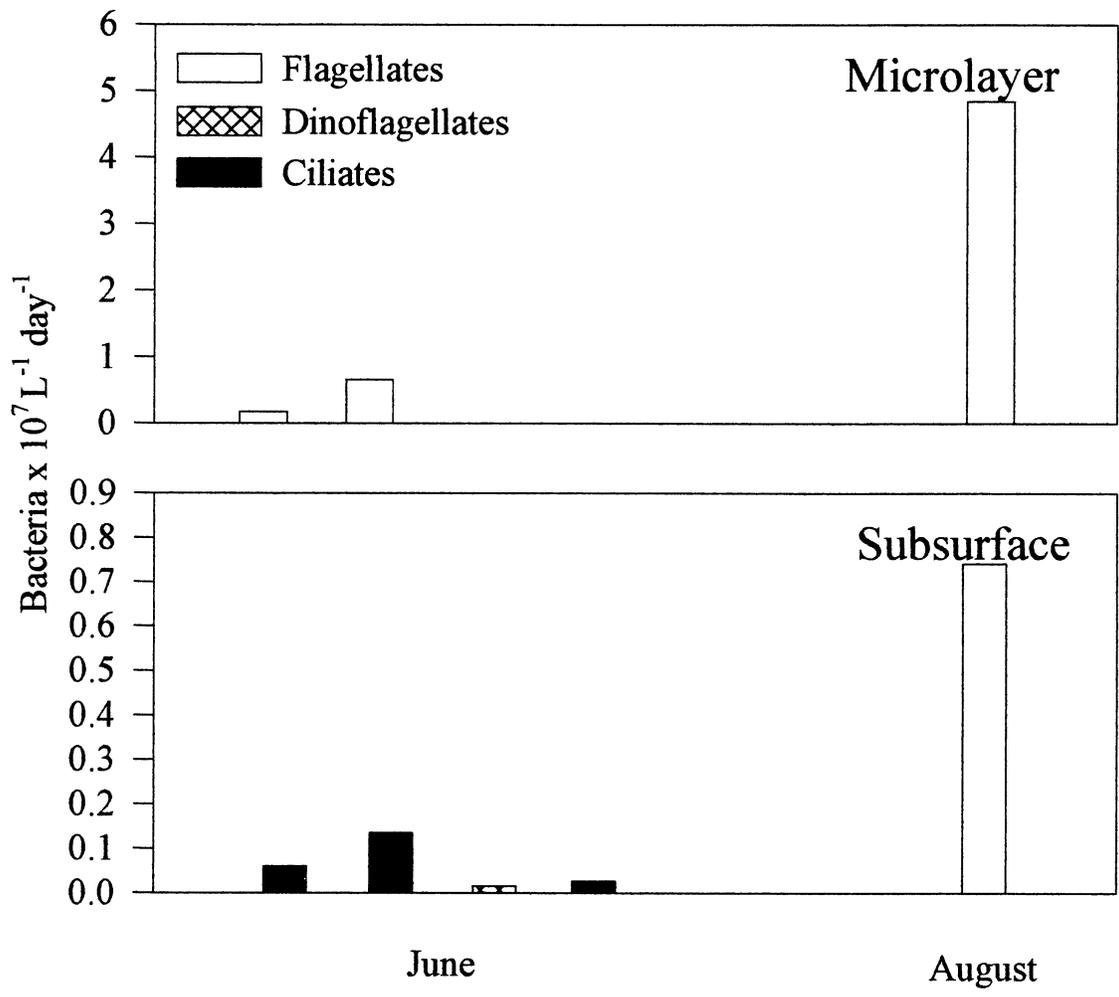


Fig. 12. Bacterial consumption by grazers in microlayer and subsurface waters. Each bar represents one sample in which significant grazing rates were measured. Samples were taken on 7 June and 24 August 1994.

Although grazing rates were significant in only eight out of 16 experiments, grazing was detected in all experiments. Positive slopes, as determined from X-Y plots of the number of FLB ingested vs. time, were obtained in most samples. Given that, estimates of grazing rates as determined for all samples are shown in Table 6. Heterotrophic flagellates appeared to be the primary consumers of FLB. This was most apparent in the microlayer in August. Ciliates were more important as grazers in the subsurface, especially in June. Although heterotrophic dinoflagellates were not the primary consumers either in the microlayer or in the subsurface, they grazed FLB in microlayer and subsurface waters in both June and August.

Production of bacterial cells was compared to grazing rates in the eight samples determined to have significant grazing rates. A conversion factor reported by Fuhrman and Azam (1982) was used to convert TdR incorporation rates to bacterial production rates (1.4×10^{18} cells produced per mole of TdR incorporated). Grazing was <1% of bacterial production in five of the eight samples, including microlayer and subsurface samples (Fig. 13). The highest ratio of grazing and bacterial production was measured in August (32.8%) in a microlayer sample.

Table 6. Bacterial consumption by grazers in microayer and subsurface waters, including all experiments. Samples were taken on 7 June and 24 August 1994. Mean values ($\times 10^7$ bacteria $L^{-1} hr^{-1}$) and standard errors are shown (n=4).

