ACTIVITIES AND BIOMASSES OF BACTERIOPLANKTON
WITHIN THE FRASER RIVER ESTUARY

by

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Activities and biomasses of bacterioplankton within the Fraser River Estuary

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ABSTRACT

Direct observation of Fraser River estuary bacterioplankton in water of various salinities demonstrated changes in bacterial shapes and decrease in average bacterial size from freshwater towards 24 °/oo salinity water. In addition, within the estuarine (plume) water which results when the freshwater of the Fraser River mixes with water (24 °/oo salinity) of Georgia Strait an increase in bacterial numbers and a stimulation in glucose heterotrophic activities occurs in the salinity range of approximately 2-18 °/oo. Even if the majority of indigenous heterotrophic bacterioplankton of the lower Fraser River appear to be deactivated when contained in membrane diffusion chambers immersed in Georgia Strait 24 °/oo salinity water, their marine (24 °/oo salinity) counterparts in Georgia Strait surface water remain viable with reduced activities when contained in membrane diffusion apparatus immersed in Fraser River freshwater.
A MIS QUERIDOS PADRES
A MI PADRINO, MESCENAS DEL SIGLO XX
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INTRODUCTION

Estuaries are often characterized by fluctuations in salt content as well as chemical and biological species composition. This variability is caused by varying oceanic and freshwater influence in near coast regions (Remane and Schlieper, 1971). Bacteria in coastal waters are not dispersed evenly throughout the water column. The populations include bacteria uniquely adapted to function in an estuarine environment together with those that are simply surviving (Jannasch, 1967). The presence of large numbers of heterotrophic bacteria in estuarine systems suggests that the organisms are well adapted to cope with the broad range of fluctuations in environmental conditions (Alexander, 1971). Bacterial populations in estuarine water therefore are composed of true, well adapted, estuarine water bacteria (Meyer-Reil, 1974) and fluctuating numbers of freshwater or marine bacteria. However, there is no definition of an estuarine bacterium similar to the one proposed for marine bacteria by MacLeod (1965).

Water in near-shore and estuarine environments characteristically contains greater concentrations of bacteria than most oceanic and inland waters; likewise, the activity of bacteria in estuarine systems is reported to be higher than observed in most other aquatic systems (Stevenson et al., 1974). The metabolic activities of bacteria not adapted to brackish water conditions may be reduced in this environment; some bacteria will die after a survival period.
On the average, the movement of dissolved substances and suspended materials, including plankton, is a slow process in estuarine environments. In many instances the time is sufficient for biological events, important in maintaining the integrity of the estuarine community, to occur (Reid, 1961). Bacteria in these estuarine environments must tolerate intrusion by seawater, therefore the bacteria inhabiting estuarine areas must be capable of a variety of physiological adaptations in response to fluctuating physical and chemical conditions. The actual tolerance to changes in salinity, nutrients concentration, temperature and other parameters by freshwater and marine bacteria inhabiting estuarine areas has yet to be elucidated.

The survival of heterotrophic nonhalophilic bacteria in the marine environment is influenced by various factors including the amount of organic material present (Seki et al., 1969). Heterotrophic potentials ($V_{\text{max}}$) of estuarine (Fraser River estuary) waters are associated with the amount of river discharge and heterotrophic bacterial activities increase with increased temperature in the Fraser River estuary (Albright, 1977). However, there is not information on the survival and activities of heterotrophic bacteria of the Fraser River or Georgia Strait when they mix into Fraser River estuary water. There is a paucity of information on bacterial numbers and activity changes within water masses as they move in and out of estuaries. Since bacteria are important in mineralizing organic matter, mobilizing minerals and as food source for some animals, questions such as the survival, dormancy, growth and activity of
the bacteria of the Fraser River and Georgia Strait entering the estuary (plume) water are of importance.

McFeters and Stuart (1972) used membrane diffusion chambers to determine survival of coliform bacteria in freshwaters. Fliermans and Gorden (1977) used modified diffusion chambers to determine bacterial survival in deep water studies. Sieburth et al (1977) developed a diffusion culture chamber to monitor changes in picoplankton as influenced by dissolved nutrients input. I have used membrane diffusion chambers to monitor changes in numbers and activities of bacteria native to the Fraser River and Georgia Strait, as they are diluted in each others water, in an effort to determine the extent to which the numbers and activities of freshwater and marine bacteria persist in the estuarine environment. Experiments-using membrane diffusion chambers (hereafter referred to as MDCh) were designed to follow changes in salinity, particulate organic carbon and nitrogen, dissolved organic carbon, as well as bacterioplankton biomasses and activities within the contained waters. The results of these membrane diffusion model experiments were compared with observed changes in bacterioplankton activities and numbers which naturally occur within the Fraser River plume.
MATERIALS AND METHODS

Sample collection:

Subsurface (1 to 7 m depth) water samples were taken, using a 6 L Van Dorn sampler, from various locations in the Fraser River, its plume and the Strait of Georgia (which form the Fraser River estuary) and used for experimental purposes within one hour of sampling. Water temperatures and salinities were determined at the time of sampling using a field thermometer and salinometer (Yellow Springs Instrument Co., Yellow Springs OH, model 33 S-C-T) respectively. Figure 1 denotes the approximate position of each sample site and the dates of sampling trips related to Fraser River discharge at Hope, British Columbia. The latitude and longitude of each sample site are presented in Table I.

Membrane diffusion chambers:

Membrane diffusion chambers were constructed from 11 cm I.D glass cylinders of 5 mm wall thickness and stoppered at each end with 0.2 μm pore size Nuclepore membranes (100 mm diameter, Nuclepore Corp., Pleasanton, CA) (Fig. 2). The relatively large pore size of the Nuclepore (0.2 μm) membrane permits diffusion of dissolved substances into or out of chambers (see Lavoie, 1975). Each membrane was held in place with two teflon retainers (see Fig. 2). Access to each chamber's contents was by a rubber serum stoppered 1.5 cm orifice, midway along the cylinder. Each chamber held 3 L of water and since
Figure 1

Chart of Southwestern British Columbia region of investigation; and dates of sampling trips related to Fraser River discharge at Hope, British Columbia.

Sampling stations are noted in chart as:
1, 3, 5, 8, 11, 12.......... Fraser River;
2, 13.......................... vicinity Bowen Island;
4................................. vicinity Nanoose Bay;
6, 9............................... Georgia Strait;
7, 10............................ plume waters of Fraser River.