Protist	Microlayer		Subsurface	
	June	August	June	August
Flagellates <5 μm	0.12 \pm 0.045	1.5 \pm 0.67	0.041 \pm 0.0089	0.32 \pm 0.14
Flagellates 5-20 μm	0.070 \pm 0.046	0.59 \pm 0.30	0.028 \pm 0.024	0.038 \pm 0.032
Dinoflagellates	0.0068 \pm 0.0034	0.017 \pm 0.0089	0.0063 \pm 0.0029	0.0076 \pm 0.0035
Ciliates	0.0017 \pm 0.0014	0.00069 \pm 0.00069	0.016 \pm 0.0088	0.0042 \pm 0.0024
Total	0.20 \pm 0.085	2.1 \pm 0.79	0.091 \pm 0.025	0.37 \pm 0.13

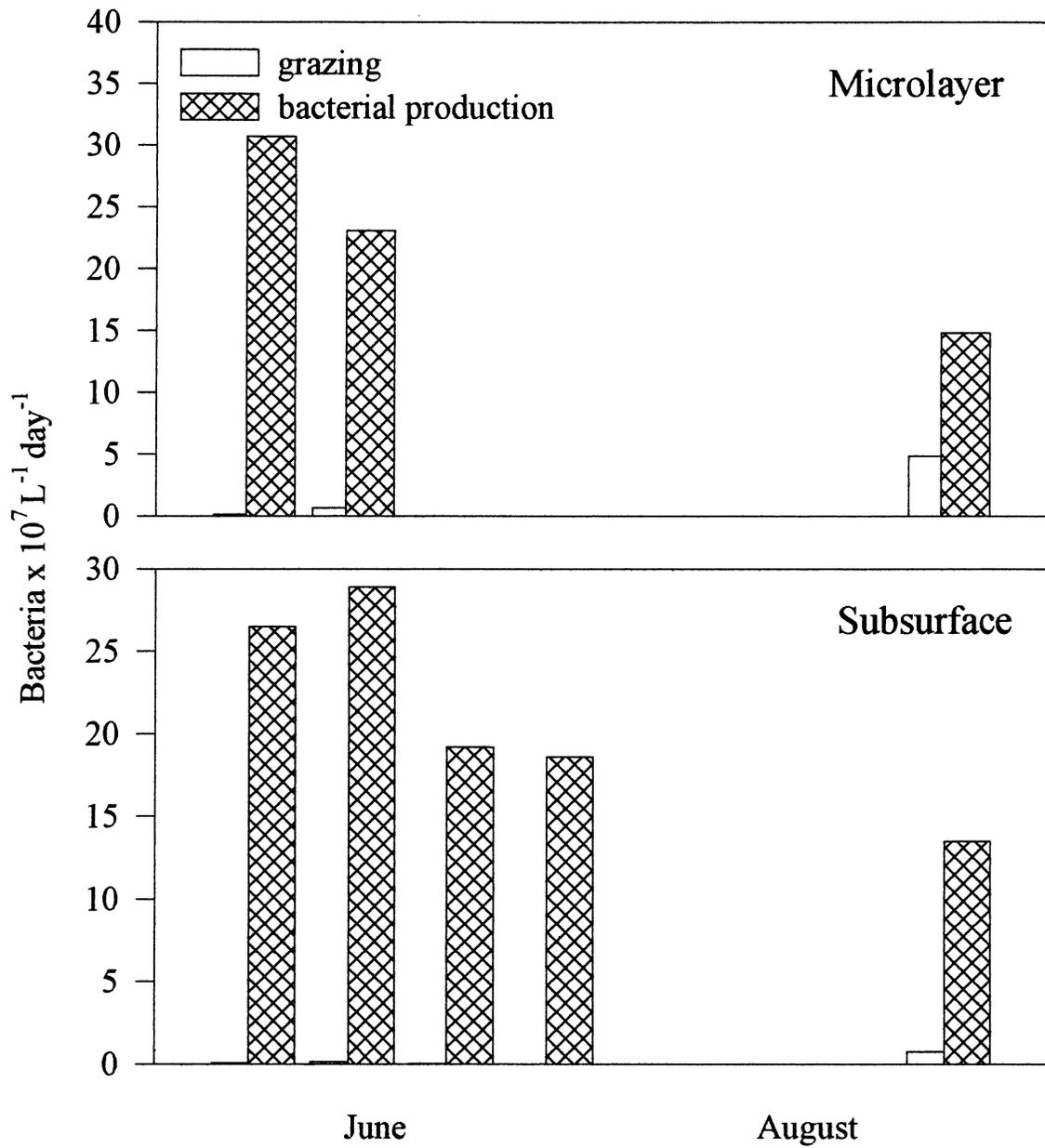


Fig. 13. Comparison of rates of bacterivory with rates of bacterial production in microlayer (A) and subsurface (B) waters. Each bar represents one sample in which significant grazing rates were measured. Samples were taken on 7 June and 24 August 1994.

DISCUSSION

Bacterial abundance and activity

Bacterial densities were usually higher in microlayer waters of Padilla Bay than in subsurface waters. Enrichments of bacterioneuston in sea-surface films have been reported in other coastal systems (Table 7). The density of bacterioneuston in Sequim Bay, another coastal estuary in Washington, was as much as two orders of magnitude higher than maximum enrichments measured in Padilla Bay. This may reflect the shallow microlayer depth (~20 μm) measured in Sequim Bay. Highest concentrations of bacterioneuston have been measured in depths of one to ten μm , and are apparently associated with the lipid film at the air-water interface (Norkrans, 1980; Sieburth, 1983). Subsurface bacterial densities in Padilla Bay are within the range of densities reported for other coastal waters (Table 8), including previous measurements within Padilla Bay (Ostendorff and Peele, 1993).

Enrichment of bacteria in Padilla Bay microlayers may be a result of many factors, including the passive accumulation of cells via various transport mechanisms such as rising air bubbles and upwellings (Norkrans, 1980; Hardy, 1982; Sieburth, 1983). The upward transport of particulate organic matter and associated microorganisms during the tidal cycle may also be a source of bacterioneuston. Slicks appeared to form in Padilla Bay when blades of eelgrass contacted the surface during a low tide. It is likely that when tidal flooding occurs, organic matter and associated microorganisms are lifted from the eelgrass, contributing to the accumulation of bacterioneuston at the surface.

Passive accumulation is not the only explanation for high concentrations of

Table 7. Comparison of bacterial numbers and [³H]thymidine incorporation rates in microlayer and subsurface waters of Padilla Bay with literature values.