Trips are noted in hydrograph as:
1.............. Fraser River surface water enclosed in membrane diffusion chambers immersed in the Georgia Strait in February 1979;
2.............. linear transect sampling of the Fraser River estuary in March 1979;
3.............. linear transect sampling of the Fraser River estuary in July 1979;
4.............. Fraser River surface water enclosed in membrane diffusion chambers immersed in the Georgia Strait in September 1979;
5.............. Georgia Strait surface water enclosed in membrane diffusion chambers immersed in the Fraser River in February 1980.
Table I. Date and location of each station sampled.

<table>
<thead>
<tr>
<th>Station number</th>
<th>Sample date</th>
<th>Location</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Temperature (°C)</th>
<th>Salinity (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7/1/79</td>
<td>Fraser River</td>
<td>122° 50' 13&quot; W</td>
<td>49° 08' 58&quot; N</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7/1/79</td>
<td>Bowen Island</td>
<td>123° 23' 52&quot; W</td>
<td>49° 19' 13&quot; N</td>
<td>5-6.5</td>
<td>21-25</td>
</tr>
<tr>
<td>3</td>
<td>12/2/79</td>
<td>Fraser River</td>
<td>122° 42' 50&quot; W</td>
<td>49° 09' 45&quot; N</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>15/2/79</td>
<td>Nanoose Bay</td>
<td>124° 03' 24&quot; W</td>
<td>49° 13' 29&quot; N</td>
<td>6.5-8.5</td>
<td>21-27.5</td>
</tr>
<tr>
<td>5</td>
<td>26/3/79</td>
<td>Fraser River</td>
<td>122° 42' 35&quot; W</td>
<td>49° 09' 36&quot; N</td>
<td>8.5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>26/3/79</td>
<td>Georgia Strait</td>
<td>123° 45' W</td>
<td>49° 13' 03&quot; N</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>26/3/79</td>
<td>Fraser River plume</td>
<td>123° 22' 09&quot; W</td>
<td>49° 07' 31&quot; N</td>
<td>8.5</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>26/3/79</td>
<td>Fraser River plume</td>
<td>123° 22' 09&quot; W</td>
<td>49° 07' 31&quot; N</td>
<td>8.5</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>26/3/79</td>
<td>Fraser River plume</td>
<td>123° 22' 09&quot; W</td>
<td>49° 07' 31&quot; N</td>
<td>8.5</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>17/7/79</td>
<td>Fraser River</td>
<td>122° 40' 16&quot; W</td>
<td>49° 09' 30&quot; N</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>17/7/79</td>
<td>Georgia Strait</td>
<td>123° 30' 54&quot; W</td>
<td>49° 14' 01&quot; N</td>
<td>15.5</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>17/7/79</td>
<td>Fraser River plume</td>
<td>123° 20' 08&quot; W</td>
<td>49° 12' 56&quot; N</td>
<td>15</td>
<td>9</td>
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<td>10</td>
<td>17/7/79</td>
<td>Fraser River plume</td>
<td>123° 20' 08&quot; W</td>
<td>49° 12' 56&quot; N</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>17/7/79</td>
<td>Fraser River plume</td>
<td>123° 20' 08&quot; W</td>
<td>49° 12' 56&quot; N</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>25/9/79</td>
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<td>122° 56' 07&quot; W</td>
<td>49° 08' 15&quot; N</td>
<td>2.5-3.5</td>
<td>0-1</td>
</tr>
<tr>
<td>12</td>
<td>12/2/80</td>
<td>Fraser River</td>
<td>123° 26' 15&quot; W</td>
<td>49° 19' 34&quot; N</td>
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<td>23</td>
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<tr>
<td>13</td>
<td>12/2/80</td>
<td>Bowen Island</td>
<td>123° 26' 15&quot; W</td>
<td>49° 19' 34&quot; N</td>
<td>5</td>
<td>23</td>
</tr>
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</table>
Figure 2

Membrane diffusion chamber apparatus diagram.

Parts are noted in diagram as:
1. ........ glass cylinder;
2. ........ rubber serum;
3, 6. ..... teflon retainers;
4. ........ rubber ring seal;
5. ........ Nuclepore (0.2 μm pore size) membrane.
the total surface area of each membrane was 95.8 cm$^2$ the surface to volume ratio of a filled cylinder was 63.8 cm$^2$/L. Each autoclave sterilized (30 min at 121°C) chamber was filled with 30 μm prefiltered water (to remove zooplankton and larger microplankton) and placed for incubation in situ equilibration water (either Fraser River or Georgia Strait). As equilibration of particles less than 0.2 μm and solutes occurred between the two waters, each membrane become coated with detritus and colonized by microorganisms, particularly bacteria. Hence, membrane filters were replaced at 8 hour intervals.

Assay of naturally occurring nutrients:

Dissolved organic carbon (DOC) was determined using a Beckman IR gas analyzer (Beckman Instruments, Fullerton, CA) and the technique described by Strickland and Parsons (1972). Particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined on a Beckman CNH analyzer (Beckman Instruments, Fullerton, CA) using a dry combustion technique.

Determination of heterotrophic activities:

Glucose heterotrophic activities were determined using the techniques described by Azam and Holm-Hansen (1973) and modified as follows. Aliquots of sample water were aspirated into 10 mL disposable plastic syringes. This was immediately followed by the addition of tritiated glucose (D-6-3H glucose, specific activity 22.5 Ci/mM, Amersham) to yield a set of five
syringes (each in duplicate) containing glucose levels of from 0.004 uCi/mL (2 x 10^{-10} M of glucose/mL) to 0.2 uCi/mL (1.2 x 10^{-8} M glucose/mL), tritiated glucose was diluted prior to use in carbon-free water prepared by the method of Strickland and Parsons (1972). Duplicate live and killed (treated with 0.2 mL of 2% glutaraldehyde) syringes were prepared for each incubation period (uptake versus time) and substrate concentration (Lineweaver-Burke plots). Care was taken to ensure that substrate uptake was linear with time, hence all incubation times (for Lineweaver-Burke plots) were 60 min at ambient water temperature. Following this incubation time the reactions were stopped by adding 0.2 mL of 2% glutaraldehyde to the active water. All samples were then filtered through wetted Millipore filters (0.2 μm pore size) at a vacuum of 0.5 atm, rinsed once with 15 mL of 0°C membrane prefiltered water. Each filter was then placed in a scintillation vial containing 15 mL of Beckman Filter-Solv scintillation cocktail, and counted in a Beckman LSC-8000 scintillation spectrometer. Counts per minute (cpm) were corrected for quench, machine efficiency and half-life decay and reported as disintegrations per minute (dpm). \( V_{\text{max}}, K_t + S_n \) and \( T_t \) calculations were as per Wright and Hobbie (1966).