Reference	Position in water column	Bacteria (cells/L)	TdR incorporation (pmol L ⁻¹ hr ⁻¹)	Location
Hardy and Apts (1984)	20 µm (filter)	0.0014x-30.5x10 ¹²	-----	Sequim Bay, WA
	subsurface	4.67- 34.5x10 ⁸	-----	
Carlucci <i>et al.</i> (1986)	150 µm (screen)	0.6-8.4x10 ⁸	2.4-3.8	Baja California
	10 cm	0.4-6.7x10 ⁸	2.7-6.6	
Carlucci <i>et al.</i> (1991)	150 µm (screen)	0.50-2.64x10 ⁹	0.4-6.4	Damariscotta estuary, ME
	10 cm	1.20-3.46x10 ⁹	1.7-12.0	
Carlucci <i>et al.</i> (1992)	150 µm (screen)	1.00-1.32x10 ⁹	-----	Southern California, nearshore
	10 cm	0.49-0.87x10 ⁹	-----	
Carlucci and Wolgast (1992)	150 µm (screen)	0.97-2.26x10 ⁹	-----	Southern California, offshore
	10 cm	0.49-1.39x10 ⁹	-----	
Ostendorff and Peele (1993)	subsurface	0.6-6.5x10 ⁹	130-245	Padilla Bay, Washington
Thompson (1995)	100 µm (glass plate)	1.8-20.9x10 ⁹	33-219	Padilla Bay, Washington
	25 cm	0.6-4.8x10 ⁹	37-207	

Table 8. Comparison of subsurface bacterial abundance in Padilla Bay with literature values of bacterial abundance in other estuaries (from Ducklow and Shiah, 1993).

Location	Abundance (10^9 cells L ⁻¹)	Reference
Sapelo Island, GA	5	Wiebe and Pomeroy (1972)
Sapelo Island, GA	5.3	Sherr <i>et al.</i> (1989a)
Woods Hole ponds	1.8-14	Kirchman (1983)
Delaware Bay	0.1-8	Coffin and Sharp (1987)
Hudson Estuary	2.5-8	Hudak <i>et al.</i> (1988)
Chesapeake Bay	0.5-25	Ducklow and Shiah (1993)
Damariscotta estuary, ME	0.5-2.6	Carlucci <i>et al.</i> (1991)
Baja California	0.06-0.84	Carlucci <i>et al.</i> (1986)
Vineyard Sound, MA	1-3	Marrasé <i>et al.</i> (1992)
Padilla Bay, WA	0.6-6.5	Ostendorff and Peele (1993)
Padilla Bay, WA	0.6-4.8	Thompson (this study)

bacteria in the microlayer. Given the enrichments of bacterioneuston and organic matter in sea-surface films, many researchers have suggested *in situ* growth of microorganisms is also taking place on the nutrient-rich organic film (Sieburth, 1976). Higher rates of TdR incorporation in the microlayer relative to subsurface waters during August in Padilla Bay support the hypothesis that microlayers can support bacterial populations that are metabolically more active than populations in subsurface waters. Enhanced heterotrophic activity in the microlayer in August was likely due to the significant enrichment of bacterial numbers relative to subsurface waters.

Microlayer and subsurface TdR incorporation rates were positively and significantly correlated with chlorophyll *a* and ammonium concentrations. The positive relationship between chlorophyll *a* concentrations and TdR incorporation rates in Padilla Bay indicates that organic substrates of phytoplankton or phytoneuston origin may be available for bacterial utilization. Concentrations of organic matter at the air-water interface, fixed by phytoneuston and phytoplankton, and the release of fixed carbon by migrating phagotrophic protists provide an important energy source for bacterial production. Bacteria readily assimilate ammonium (Kirchman, 1994), so it is not surprising that rates of TdR incorporation are positively related to ammonium concentration. Higher TdR incorporation rates also corresponded with higher temperatures in microlayer and subsurface waters. Temperature is a major factor regulating bacterial growth in temperate estuaries (Shiah and Ducklow, 1994). The relationship between temperature and bacteria may explain higher bacterial numbers in Padilla Bay microlayers relative to subsurface waters, and the seasonal increase in rates of

TdR incorporation in both environments.

While rates of TdR incorporation were higher in the microlayer than in subsurface waters in August, bacterial metabolic activity was not enhanced in April or June. Cell-specific TdR incorporation rates were always higher in the subsurface than in the microlayer. The apparent paradox between enriched bacterial numbers and low TdR incorporation rates has been shown in other studies (Table 7). In subsurface waters of Padilla Bay, TdR incorporation rates were within the range of rates observed in Chesapeake Bay (Table 9). TdR incorporation rates in this study overlapped, but were slightly lower than previous subsurface measurements in Padilla Bay (Ostendorff and Peele, 1993).

The observation that the microlayer in Padilla Bay is enriched with bacterioneuston, but that subsurface populations are metabolically more active may be explained by differences in substrate composition. In addition to organic carbon sources derived *in situ*, much of the organic matter found in estuarine environments is allochthonous (Ducklow and Shiah, 1993). This may be true in Padilla Bay, although allochthonous inputs from local sloughs are controlled by tidal gates and enter the bay in pulses rather than as a continuous supply. The Swinomish Channel may provide a more continuous source of allochthonous organic carbon from the Skagit River. It is usually assumed that allochthonous material consists primarily of refractory dissolved and particulate organic carbon (DOM and POM), since labile compounds are utilized rapidly by microorganisms *in situ*. Microlayers are enriched in particulate organic carbon (POC), and carbon to nitrogen ratios are usually greater in the microlayer than in subsurface

Table 9. Comparison of subsurface [³H]thymidine incorporation rates in Padilla Bay with literature values.