Determination of actively metabolizing bacteria:

Numbers of actively metabolizing bacteria were determined using the technique developed by Hoppe (1976). An aliquot of sample water was aspirated into a disposable plastic syringe.
This was immediately followed by the addition of radioactively labeled substrate (D-6-3H glucose, 10 Ci/mM, Amersham) into experimental and control syringes (in duplicate) to a final concentration of 1.2 x 10^{-6} mg/L; control syringes were killed with 0.2 mL of 2% glutaraldehyde. Controls were necessary to quantitatively determine non-specific sorption. All samples were incubated for 3 hours at in situ temperature, when the contents of the experimental syringes were killed with 2% glutaraldehyde. Each sample was then filtered through wetted Nuclepore filters (25 mm diameter, 0.2 µm pore size) at 0.2 atm. Following air drying, each filter was fixed to a gummed microscope slide and coated with Kodak NTB-2 emulsion as described by Faust and Correl (1977). After drying, the slides were stored in light-proof boxes at 4°C for 3 to 5 days. After exposure, the emulsion was developed in Dektol developer at 15°C for 2 min, followed by a 10 sec distilled water rinse, 5 min in acid fixer and 5 to 10 min washing in running tap water (Rogers, 1973). After drying the emulsion was cleared for one hour in methylsalicylate and mounted in Permount with a cover slip (Watt, 1971). Loci were counted using a Zeiss phase contrast microscope (Model Standard WL) at nominal 1200x magnification. The number of actively metabolizing bacteria per mL were calculated following Hoppe (1976).

Determination of microbial numbers:

Marine heterotrophic bacterial numbers were determined by spread plating water samples onto bacto peptone (Difco), 2 g;
bacto yeast extract (Difco), 3 g; D-glucose, 2 g; synthetic seawater salts (Rila Marine Mix, Teaneck, NJ), 25 g; distilled water, 1000 mL and the pH adjusted to 7.4 with NaOH (hereafter referred to as marine nutrient media), and incubating these plates at 4, 15, 17 or 22°C (depending upon the temperature closest to the water at the time of sampling) for 7-10 days.

Heterotrophic freshwater bacterial numbers were determined by plating water samples in the above medium without the seawater salts addition (hereafter referred to as freshwater nutrient media) and incubating them under similar temperature and time conditions.

Numbers of bacterioplankton were determined using the acridine orange direct count technique of Hobbie et al. (1977); subsamples for the AODC determinations were concentrated on 25 mm diameter, 0.2 μm pore size irgalan black stained Nuclepore filters using a membrane filter assembly (Millipore, Corp.), at a vacuum head of 150 mm Hg. Each sample filter was placed over an underfilter which had been soaked thoroughly with a surfactant (WAYFOS, Phillip Hunt Corp., East Providence, RI) to prevent clumping of particles (Bowden, 1977); to estimate number of bacteria clumps, control filters were not treated with surfactant. The volume of sample to be filtered was adjusted to give between 15 and 80 bacteria per viewing field (Daley and Hobbie, 1975). After filtration 0.2 mL of a fluorochrome solution (acridine orange, 1:10⁴) was pipetted on to the filter and removed by vacuum after five min; the 0.2 μm membrane filters were rinsed with prefiltered distilled water. The
bacteria on damp filters were viewed and ten randomly selected fields were counted with a Carl-Zeiss epi-illuminated microscope (1200x magnification) using blue light excitation. Details of the method are given in Daley and Hobbie (1975) and Hobbie et al (1977).

To calculate bacterial biomass a conversion factor based on the average size of aquatic bacteria was estimated using scanning electron microscopy. Subsamples were concentrated on 25 mm diameter 0.2 μm Nuclepore filters using a membrane filter assembly (Millipore, Corp.). A small volume of 2% filtered glutaraldehyde (E.M. grade, Polysciences, Inc.) was added to the subsample before it completely passed through the filter. Then each sample filter was placed in an aluminum-foil boat and covered by a clean 0.2 μm Nuclepore filter to reduce the possibility of contamination or loss of bacteria in subsequent steps (Kurtzman et al, 1974). Next, the boats were transferred to a filtered buffer solution of cacodylic acid and HCl (0.2 M) with 5% (wt/vol) sucrose to stabilize cell membranes. The boats were left in this buffer wash for about 24 h . Detailed description of the fixative and buffer are found in Hayat (1976). Residual water in the samples was removed by dehydration in water-acetone and acetone-amyl acetate solutions, followed by critical-point drying with CO₂ (Albrecht et al, 1976). The boats were rinsed for 10 min in solutions of 15, 45, 70, 90 and 100% pure acetone in distilled water, followed by solutions of 15, 45, 70, 90 and 100% amyl acetate in pure acetone. After dehydration the boats were critical-point dried with CO₂. The
boats were disassembled and the dried filters, their cover filters, and blank control filters were mounted using a non-conductive high viscosity adhesive (fast-setting epoxy resin) onto a specimen stub to avoid re-wetting the sample (Hayes and Pawley, 1976). Specimen stubs were mounted in the vacuum coater at an angle and sputtered with gold-palladium alloy (60/40%) in vacuum evaporator rotating while coating to obtain a uniform (100 Å thick) coating. Viewing was performed with a ETEC Autoscan scanning electron microscope at 0° angle and 2000 to 10000x magnification. Estimates of bacterial shapes and sizes were taken from micrographs of ten randomly selected fields on the filters, using Ilford FP4 120 film. Cell volumes were increased by 5% to correct for the 3 to 5% shrinkage caused by glutaraldehyde (Bowden, 1977). Biomass calculation was based on data for bacterial numbers (AODC) and classification with regard to cell lengths (Boyde and Williams, 1971; Zimmermann, 1977).
RESULTS

Linear transect sampling of the Fraser River plume:

Data for numbers of colony forming units (CFU), acridine orange direct counts (AODC) and numbers of actively metabolizing bacteria (NAB) as determined by microautoradiography versus sample salinity (triplicate analysis of duplicate samples of each salinity water) are illustrated in Fig. 3. Total CFU/mL determined using freshwater and marine nutrient media (see materials and methods) decreased as the plume water became more saline. Although the freshwater bacteria outnumbered the marine forms within this estuary, there was a significant shift of mean values (analysis of variance; $F=8.63$, $df=4/10$, $p<.05$) towards a greater proportion of the bacteria being marine at increased plume water salinities. Bacterial numbers, as determined by AODC in samples of plume waters sampled in March and July 1979, showed an approximately two-third reduction as the salinity increased from 7 to 25 °/oo. The NAB/mL fluctuated from $1 \times 10^5$ to $9.5 \times 10^5$. In March 1979, 31% of the bacteria (based in AODC) were actively metabolizing glucose in freshwater (0 °/oo) whereas the percent active bacteria decreased to 8.6% in 25 °/oo salinity water. In July 1979 a significant (analysis of variance; $F=6.01$, $df=4/10$, $p<.05$) reduction of mean values of active bacteria also occurred (34.4% in fresh Fraser River water to 11.1% in surface Georgia Strait water of 25 °/oo salinity).