Location	Bacterial production pmol L ⁻¹ hr ⁻¹	Reference
Chesapeake Bay	0.5-500	Ducklow and Shiah (1993)
Sapelo Island, GA	79.5	Sherr <i>et al.</i> (1989a)
Damariscotta estuary, ME	1.7-12.0	Carlucci <i>et al.</i> (1991)
Baja California	2.7-6.6	Carlucci <i>et al.</i> (1986)
Padilla Bay, WA	130-245	Ostendorff and Peele (1993)
Padilla Bay, WA	37-207	Thompson (this study)

waters (Carlson, 1983; Garabetian *et al.*, 1991). High C:N ratios indicate allochthonous origin (Wetzel, 1983), which suggests that microlayer DOM and POM are more refractory organic matter in subsurface waters. The potential difference in the composition of DOM between microlayer and subsurface waters may affect bacterial community structure. Tranvik (1992) suggested that bacterioplankton are a heterogeneous mixture of slow-growing degraders of refractory substrates and fast-growing degraders of labile substrates. Bacterioneuston in Padilla Bay, with lower metabolic activity than bacterioplankton, may be an assemblage of slower growing decomposers of refractory material, assuming that much of the organic matter in the microlayer is refractory.

Inhibitory effects of pollutants may help explain the lower rates of TdR incorporation in the microlayer. Chlorinated hydrocarbons, pesticides and heavy metals can be concentrated in microlayers, and negatively affect growth and metabolism (Hardy, 1982; Hardy *et al.*, 1985; Maki, 1993). Toxicity toward echinoderm larvae exposed to microlayers deposited onto intertidal substrates in Padilla Bay has been shown (Gardiner, 1992). The potential for anthropogenic contamination of Padilla Bay exists because of local land use patterns that include agriculture, industry and urbanization. Furthermore, the expansive eelgrass beds in Padilla Bay may be a source of natural toxins such as phenols that can be concentrated in microlayers.

Bacterial growth rates in sea-surface films may also be affected by exposure to intense solar radiation. Photoinhibition in phytoneston, resulting in lower levels of photosynthesis (Hardy and Apts, 1984), may result in differences in heterotrophic activity between microlayer and subsurface waters. Bacterial processes in microlayer and

subsurface waters may be affected by growth delays caused by solar radiation (Sieracki and Sieburth, 1986), though it is unclear whether or not bacterioneuston at a depth of approximately 100 μm would be affected differently than bacterioplankton at a depth of 25 cm. Bacterioneuston possess various adaptations that may increase resistance to the negative effects of solar radiation (Maki, 1993). If the production of cells with various adaptive mechanisms delays growth, lower activity in the microlayer relative to subsurface waters may result.

Bacterial community structure and production in sea-surface microlayers and subsurface waters may be regulated by the variable feeding strategies of predators (Turley *et al.*, 1986; Albright *et al.*, 1987). While the nutritive status of bacterioneuston of Padilla Bay is not known, they are clearly less active than bacterioplankton on a per cell basis. Selective grazing of faster growing cells (González *et al.*, 1990; Sherr and Sherr, 1994) and slower grazing on less active cells (González *et al.*, 1993) may simultaneously explain the high numbers and low activity of microlayer bacterial populations.

Protist abundance and grazing activity

This is the first known study to describe the heterotrophic protist community in sea-surface microlayers, so estimates of abundance and grazing activities have been compared to subsurface studies. The number of heterotrophic flagellates in the microlayer is higher than the subsurface densities reported for other coastal environments (Table 10). The number of heterotrophic flagellates, dinoflagellates and ciliates in subsurface waters are similar to protist densities in other estuarine environments (Table 10). The number of

Table 10. Comparison of protist numbers in Padilla Bay with literature values.

Reference	Protists	(cells L ⁻¹)
Marrasé <i>et al.</i> (1992) Vineyard Sound MA	HNF	0.6-4x10 ⁶
Sherr <i>et al.</i> (1989a) Sapelo Is., GA	Hflag	2.1±0.6x10 ⁶
	Ciliates	1.4±0.5x10 ⁴
Sherr and Sherr (1991) SE US cont. shelf Salt marsh estuary, GA Great Barrier Reef	HNF (2-20 µm)	3.2-6.7x10 ⁵
		1.1-4.0x10 ⁶
		1.9±0.5x10 ⁵
Haigh and Taylor (1991) N. St. of Georgia, BC	Nanoflagellates	1.9-3.4x10 ⁶
	Ciliates	1.4-3.6x10 ⁴
Montagnes <i>et al.</i> (1988) Gulf of Maine	Ciliates	0.35-6.0x10 ³
Dolan and Coats (1990) Chesapeake Bay	Hflag	1.1-3.7x10 ⁶
	Ciliates	0.18-1.7x10 ⁴
Lessard (1991) Chesapeake Bay	Hdino	0.03-4.1x10 ⁴
	Ciliates	0.04-2.8x10 ⁴
North Atlantic, Gulf Stream	Hdino	0.5-1.6x10 ³
	Ciliates	0.5-1.2x10 ³
Sargasso Sea	Hdino	0.1-1.0x10 ³
	Ciliates	1.0-9.0x10 ²
Thompson (this study) Microlayer	Hflag	9.9-96.4x10 ⁵
	Hdino	2.4-2.5x10 ⁴
	Ciliates	1.6-2.3x10 ³
Subsurface	Hflag	6.4-44.3x10 ⁵
	Hdino	1.6-2.2x10 ⁴
	Ciliates	4.6-9.7x10 ³

heterotrophic flagellates in the microlayer of Padilla Bay was positively correlated with bacterial numbers. In subsurface waters, ciliate abundance was positively correlated with bacterial numbers. Other investigators have shown a similar relationship between the abundance of heterotrophic flagellates and bacterial numbers (Azam *et al.*, 1983; Andersen and Sørensen 1986; Sherr *et al.*, 1989a).

The composition of the heterotrophic protist community in Padilla Bay changed over time. Heterotrophic flagellates <20 μm were more abundant in summer in microlayer and subsurface waters, whereas the maximum number of ciliates was found during June in subsurface waters. Similar distribution patterns are found in the Gulf of Maine, where larger protists that feed on large phytoplankton are more common during spring, and smaller protists, including small ciliates that graze on small flagellates and bacterial particles, are more abundant in the summer (Montagnes *et al.*, 1988). Circulation in Padilla Bay is not well described, but may help explain distribution patterns of heterotrophic protists and phytoplankton (Haigh and Taylor, 1991).

While community grazing rates were not obtained during this study, bacterivory as estimated by the ingestion of fluorescently labelled bacteria was documented in microlayer and subsurface waters. This study is the first to investigate bacterivory in sea-surface microlayers. The highest measured grazing rate occurred in microlayer samples, indicating the potential importance of bacterioneuston as a carbon source for higher trophic levels in the surface film. Interestingly, flagellates <20 μm were the only heterotrophic protists with significant grazing in the microlayer, and ciliate grazing was most prevalent in the subsurface in June. These results correspond to the spatial distribution of protists, and

with the positive relationships between bacterial density and heterotrophic protist abundance. In samples where grazing rates were significant, bacterivory by heterotrophic flagellates was similar and bacterivory by ciliates was lower to rates measured in other estuarine environments (Table 11).

Bacterial production and bacterivory

Measured rates of bacterivory rarely match rates of bacterial production in estuaries (McManus and Fuhrman, 1988; Sherr and Sherr, 1994). In this study grazing rates in both the microlayer and subsurface waters of Padilla Bay were usually less than 1% and not more than 33% of the estimated bacterial production.

One explanation for the lack of balance between grazing rates and bacterial production is the possibility that experimental conditions do not simulate *in situ* conditions. Methods may either overestimate production or underestimate grazing. Bacterivorous protists can select against artificial or heat-killed particles, and egestion can occur upon fixation of samples (McManus and Fuhrman, 1988). Recent evidence suggests that bacterivorous protists preferentially graze motile bacteria (González *et al.*, 1993). Community grazing rates based on particle uptake methods may be underestimated by as much as 50%. The uptake of fluorescent particles gives consistently lower rates of bacterivory than dilution, inhibition, filtration or the uptake of genetically marked bacteria (Vaqué *et al.*, 1994). However, overestimates of bacterial production can occur using dilution, inhibition, filtration or the uptake of genetically marked bacteria because these methods usually have longer incubation periods, and samples are handled

Table 11. Comparison of subsurface ingestion rates in Padilla Bay with literature values. Percentages represent percent of total daily bacterial production, if available.

Reference	Grazers	Method	Grazing rate (bacteria L ⁻¹ d ⁻¹)
Sherr and Sherr (1987) Sapelo Is., GA	Ciliates	FLB	0.7-4.1x10 ⁹
Vaque <i>et al.</i> (1994) (review)	Total grazing, seawater	(particle uptake methods, n=19)	2.7 x 10 ⁸
	HNF, seawater	(particle uptake methods, n=21)	1.4x10 ⁵
	Total grazing, lakes	(particle uptake methods, n=32)	4.3x10 ⁸
	HNF, lakes	(particle uptake methods, n=122)	1.4x10 ⁶
Marrasé <i>et al.</i> (1992) Vineyard Sound MA	HNF	FLB	4x10 ⁷ -2x10 ⁹ (3-41%)
Sherr <i>et al.</i> (1989a) Sapelo Is., GA	HNF Ciliates	FLB	4.3x10 ⁸ 2.5x10 ⁸ (50%)
Thompson (this study) Microlayer Subsurface	Hflag	FLB	0-11.6x10 ⁸ (0-32.8%)
	Hdino	FLB	0-1.7x10 ⁸ (0-5.5%)
	Ciliates	FLB	0-8.8x10 ⁵ (0-0.1%) 0-3.2x10 ⁷ (0-0.47%)

more frequently.

Sample size is a problem often encountered when sampling the sea-surface film. Small bottles increase the adsorptive surface area per unit volume of a sample, thereby increasing the potential for nutrients to concentrate and microorganisms to increase in number. Potential bottle effects on bacterivory include lower grazing rates and higher variability in small-volume samples (<500 ml) (Marrasé *et al.*, 1992). In this study, sample volume was limited to approximately 150 ml. Therefore, the estimates of bacterivory in both microlayer and subsurface waters of Padilla Bay are conservative.

Other reasons for the lack of balance between bacterivory and bacterial production include bacterial mortality due to grazing by mixotrophic phytoflagellates (Porter, 1988; Haigh and Taylor, 1991), predation by metazooplankton (Turner and Tester, 1992) and viral lysis (Bergh *et al.*, 1989; Proctor and Furhman, 1990 and 1992). Mixotrophy by phytoflagellates was suspected during this study, and was detected in chloroplast-retaining ciliates. However, ingestion of FLB by phytoflagellates was not measured. Nevertheless, estimated bacterivory by phytoflagellates may be nearly equivalent to bacterivory by heterotrophic flagellates (Porter, 1988). While predation on bacteria by metazoans has been documented in other estuaries, the total impact of metazoan grazers on bacterial production is not known (Turner and Tester, 1992) and has not been measured in Padilla Bay. Infection of marine procaryotes by viruses has also been suggested as a significant cause of bacterial mortality. In a recent review, Suttle (1994) suggests that 10 to 20% of the bacterial population could be lysed per day. No information is available on the density or infectivity of viral particles in Padilla Bay.

Padilla Bay is a shallow-water estuary dissected by a number of channels that empty the bay during tidal exchanges. The residence time and movement of water in Padilla Bay have not been investigated. However, physical processes including the dilution of seawater in estuaries and seaward advection of surface waters can strongly affect bacterial abundance patterns and production (Ducklow and Shiah, 1993). The distribution of microorganisms can be determined by circulation patterns created by tidal mixing, stratification and turbulence, resulting in a mosaic (Haigh and Taylor, 1991). Circulation patterns may contribute to the patchy nature of biological, physical and chemical measurements in Padilla Bay.