Data upon the size distribution of the "average" bacterium within plume waters of various salinities are presented in
Figure 3

Bacteria/mL of Fraser River estuary water (1 m depth) sampled along a transect from New Westminster (0 °/oo salinity) to Georgia Strait (25 °/oo salinity) in March 1979 (solid lines) and July 1979 (broken lines). Symbols denote the following; open triangles (AODC), closed triangles (NAB, actively metabolizing bacteria as determined by microautoradiography), open and closed circles refer to CFU based upon nutrient media (see methods) without and with marine salts added respectively.
Table II. Size distribution, biomass of bacteria (mean values, March 1979) and percentage of different cell types on total numbers of bacteria of Fraser River estuary water sampled along a transect from New Westminster (0 °/oo salinity) to Georgia Strait (25 °/oo salinity).

<table>
<thead>
<tr>
<th>Size fractions</th>
<th>Average volume of bacteria</th>
<th>Biomass based in AODC counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cell length μm rods</td>
<td>0.4 0.8 1.2 1.8 &gt;2.4</td>
<td>-</td>
</tr>
<tr>
<td>Mean cell diameter μm cocci</td>
<td>0.3 0.7 - - -</td>
<td>-</td>
</tr>
<tr>
<td>Mean averaged volume μm³</td>
<td>0.018 0.13 0.18 0.29 0.50</td>
<td>μm³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Numbers %</th>
<th>0 °/oo salinity sample</th>
<th>7 °/oo salinity sample</th>
<th>15 °/oo salinity sample</th>
<th>19 °/oo salinity sample</th>
<th>25 °/oo salinity sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 °/oo salinity sample</td>
<td>60.4 28.1 6.2 3.1 2.2</td>
<td>0.080</td>
<td>5.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 °/oo salinity sample</td>
<td>64.1 26.2 5.9 2.3 1.5</td>
<td>0.072</td>
<td>12.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 °/oo salinity sample</td>
<td>62.9 27.7 4.3 3.0 2.1</td>
<td>0.076</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 °/oo salinity sample</td>
<td>66.2 25.3 4.1 2.6 1.8</td>
<td>0.070</td>
<td>6.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °/oo salinity sample</td>
<td>74.2 18.4 3.3 1.9 2.2</td>
<td>0.061</td>
<td>9.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table II, where it is shown that the volume of the "average" bacterium decreased (based on analysis of variance; $F_{4,10}=16.35$, was significant at 5%) in the more saline waters as compared to the lower Fraser River freshwater. In all samples however, a high percentage of the cells were very small "mini" cells. The mean bacterial volume of samples removed from the Fraser River estuary was 0.072 $\mu$m$^3$/cell. Bacterial biomass data are presented in Table II. In the Fraser River estuary the bacterial biomasses sampled during March and July 1979 varied from 5.54 to 17.19 mgC/m$^3$.

Figs. 4 and 5 illustrate the glucose heterotrophic activities of the bacterioplankton within plume waters during the March 1979 sampling trip. Glucose uptake versus time by the bacterioplankton was linear in all instances with the greatest activity found in salinity 15 $^\circ$/oo water and the lowest in the surface (25 $^\circ$/oo salinity) water of the Strait of Georgia adjacent to the plume. Fig. 5 illustrates the glucose heterotrophic activities of three samples of plume water as well as one of each of Fraser River and Georgia Strait fresh and 25 $^\circ$/oo salinity surface waters respectively. Clearly, as the water flows from the Fraser River into the Strait of Georgia, there was increased glucose heterotrophic activities which were maximal in 7-15 $^\circ$/oo salinity water and which decreased to minimal values in surface Strait of Georgia water of 25 $^\circ$/oo salinity. Fig. 6 illustrates the glucose heterotrophic potentials ($V_{\text{max}}$), turnover times ($T_t$) and $K_t+S_n$ values as assayed in duplicate for each of three water samples removed at
Net uptake of $^3$H-glucose as a function of time by the native microflora of Fraser River estuary surface (1 m depth) water sampled along a transect from New Westminster (0 °/oo salinity) to Georgia Strait (25 °/oo salinity) in March 1979. Symbols denote the following; open triangles (0 °/oo salinity water), open circles (7 °/oo salinity water), open squares (15 °/oo salinity water), closed triangles (19 °/oo salinity water) and closed circles (25 °/oo salinity water).
Modified Lineweaver-Burke plot of heterotrophic activity by the native microflora of surface (1 m depth) water of the Fraser River estuary (March 1979) sampled along a transect from New Westminster (0 °/oo salinity) to Georgia Strait (25 °/oo salinity). Symbols denote the following; open triangles (0 °/oo salinity water), open circles (7 °/oo salinity water), open squares (15 °/oo salinity water), closed triangles (19 °/oo salinity water) and closed circles (25 °/oo salinity water); T refers to the incubation time in hours and f to the fraction of available substrate utilized.
Figure 6

Variation of glucose heterotrophic potential ($V_{\text{max}}$) (open circles), $K_{t+S_n}$ values (closed circles) and turnover times ($T_t$) (open triangles) in Fraser River estuary surface (1 m depth) water sampled along a transect from New Westminster (0 °/oo salinity) to Georgia Strait (25 °/oo salinity) in March 1979 versus the sample salinity.
each salinity. This figure clearly shows that $K_{t+S_n}$ values closely followed changes in $V_{\text{max}}$ values. The $V_{\text{max}}$ and $K_{t+S_n}$ ratios of Fraser River (0 $^\circ$/oo) to Strait of Georgia (25 $^\circ$/oo) waters were 2.3 and 2.7:1 respectively with lower ratios noted at intermediate salinities. The lowest ratios were noted at salinity 19 $^\circ$/oo water. The turnover times of glucose by the plume bacterioplankton exhibited a continuous decrease from 0 to 19 $^\circ$/oo salinity water and then increased in 25 $^\circ$/oo salinity water to a level approximately equal to that of the Fraser River freshwater.