Temperature and substrate availability are important abiotic factors in the regulation of bacterial populations (Pace and Cole, 1994; Shiah and Ducklow, 1994). It is conceivable that bacterial growth in Padilla Bay was limited by temperature in April, allowing a significant response to higher temperatures in June. Top-down factors such as mortality due to grazing or viral lysis may limit production in August, when growth rates were lower despite higher temperatures. The availability of labile substrates is also a factor that may limit bacterial growth.

A conservative estimate of the relative importance of various loss processes on bacteria in Padilla Bay would include grazing by heterotrophic protists (<1%), phytoflagellate bacterivory (<1%; equal to heterotrophic bacterivory) and viral lysis (10%). In this most conservative scenario, bottom-up factors such as temperature and substrate limitation may be more important in the regulation of bacterial production in Padilla Bay than top-down factors, including bacterivory and viral lysis. However, if the

highest rates of bacterivory measured in Padilla Bay are coupled with the highest estimates for other bacterial loss processes, removal of bacteria would approach bacterial production. In this scenario, heterotrophic protists (33%) and phytoflagellates (33%) would remove about 66% of bacterial production daily and viral lysis would remove 20%. If grazing rates based on particle uptake methods are actually underestimated by 50%, then bacterivory alone would equal or exceed bacterial production.

Conclusion

The sea-surface microlayer in Padilla Bay is a patchy and highly variable environment. The patchiness in biological parameters is likely due to extreme variations in environmental parameters, seasonal changes in the microbial assemblage, and physical factors including circulation patterns.

Although patchiness was evident, some conclusions can be drawn that may explain heterotrophic community structure and dynamics in the microlayer of Padilla Bay.

Bacterial abundance was generally higher and more variable in the microlayers than in subsurface waters. In contrast, subsurface bacterial communities were metabolically more active than microlayer communities. Bacterial production in the microlayer may have been limited by substrate composition or inhibited by pollutants concentrated in the surface film.

Differences in grazing pressure may also explain lower metabolic activity in microlayers. Heterotrophic activity in the microlayer can exceed subsurface activity if enrichment of bacteria in the surface film is significant. Small flagellates were the most abundant members of the heterotrophic protistan community in both the microlayer and the subsurface. Other heterotrophic protists included flagellates 5 to 20 μm , dinoflagellates, and ciliates. Bacterivory occurred in both microlayer and subsurface waters and was attributed largely to flagellates. Grazing rates were not equivalent to rates of bacterial cell production, and usually were less than 1% of the estimated bacterial cell production. It appears that bacterivory by heterotrophic protists is not a major path of energy transfer in microlayer or subsurface waters of Padilla Bay. Other factors may be more important in balancing bacterial growth, such as physical advection and viral lysis.

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APPENDIX A

Environmental conditions on day two of each sampling period.

Date	Time	Tidal conditions*	Weather	Slicks
12 Apr	0948-1212	Ebb→slack 0.6 m to -0.1 m	Followed rainy period; some wind	-----
7 June	1130-1430	Flood -0.2 m to 1.1 m	Followed rainy period; mostly calm	stations 2, 10
24 Aug	0830-1100	Ebb 1.8 m to 1.0 m	Followed warm dry period; calm	stations 5, 9

* Direction of flow (flood or ebb) and tidal height in meters at nearest tidal height station (Guemes Channel near Cap Sante, Anacortes, WA).

APPENDIX B

Analysis of variance tables for physical parameters

B1. Two-way analysis of variance for temperature.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x position	2	3.6776	*0.0458
Error	18		
Total	23		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

B2. Two-way analysis of variance for salinity.

Source of variation	df	F	p
Main effects			
Date	2	35.0727	*0.0000
Position	1	5.1229	*0.0362
Interaction terms:			
Date x position	2	0.5132	0.6071
Error	18		
Total	23		

* Significant main effect; see Appendix C for the results of Tukey's pairwise comparison.

B3. Two-way analysis of variance for microlayer depth.

Source of variation	df	F	p
Main effects			
Date	2		
Interaction terms:			
Date x station	9	3.3231	*0.0090
Error	24		
Total	35		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

Analysis of variance tables for water quality parameters

B4. Three-way analysis of variance for chlorophyll *a* concentration.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9		
Date x position	2		
Date x position x station	9	2.5783	*0.0168
Error	47		
Total	*70		

* Significant three-way interaction present; see Appendix C for the results of simple main effects evaluation.

**One missing data point. Exact F-test constructed as described by Siegel (1992).

B5. Three-way analysis of variance for pheopigment concentration.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9	1.0422	0.4219
Date x position	2	7.6553	*0.0013
Date x position x station	9	1.4168	0.2083
Error	47		
Total	**70		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

**One missing data point. Exact F-test constructed as described by Siegel (1992).

B6. Three-way analysis of variance for ammonium concentration.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9	3.0027	*0.0064
Date x position	2	0.7340	0.5067
Date x position x station	9	1.7134	0.1118
Error	48		
Total	71		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

B7. Three-way analysis of variance for nitrate (nitrate+nitrite) concentration.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9		
Date x position	2		
Date x position x station	9	11.2272	*0.0000
Error	47		
Total	71		

* Significant three-way interaction present; see Appendix C for the results of simple main effects evaluation.

B8. Three-way analysis of variance for phosphate concentration.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9		
Date x position	2		
Date x position x station	9	2.2071	*0.0378
Error	47		
Total	71		

* Significant three-way interaction present; see Appendix C for the results of simple main effects evaluation.

Analysis of variance tables for bacterial measurements

B9. Three-way analysis of variance for bacterial numbers, day one (sixteen station sampling).