When expressed in a per unit biomass (CFU, AODC, NAB) basis glucose heterotrophic potentials for triplicate analysis of duplicate samples at each salinity are illustrated in Fig. 7. Glucose $V_{\text{max}}$/CFU (freshwater medium) indicated a 9.6-fold increase from 0 to 25 $^\circ$/oo salinity water. Whereas the $V_{\text{max}}$/CFU determined using the marine nutrient medium displayed a 2.8-fold significant increase of mean values (analysis of variance; $F=13.6$, df=4/10, $p<.05$) from freshwater to the 25 $^\circ$/oo salinity water during March 1979. Glucose $V_{\text{max}}$/AODC showed a significant decrease of mean values (analysis of variance; $F=10.07$, df=4/10, $p<.05$) of over 75% in 25 $^\circ$/oo salinity water as compared to the Fraser River freshwater. However, $V_{\text{max}}$/AODC values were enhanced at 7 and 15 $^\circ$/oo salinity (Fig. 7) but decreased at 19 $^\circ$/oo. Glucose $V_{\text{max}}$/NAB showed a similar significant pattern (analysis of variance; $F=17.1$, df=4/10, $p<.01$) of enhanced specific activities within 7 and 15 $^\circ$/oo salinity waters as well.

Fig. 8 illustrates significant variations of mean values
Figure 7

Bacterial specific activities of Fraser River estuary water (1 m depth) sampled along a transect from New Westminster (0 °/oo salinity) to Georgia Strait (25 °/oo salinity) in March 1979. Symbols denote the following; closed triangles (cell counts as determined by microautoradiography), open triangles (cells as counted by AODC) and open and closed circles refer to CFU determinations on nutrient media (see methods) without and with marine salts respectively.
Figure 8

DOC (open triangles), POC (open circles) and PON (closed circles) concentrations per mL of Fraser River estuary surface (1 m depth) water sampled along a transect from New Westminster (0 °/oo) to Georgia Strait (25 °/oo) in March 1979 versus the sample salinity.
in POC (analysis of variance; $F=9.15$, $df=4/10$, $p < 0.05$) and PON (analysis of variance; $F=6.71$, $df=4/10$, $p < 0.05$) as well as DOC (analysis of variance; $F=13.98$, $df=4/10$, $p < 0.05$) versus sample salinity (as averages of triplicate assays of two water samples at each salinity). The POC at 25 $^\circ$/oo salinity was 1.6-fold and 1.4-fold that of freshwater, in March and July 1979 respectively, whereas the PON values were 3-fold and 1.8-fold respectively. The DOC at 25 $^\circ$/oo salinity was 1.7-fold and 2.6-fold greater than in freshwater, in March and July 1979 respectively.

Fraser River water contained in membrane diffusion chambers and placed in Georgia Strait:

Fig. 9 illustrates salinity changes inside MDCh containing Fraser River freshwater removed adjacent to New Westminster as it equilibrated with the surrounding Georgia Strait surface (1 m depth) water. During the subsequent 48 h incubation the salinity of the contained water equilibrated to 24 $^\circ$/oo across the 0.2 um pore size Nuclepore membranes.

Data for CFU, AODC and NAB values of native Fraser River bacteria (as averages of triplicate assays of one water sample at each salinity from two MDCh experiments) versus the sample salinity are illustrated in Fig. 10. When assayed using freshwater nutrient media, there was an approximately 98% kill of the contained freshwater viable bacteria (CFU) by the time the salinity reached 24 $^\circ$/oo within the contained water. However, the small proportion (2%) of the salinity tolerant
Salinity equilibration curves of Fraser River surface (1 m depth) water (0 °/oo salinity) (open circles, September 1979 and closed circles, February 1979) enclosed in membrane diffusion chambers immersed in the Georgia Strait surface (1 m depth) water. And salinity equilibration curve of Georgia Strait surface (1 m depth) water (23 °/oo salinity) (open triangles) enclosed in membrane diffusion chambers immersed in the Fraser River in February 1980.
Changes in bacterial numbers of Fraser River freshwater enclosed in membrane diffusion chambers immersed in surface (1 m depth) Georgia Strait saline water as salinity equilibration occurs (solid lines, February 1979 and broken lines, September 1979). Symbols denote the following; open triangles (cells as counted by AODC), closed triangles (cell counts as determined by microautoradiography) and open and closed circles refer to CFU determinations on nutrient media (see methods) without and with marine salts addition respectively.
Sample salinity (‰) vs. Bacteria/mL

- Sample salinity values: 0, 8, 16, 24
- Bacteria/mL values: 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7
bacteria which grew on marine nutrient medium remained at approximately $10^3$ cells/mL during this equilibration period. By the time the salinity equilibrated to 24 $\%$ the contained seawater tolerant marine bacteria outnumbered the freshwater cells. Based upon total CFU (bacterial counts on freshwater and marine nutrient media) present in freshwater and after equilibration to 24 $\%$ salinity, there was a 94% decrease in numbers of viable Fraser River bacterioplankton in September, 1979. The AODC and NAB assays were also indicative of injury to the contained Fraser River bacterioplankton. Although the AODC decreased in one experiment but increased slightly in the other there was a 73% and 32% significant reduction of mean values (analysis of variance; $F=8.17$, $df=4/10$, $p<.05$) in number of active glucose metabolizing bacteria.

Data on average volume of bacteria are presented in Table III. The average volume of bacteria diminished (based on analysis of variance; $F_{4,10}=12.2$, was significant at 5%) with increasing salinity. The mean volume of a bacterial cell for samples from Fraser River water incubated inside MDCh was 0.075 $\mu m^3$. The "average" bacterial shape changed from bacillus at 0 $\%$ salinity to short rods and a population dominated by very small bacteria at 20 $\%$ salinity. In freshwater many attached forms (clumps) were found and when the salinity reached 20 $\%$ there were fewer attached forms and large numbers of unattached forms. Although the free cells of the bacterioplankton had a small average volume, the small cells outnumbered the larger cells 8-fold at salinity 20 $\%$ and 2-fold in freshwater that
Table III. Bacterial shape, size distribution and percentage of different cell types on total numbers of bacteria in Fraser River surface (1 m depth) freshwater enclosed in membrane diffusion chambers immersed in Georgia Strait in February 1979.