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9		
Date x position	2		
Date x position x station	9	2.8258	*0.0000
Error	48		
Total	71		

* Significant three-way interaction present; see Appendix C for the results of simple main effects evaluation.

B10. Three-way analysis of variance for bacterial numbers, day two (four station sampling).

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9	0.7132	0.6941
Date x position	2	17.9112	*0.0007
Date x position x station	9	1.3094	0.2571
Error	48		
Total	71		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

B11. Three-way analysis of variance for [³H]thymidine incorporation rates.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9	18.3034	*0.0000
Date x position	2	19.8645	*0.0005
Date x position x station	9	1.1892	0.3241
Error	48		
Total	71		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

B12. Three-way analysis of variance for [³H]thymidine incorporated per bacterial cell.

Source of variation	df	F	p
Main effects			
Date	2		
Position	1		
Interaction terms:			
Date x station	9	5.6825	*0.0000
Date x position	2	0.6526	0.5251
Date x position x station	9	1.6015	0.1462
Error	48		
Total	**70		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

** One missing data point. Exact F-test constructed as described by Siegel (1992).

Analysis of variance tables for heterotrophic protist numbers

B13. Three-way analysis of variance for total heterotrophic protist numbers.

Source of variation	df	F	p
Main effects			
Date	1		
Position	1		
Interaction terms:			
Date x station	2	0.3446	0.7183
Date x position	1	756.5495	*0.0013
Date x position x station	2	0.0060	0.9940
Error	8		
Total	15		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

B14. Three-way analysis of variance for total numbers of heterotrophic flagellates, <5 μm and 5-20 μm .

Source of variation	df	F	p
Main effects			
Date	1		
Position	1		
Interaction terms:			
Date x station	2	0.3435	0.7193
Date x position	1	761.8281	*0.0013
Date x position x station	2	0.0060	0.9940
Error	8		
Total	15		

* Significant two-way interaction present; see Appendix C for the results of simple main effects evaluation.

B15. Three-way analysis of variance for numbers of heterotrophic flagellates <5 μm .

Source of variation	df	F	p
Main effects			
Date	1	16.6825	0.0550
Position	1	9.4595	0.0915
Interaction terms:			
Date x station	2	1.1699	0.3583
Date x position	1	6.4803	0.1258
Date x position x station	2	0.2403	0.7921
Error	8		
Total	15		

B16. Three-way analysis of variance for numbers of heterotrophic flagellates 5-20 μm .

Source of variation	df	F	p
Main effects			
Date	1		
Position	1		
Interaction terms:			
Date x station	2		
Date x position	1		
Date x position x station	2	5.0848	*0.0376
Error	8		
Total	15		

* Significant three-way interaction present; see Appendix C for the results of simple main effects evaluation.

B17. Three-way analysis of variance for numbers of heterotrophic dinoflagellates.

Source of variation	df	F	p
Main effects			
Date	1	3.4208	0.2056
Position	1	6.1737	0.1309
Interaction terms:			
Date x station	2	0.2399	0.7921
Date x position	1	1.0117	0.4204
Date x position x station	2	0.1369	0.8740
Error	8		
Total	15		

B18. Three-way analysis of variance for numbers of ciliates.

Source of variation	df	F	p
Main effects			
Date	1	10.5131	0.0834
Position	1	14.5274	0.0625
Interaction terms:			
Date x station	2	0.8664	0.4566
Date x position	1	2.6504	0.2451
Date x position x station	2	1.9824	0.1999
Error	8		
Total	15		

APPENDIX C

C1. Evaluation of simple main effects between time periods for physical parameters (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface
Temperature	(air temperature)	
April-June	0.0006	0.4714
April-August	0.0002	0.2009
June-August	0.6591	0.5612
 Salinity		
April-June	*	*
April-August	*	*
June-August		*
 Microlayer depth		
April-June	0.0965	
April-August	0.0000	
June-August	0.0000	

* Tukey (HSD) pairwise comparison of salinity means between microlayer and subsurface show a significant difference between the two means.

C2. Mean values (\pm SD) of physical measurements in microlayer and subsurface waters and evaluation of simple main effects between microlayer and subsurface waters (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface	p
Temperature ($^{\circ}$ C)	(air temperature)		
April	13.8 \pm 2.22	11.7 \pm 0.342	0.1491
June	19.5 \pm 3.11	12.7 \pm 0.556	0.0001
August	20.1 \pm 2.87	13.5 \pm 0.123	0.0002
Salinity (‰)			
All time periods	28.1 \pm 1.03	28.6 \pm 1.08	*
Microlayer depth (μ m)			
April	123.6 \pm 11.0		
June	118.1 \pm 12.3		
August	95.0 \pm 4.61		

* Tukey (HSD) pairwise comparison of salinity means between microlayer and subsurface show a significant difference between the two means.

C3. Evaluation of simple main effects between time periods for water quality parameters. (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface
Chlorophyll α		
April-June	0.0000	0.0000
April-August	0.0000	0.0000
June-August	0.9124	0.0948
Pheopigment		
April-June	0.7870	0.0134
April-August	0.0000	0.0174
June-August	0.0000	0.9175
Ammonium		
April-June	0.0000	0.0000
April-August	0.0000	0.0000
June-August	0.0531	0.1540
Nitrate + nitrite		
April-June	0.0000	0.0000
April-August	0.0000	0.0000
June-August	0.0000	0.0000
Phosphate		
April-June	0.0429	0.3082
April-August	0.0000	0.0000
June-August	0.0083	0.0000