<table>
<thead>
<tr>
<th>Mean cell length μm rods</th>
<th>Size fractions</th>
<th>Average volume of bacterial numbers (AODC) μm³</th>
<th>Cell shape distribution in % of direct counts cocci and very small rods attached bacteria rods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cell diameter μm cocci</td>
<td>0.4 0.8 1.2 1.8 &gt;2.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean averaged volume μm³</td>
<td>0.018 0.13 0.18 0.29 0.50</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Numbers %</th>
<th>0 °/oo salinity sample</th>
<th>9 °/oo salinity sample</th>
<th>15 °/oo salinity sample</th>
<th>19 °/oo salinity sample</th>
<th>24 °/oo salinity sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>61.0 25.3 7.9 3.1 2.7</td>
<td>0.082 19.7 38.2 42.1</td>
<td>61.2 27.7 6.1 2.7 2.3</td>
<td>0.079 28.1 41.7 30.2</td>
<td>63.3 25.2 5.6 3.3 2.6</td>
<td>0.078 22.2 57.6 20.2</td>
</tr>
<tr>
<td>65.4 25.3 5.9 1.6 1.8</td>
<td>0.070 17.3 68.8 13.9</td>
<td>68.9 24.0 4.2 1.3 1.6</td>
<td>0.064 10.8 80.0 9.2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

28
was contained in the MDCh. Numbers of very small bacteria and cocci increased with salinity. Bacterial cells within and on detritus were somewhat larger than free bacteria and thus made a larger contribution to the total biomass. The bacterial biomass represented from 11.4 mgC/m$^3$ to 63.6 mgC/m$^3$ in Fraser River water contained in MDCh during salinity equilibration.

Fig. 11 illustrates the linear glucose uptake as a function of time by bacterioplankton in Fraser River water contained in MDCh and incubated in Georgia Strait in February 1979. The linear uptake of glucose decreased with increased salinity equilibration. Fig. 12 demonstrates that the heterotrophic activities of the contained Fraser River bacterioplankton are rapidly destroyed by increased salinity inside the chambers.

Data for DOC, POC and PON concentrations, in Fraser River water contained in MDCh, versus the sample salinity are illustrated in Fig. 13 for triplicate assays of one water sample at each salinity. POC exhibited 1.6 and 1.7-fold increases from the start to the end of the equilibration during February and September 1979 respectively. PON decreased during equilibration in September, but in February initial and final values were equal. DOC values displayed an increase in both dates, of 1.4-fold in February and 1.2-fold in September 1979.

Georgia Strait water contained in membrane diffusion chambers and placed in the Fraser River:

Data for salinity changes inside MDCh containing Georgia
Figure 11

Net uptake of $^3$H-glucose as a function of time by the native microflora of Fraser River freshwater enclosed in membrane diffusion chambers immersed in the Georgia Strait as salinity equilibration occurs (February 1979). Symbols denote the following; open triangles (0 °/oo salinity water), open circles (9 °/oo salinity water), open squares (13 °/oo salinity water), closed triangles (15 °/oo salinity water) and closed circles (20 °/oo salinity water).
Figure 12

Modified Lineweaver-Burke plot of heterotrophic activity by the microflora of Fraser River freshwater enclosed in membrane diffusion chambers immersed in the Georgia Strait as salinity equilibration occurs (February 1979). Symbols denote the following; open triangles (0 °/oo salinity water), open circles (12 °/oo salinity water), open squares (17.5 °/oo salinity water) and closed triangles (22 °/oo salinity water); T refers to the incubation time in hours and f to the fraction of available substrate utilized.
$^3$H-glucose added ($10^{-9}$ M)

$T/f$
Changes in DOC (open triangles), POC (open circles) and PON (closed circles) concentrations per mL in Fraser River surface (1 m depth) freshwater (February 1979 solid lines, September 1979 broken lines) enclosed in membrane diffusion chambers immersed in the Georgia Strait as salinity equilibration occurs.
Sample salinity (%₀)

DOC (mg/L)
Strait surface water (1 m depth) of salinity 23 °/oo as it equilibrated with the surrounding Fraser River surface (1 m depth) freshwater are illustrated in Fig. 9. During the 55 h incubation the salinity of the contained water equilibrated to 2 °/oo across the 0.2 μm Nuclepore membranes.

Data for CFU, AODC and NAB values of native Georgia Strait bacteria versus the sample salinity are illustrated in Fig. 14, triplicate assays of two subsamples were performed. As expected viable marine bacterioplankton (grown on marine nutrient media) greatly outnumbered the viable freshwater bacteria (grown on freshwater nutrient media) which exhibited a 2-fold significant increase (analysis of variance; F=11.07, df=4/10, p<.05) in numbers with salinity equilibration to 2 °/oo. Little apparent effect upon AODC cell numbers/mL was noted (Fig. 14). As salinity decreased NAB/mL also decreased (based on analysis of variance; F4,10 =15.24, was significant at 1%). The bacterial biomass represented from 9.5 mgC/m³ to 15.2 mgC/m³ in Georgia Strait water contained in MDCh during salinity equilibration.

Fig. 15 illustrates modified Lineweaver-Burke plots of contained Georgia Strait bacterioplankton subjected to analysis by the heterotrophic potential technique; triplicate assays of two subsamples of each salinity were performed. In Fig. 16 the heterotrophic potential (Vmax), as total activity, of contained Georgia Strait water bacterioplankton decreased 45% with reduced salinity whereas, the Kt+S values increased by 30% from 23 °/oo to 2 °/oo salinity water. Fig. 16 also illustrates an increase (2.3-fold) of turnover time (Tt) of glucose by the Georgia
Figure 14

Changes in bacterial numbers of surface (1 m depth) Georgia Strait 23 °/oo salinity water enclosed in membrane diffusion chambers immersed in the surface of the Fraser River as salinity equilibration occurs (February 1980). Symbols denote the following; open triangles (acridine orange direct counts), closed triangles (NAB as determined by microautoradiography), open and closed circles refer to CFU/mL counted on nutrient media (see methods) without and with marine salts addition respectively.
Sample salinity (%o)

Bacteria/mL

Sample salinity (%o)
Modified Lineweaver-Burke plot of heterotrophic activity by the native microflora of Georgia Strait surface (1 m depth) water (23 °/oo salinity) enclosed in membrane diffusion chambers immersed in the Fraser River as salinity equilibration occurs (February 1980). Symbols denote the following; open circles (23 °/oo salinity water), open triangles (18 °/oo salinity water), open squares (11 °/oo salinity water), closed triangles (6 °/oo salinity water) and closed circles (2 °/oo salinity water); T refers to the incubation time in hours and f to the fraction of available substrate utilized.
$\text{3H-glucose added } \times 10^{-9} \text{ M}$
Figure 16

Variation of glucose heterotrophic potential ($V_{\text{max}}$) (open circles), $K_t + S_n$ values (closed circles) and turnover times ($T_t$) (open triangles) in Georgia Strait surface (1 m depth) water enclosed in membrane diffusion chambers immersed in the Fraser River as salinity equilibration occurs.
Strait microflora contained in MDCh.

Data for heterotrophic activity per unit of biomass (CFU, AODC, NAB basis) for Georgia Strait bacteria contained in MDCh versus the sample salinity are illustrated in Fig. 17. The heterotrophic activity per CFU decreased for viable bacteria grown on both freshwater and marine nutrient media, the heterotrophic activity per AODC also decreased as salinity equilibrated from 23 °/oo to 2 °/oo. The glucose activity per NAB showed an increase with salinity reduction.