C4. Mean values (\pm SD) of water quality parameters in microlayer and subsurface waters and evaluation of simple main effects between microlayer and subsurface waters (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface	p
Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)			
April	1.73 \pm 0.448	1.06 \pm 0.694	0.9874
June	3.49 \pm 0.805	3.07 \pm 0.689	0.2261
August	3.46 \pm 1.90	2.59 \pm 0.523	0.0021
Pheopigment ($\mu\text{g L}^{-1}$)			
April	3.30 \pm 1.32	1.29 \pm 0.652	0.0003
June	3.16 \pm 0.820	2.61 \pm 0.239	0.3107
August	6.59 \pm 2.68	2.57 \pm 0.333	0.0000
Ammonium ($\mu\text{g-at L}^{-1}$)			
April	0.353 \pm 0.220	0.294 \pm 0.266	0.4795
June	1.46 \pm 0.342	1.23 \pm 0.121	0.0061
August	1.30 \pm 0.202	1.11 \pm 0.197	0.0240
Nitrate + nitrite ($\mu\text{g-at L}^{-1}$)			
April	11.18 \pm 1.65	11.16 \pm 2.47	0.0000
June	7.76 \pm 2.14	6.91 \pm 1.05	0.0000
August	13.46 \pm 0.807	14.067 \pm 0.711	0.0808
Phosphate ($\mu\text{g-at L}^{-1}$)			
April	1.74 \pm 0.433	1.73 \pm 0.394	0.9333
June	2.16 \pm 0.783	1.93 \pm 0.613	0.0442
August	2.70 \pm 0.420	2.88 \pm 0.567	0.0936

C5. Evaluation of simple main effects between time periods for bacterial numbers (day one; t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface
Bacterial numbers		
April-June	0.0697	0.2072
April-August	0.0000	0.4212
June-August	0.0000	0.0404

C6. Evaluation of simple main effects between time periods for bacterial measurements (day two; t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface
Bacterial numbers		
April-June	0.1256	0.8361
April-August	0.0000	0.3879
June-August	0.0000	0.2858
TdR incorporation		
April-June	0.0000	0.0000
April-August	0.0000	0.0000
June-August	0.0116	0.0000
TdR incorp. per cell		
April-June	0.0000	0.0000
April-August	0.0049	0.0000
June-August	0.0000	0.0000

C7. Mean values \pm SD of bacterial numbers (day 1) and evaluation of simple main effects between microlayer and subsurface waters (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	(CV)	Subsurface	(CV)	p
Bacterial numbers ($\times 10^9$ cells L ⁻¹)					
April	3.0 \pm 0.87	(29)	2.3 \pm 0.69	(30)	0.9305
June	3.6 \pm 0.74	(21)	2.7 \pm 0.94	(35)	0.2518
August	5.8 \pm 3.6	(62)	2.0 \pm 0.71	(35)	0.0729

C8. Mean values (\pm SD) of bacterial measurements (day 2) and evaluation of simple main effects between microlayer and subsurface waters (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface	p
Bacterial numbers ($\times 10^9$ cells L ⁻¹)			
April	3.1 \pm 0.83	2.3 \pm 0.24	0.0038
June	2.7 \pm 0.59	2.3 \pm 0.27	0.2091
August	4.9 \pm 0.13	2.0 \pm 0.25	0.0000
TdR incorporation (pmol L ⁻¹ hr ⁻¹)			
April	46.0 \pm 7.9	70.4 \pm 21.9	0.0006
June	145.87 \pm 46.33	164.9 \pm 35.9	0.0025
August	130.20 \pm 12.56	100.6 \pm 12.5	0.0000
TdR incorp. per cell (pmol $\times 10^{-8}$ cell ⁻¹ hr ⁻¹)			
April	1.6 \pm 0.49	3.1 \pm 1.0	0.0014
June	5.7 \pm 0.21	7.3 \pm 0.19	0.0007
August	2.8 \pm 0.96	5.1 \pm 0.83	0.0000

C9. Evaluation of simple main effects between June and August for protist numbers. (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface
Total protists	0.0007	0.0466
Flagellates <5 μm	*	*
Flagellates 5 to 20 μm	0.0001	0.7431
Total flagellates	0.0007	0.0465
Dinoflagellates	*	*
Ciliates	*	*

* Three-way analysis of variance showed no significant difference between time periods.

C10. Mean values (\pm SD) of protist numbers (cells L⁻¹) and evaluation of simple main effects between microlayer and subsurface waters (t-test results). Significant effects are shown in bold.

Parameter	Microlayer	Subsurface	p
Total protists			
June	1.0x10 ⁶ ±0.42x10 ⁶	0.48x10 ⁶ ±0.063x10 ⁶	0.8373
August	9.7x10 ⁶ ±2.6x10 ⁶	4.5x10 ⁶ ±2.8x10 ⁶	0.0120
Flagellates <5 µm			
June	0.78x10 ⁶ ±0.14x10 ⁶	0.48x10 ⁶ ±0.063x10 ⁶	0.8590
August	7.4x10 ⁶ ±3.4x10 ⁶	4.2x10 ⁶ ±2.8x10 ⁶	0.0872
Flagellates 5 to 20 µm			
June	2.1x10 ⁵ ±0.91x10 ⁵	1.7x10 ⁵ ±0.4x10 ⁵	0.9981
August	2.3x10 ⁶ ±1.4x10 ⁶	0.27x10 ⁶ ±0.13x10 ⁶	0.0050
Total flagellates			
June	0.99x10 ⁶ ±0.14x10 ⁶	0.64x10 ⁶ ±0.026x10 ⁶	0.8368
August	9.6x10 ⁶ ±2.6x10 ⁶	4.4x10 ⁶ ±2.8x10 ⁶	0.0119
Dinoflagellates			
June	2.2x10 ⁴ ±0.50x10 ⁴	1.6x10 ⁴ ±1.1x10 ⁴	*
August	2.5x10 ⁴ ±1.1x10 ⁴	2.2x10 ⁴ ±0.69x10 ⁴	*
Ciliates			
June	2314.0±1781.4	9736.7±3001.7	*
August	1610.3±1230.7	4590.4±1859.2	*

* Three-way analysis of variance showed no significant difference between microlayer and subsurface means.