Data for DOC, POC and PON concentrations in Georgia Strait water contained in MDCh, versus the sample salinity are illustrated in Fig. 18; triplicate assays of two subsamples of each salinity water were performed. POC exhibited a significant increase (analysis of variance; F=9.05, df=4/10, p<.05) whereas, DOC (analysis of variance; F=7.9, df=4/10, p<.05) and PON (analysis of variance; F=6.77, df=4/10, p<.05) exhibited significant decrease as salinity equilibrated to 2 °/oo.
Figure 17

Bacterial specific activities of Georgia Strait surface (1 m depth) water (23 °/oo salinity) enclosed in membrane diffusion chambers immersed in the Fraser River (at 1 m depth) as salinity equilibration occurs (February 1980). Symbols denote the following; open triangles (cells as counted by AODC), closed triangles (cell counts as determined by microautoradiography), and open and closed circles refer to CFU determinations on nutrient media without and with marine salts (see methods) respectively.
Sample salinity (%o) vs. µg glucose/h.

The graph shows the relationship between sample salinity and the rate of glucose consumption. The salinity levels range from 23% to 2%, with corresponding glucose consumption rates ranging from $10^{-10}$ to $10^{-4}$. The data points are indicated with error bars, suggesting variability or uncertainty in the measurements.
Changes in DOC (open triangles), POC (open circles) and PON (closed circles) concentrations per mL in Georgia Strait surface (1 m depth) water enclosed in membrane diffusion chambers immersed in the Fraser River (at 1 m depth) as salinity equilibration occurs.
DISCUSSION

A relatively high percentage of very small cells was found in the Fraser River estuary (Table II) when linear transects from 0 °/oo salinity freshwater to 24 °/oo salinity seawater, 1 m depth, were done. However, because of the limited resolution of light microscopy, the precise form of these cells could not be accurately determined; therefore, rods and cocci of this size range were counted together when using fluorescent microscopy (AODC). Several investigators (Wiebe et al, 1972; Ferguson and Rublee, 1976; Zimmermann, 1977) point out the presence of very small (0.3 um diameter) "coccoidal" forms when viewed with epifluorescence (AODC). With the use of scanning electron microscopy it was possible to confirm that most of the extremely small bacteria found in the Fraser River, its plume and Georgia Strait water samples at the 1 m depth were short rods rather than cocci, an observation which has also been noted by Watson et al (1977) and Zimmermann (1977) for certain American and European coastal waters respectively.

A shift in mean bacterial sizes occurred in the Fraser River estuary from freshwater towards Georgia Strait surface water (24 °/oo salinity) (Table II). In river water of 0 °/oo salinity a much greater proportion of these bacterial cells had mean volumes ≥0.13≤0.50 um³ as compared to those of Georgia Strait surface water where the majority of the bacteria had volumes ≤0.13 um³. This increase in the proportion of small bacterioplankton which occurred when the freshwater of the
Fraser River mixed with the seawater of Georgia Strait may be due to several factors, including: death of certain cells; growth and cell division of a portion of the fresh and/or 24 °/oo salinity water bacterioplankton in the plume (≥1<21 °/oo salinity) waters; an alteration in bacterial morphologies and conservative mixing of the bacterioplankton of one water with the other.

The average bacterial volume was used to calculate total bacterial biomass (Table II) with the following assumptions: bacterial density of 1.07 g/cm³ (Lamanna et al., 1973; Doetsh and Cook, 1973), dry weight to wet weight ratio of 0.22 (Jordan, 1919; Roberts et al., 1957; Luria, 1960; Lamanna and Mallete, 1965) and carbon to dry weight ratio of 0.50 (Luria, 1960). The average bacterial volume from the lower Fraser River water samples was found to be 0.072 μm³, a magnitude which is in agreement with the findings of Ferguson and Rublee (1976) and Zimmermann (1977). The slight discrepancy between the mean volume measured by these authors and that found in the present research may be due to different sample locations as well as differences in techniques. Parsons et al. (1969) from values obtained in February to May 1967 estimated planktonic carbon (mgC/m³) to average 180 mgC/m³ in the Fraser River plume. Based on Parsons et al. (1969) values it can be assumed that in the Fraser River plume the bacterial biomass represents a slight proportion (3.5% to 9.5%) of the planktonic standing stock. Zimmermann (1975) found, in the Kiel area, that the bacterial biomass represents only a small proportion (0.8% to 4.4%) of the...
plankton standing stock whereas in upwelling areas, which are rich in nutrients Watson (1975) found that bacterial biomass contribute significantly to planktonic standing stock.

Several transects were done from 0 °/oo salinity water (freshwater in the vicinity of New Westminster) to water of approximately 24 °/oo salinity (Strait of Georgia) assaying bacterial concentrations and glucose heterotrophic potentials. CFU counts (Fig. 3) indicated an increase in viable bacterial concentrations in the plume waters as compared to either parent water (plate counting greatly underestimates bacterial numbers since only a small fraction of total viable cells will grow on any single nutrient medium), and the curve for March 1979 indicated a slight increase in NAB and AODC (Fig. 3) in plume waters as compared to the Fraser River water (with the AODC technique is not possible to differentiate between live and dead bacteria). The data for the July 1979 cruise indicated conservative mixing of the two parent waters with regard to NAB and AODC. A similar pattern, of conservative mixing of river water with seawater was noted in the Newport River estuary by Palumbo and Ferguson (1978). The behaviour of glucose heterotrophic activities was different than that observed with NAB and AODC. Glucose heterotrophic potentials showed a marked stimulation in plume waters (Figs. 4 and 5). In this instance (March 1979) a glucose $V_{max}$ value of $5.1 \times 10^{-1}$ μg/L/h of the cells in 0 °/oo salinity water increased by 15.3 and 6.7-fold to 7.8 and 4.5 μg/L/h respectively in 7 and 15 °/oo salinity water. However at 19 °/oo salinity a sharp decrease in glucose $V_{max}$
(1.7 x 10^{-1} \, \mu g/L/h) was noted with a potential of 2.2 x 10^{-1} \, \mu g/L/h observed for the 24 \, ^o/oo salinity water. Environmental conditions for these bacterioplankton may in fact be more favourable within plume waters of 5 to 15 \, ^o/oo salinity as is indicated by the data in Fig. 7.

Based on CFU, NAB and AODC the glucose specific activities (calculated as described by Wright, 1978) increased within plume waters as compared to either parent water. Wright (1978) noted a similar occurrence for water collected (from 1 m depth) along a transect within the River Essex estuary of the United States east coast. Although the bacterial concentrations (AODC) decreased from 28 \, ^o/oo to 33 \, ^o/oo salinity water the greatest specific activities were noted between the estuary entrance and offshore. Although Wright (1978) indicates that these changes may be related to nutritive quality of the water the precise reasons for the stimulation in specific activity are not yet clear.

The results in Figs. 3 to 7 indicate that since the standing stocks of bacterioplankton in these plume waters do not substantially increase, the stimulation in glucose heterotrophic potentials may be a reflection of more abundant nutrient levels in plume waters. The observed increases in glucose K_{t+S_n} values may be caused by increases in the transport constant (K_t), the natural substrate concentration (S_n), or by simultaneous variations of both parameters (Gocke, 1977). Since high concentrations of dissolved organic compounds tend to increase V_{max} values, the observed parallel increase in maximum uptake
velocities and the $K_t + S_n$ values for glucose (Fig. 6) could be due to higher concentrations of this compound. However, DOC concentrations were greater in surface (1 m depth) Georgia Strait water than in plume water (Fig. 8). It is also possible that when increases of $V_{\text{max}}$ were observed distinct bacterial groups with high transport constants dominated the uptake of glucose. This suggests that it was the uptake constant ($K_t$) which was varying and carbon substrate was not the limiting nutrient factor for the bacterioplankton activities. Therefore the plume water may be more sufficient in nutritive components (other than organic carbon) than either parent water.

POC production was not found to be related to accelerated heterotrophic activity (Figs. 6 and 8). That the nutrients exported by rivers tend to be largely utilized in estuaries is reflected in Fig. 8. Since $C/N$ ratios are less than 17:1 the particulate fraction appears to be suitable as zooplankton feed. These nutritive values increase within the lower portion of the Fraser River plume. The mean $C/N$ values for March 1979 linear transect were 7.1 and 3.8 at waters of salinity 0 and 24 °/oo respectively.

A membrane diffusion chamber apparatus was developed to test the proposed possibilities (bacterial death, growth and cell division, alteration in bacterial morphologies and conservative mixing of the bacterioplankton of both parent waters) for the increase in the proportion of small bacterioplankton that occurs when freshwater mixes with seawater in the Fraser River estuary. It was additionally used to follow
changes in bacterial numbers and activities of bacterioplankton from both parent waters as freshwater and seawater mixing takes place.

When 3 L of Fraser River freshwater (0 °/oo salinity) were placed in membrane diffusion chambers which were themselves then placed in Georgia Strait surface water (1 m depth) of salinity 25 °/oo the salinity of the contained water equilibrated to 24 °/oo in 48 h (Fig. 9) during which time there was a decrease of approximately 94% of the contained viable bacteria (Fig. 10). AODC (September 1979) and NAB assays were also indicative of injury (Fig. 10) but it should be stressed that such damaged cells are not necessarily killed or destroyed. According to Sieburth (1978) the epibacteria apparently use the polymeric substances associated with organic debris and are inactive transients when free in water. Therefore the decrease in the number of active and/or viable bacteria could well be related to the decrease in the number of attached forms (Table III). Glucose heterotrophic activities of the contained Fraser River bacteria were disrupted (Fig. 12) soon after saline Georgia Strait water mixed into the Fraser River freshwater. Glucose uptake versus time plots of the contained and equilibrating bacteria remained linear, but this activity decreased with increasing salinity until by 20 °/oo salinity this activity also ceased (Fig. 11). The observed stimulation in glucose heterotrophic potentials for the Fraser River plume mixed population was not observed when Fraser River bacterioplankton was contained in the membrane diffusion chambers; this
population was inhibited when equilibrating with Georgia Strait water which may not have the components necessary for in situ stimulation in plume waters. The slight increase in AODC (Fig. 10) noted in February 1979 may have been due to cell division of the salinity tolerant bacteria. A reduction in average volume of the contained bacterioplankton (Table III) suggests alteration in bacterial morphologies with changing salinity.

Based on these results there appears to be two types of bacterioplankton populations within the freshwater of the lower Fraser River. One population, which is the more numerous, appears to be rapidly inactivated within the seawater of Georgia Strait at salinities greater than approximately 16 ‰. The second and less numerous bacterioplankton population appears to remain viable within the Georgia Strait surface water. However, whether or not they are active in Georgia Strait, is speculative.

When Georgia Strait surface water (1 m depth) of salinity 23 ‰ was placed in sterile membrane diffusion chambers which were then placed in Fraser River surface (1 m depth) freshwater, little evident effect on numbers of viable and total bacteria (AODC) was noted (Fig. 14). A sewage treatment plant and its associated outfall was 2 km south of the area where membrane diffusion chambers were incubated; however, this probably did not influence the observed results. Weiland et al. (1979) noted a similar event in South Carolina salt-marsh creeks where the penetration of freshwater had little effect on the total level
of microbial biomass. As the salinity decreased in the contained Georgia Strait water, glucose heterotrophic activities were not destroyed, although they decreased (Fig. 15). The numbers of actively metabolizing bacteria (NAB, Fig. 14) and glucose $V_{\text{max}}$ (Fig. 16) presented a similar decrease; but, the activity per NAB (Fig. 17) exhibited an increase which may be due to changes in the physiological state of the bacterial populations.

In summary, deactivation appears to occur to the majority of the Fraser River bacterioplankton in saline (>20 °/oo) Georgia Strait water; and the bacterioplankton of Georgia Strait surface water mix conservatively into Fraser River water. In connection with this point, MacLeod and Onofrey (1956) noted that bacteria isolated from Georgia Strait, grew at greater rates when half-strength seawater medium was used; however, the complete absence of cations such as Na$^+$ prevented growth. Because under natural conditions in the Fraser River plume (>1<21 °/oo salinity) water it is improbable that the salinity of the water would reach 0 °/oo it is possible that most of the Georgia Strait bacteria are not killed but remain with lowered activities.
CONCLUSIONS

This study has shown that, as Fraser River water enters Georgia Strait the bacterioplankton change from a high percentage of rods associated with particulate matter to small and unattached short rods. In addition, membrane diffusion chamber experiments indicate that deactivation of the majority of freshwater bacterioplankton of lower Fraser River probably occurs when these bacteria enter the saline water of Georgia Strait. This inhibitory influence of Georgia Strait water is greater at higher salinities. These dead or inactive Fraser River bacterioplankton then contribute to the particulate and dissolved organic matter pool, especially in plume waters. However, the native bacteria of Georgia Strait surface water seem to remain viable in plume waters with reduced activities.
LITERATURE CITED


