APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1986, p. 614–621
0099-2240/86/030614-08$02.00/0

Vol. 51, No. 3

Attached and Free-Floating Bacterioplankton in Howe Sound, British Columbia, a Coastal Marine Fjord-Embayment

L. J. ALBRIGHT,* S. K. McCRAE, AND B. E. MAY

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

Received 9 August 1985/Accepted 5 December 1985

Factors which influence the attachment of bacterioplankton to particles (including phytoplankton) were investigated by using (i) water samples removed from a coastal temperate fjord over an annual cycle and (ii) unialgal cultures of *Prorocentrum minimum*, *Dunaliella tertiolecta*, and *Skeletonema costatum*. Silt and salinity levels in this fjord seawater did not appear to influence bacterial attachment, but the percent attached bacteria was inversely related to both chlorophyll *a* concentrations and primary productivities. During periods of high primary productivities the percent attached bacteria was low, whereas during periods of low, increasing, and declining primary productivities the percent attached bacteria was high. A similar pattern of bacterial attachment was observed when the three phytoplankton were grown as batch cultures. The percent attached bacterial numbers increased upon the initiation of algal growth and after these cells stopped growing, but not while the algae were growing. We suggest that a major factor influencing the attachment of bacterioplankton is the physiological condition of their major nutrient source, the phytoplankton; mainly free-living bacteria are associated with growing phytoplankton, whereas a much greater proportion of the bacteria are attached among senescent phytoplankton populations.

Many investigators have noted that bacteria in marine and fresh waters occur as both free-living and particle-attached cells (1–3, 7, 19). Although the numbers and the proportion of bacteria which are attached tend to vary from one aquatic system to another, in general the pattern has been for free-living bacteria to predominate in open ocean surface waters (11, 22, 26), whereas in both fresh and estuarine waters the degree of bacterial attachment varies considerably (2, 5, 12, 13).

Bell and Albright (2) examined several characteristics (i.e., free and attached bacterial numbers, total particulates, chlorophyll *a* [chl *a*], particulate organic carbon, temperature, salinity, and [glucose and amino acid] heterotrophic activities) of free-floating and attached bacteria of 44 diverse freshwater and seawater environments in an attempt to determine the factors which significantly influence cell attachment in situ. Cluster analyses of their data indicated that of the factors examined three explained a significant amount (ca. 54%) of the total sample variance. These factors were (i) salinity (for the marine samples), (ii) particulate load (i.e., seston, particulate carbon, and silt), and (iii) glucose and amino acid heterotrophic uptake.

Knowledge that these three factors were significantly correlated with attachment of bacterioplankton to particles in natural waters encouraged us to further examine this problem. We therefore selected a coastal seawater site where the two features of salinity and particulate (both organic [mainly phytoplankton] and inorganic [mainly silt]) loads vary considerably with time. Such a site was in Howe Sound, a temperate fjord-embayment on the west coast of Canada where an annual spring-summer phytoplankton bloom occurs (24). The salinity and silt content of the surface water of this estuary are greatly influenced over an annual cycle mainly by Squamish River flow. This river, which enters Howe Sound at its head, is a good example of a heavily silt-laden west coast Canadian river with maximum flow during the freshet months of May through July. A salinity gradient occurs from surface to deeper water in this fjord-embayment throughout the year. The sampling program was designed to analyze water removed from several depths in Howe Sound over an annual cycle.

* Corresponding author.

MATERIALS AND METHODS

Howe Sound, which is an inlet contiguous with the Strait of Georgia, is a combined fjord-embayment. The fjord portion extends from Anvil Island north to the Squamish River (Fig. 1). On the southern end of this fjord portion a sill of between 30 and 70 m depth occurs. This results in an inner basin to the north with a maximum depth of ca. 280 m. The deeper water of this basin tends to be poorly mixed for much of the year (20). The surface water (0 to 10 m approximately) is better mixed, with the main forcing factors being the Squamish River discharge and tidal currents. During periods of high river discharge the silt content of the river water is quite high. Water samples were retrieved from each of stations 1, 2, and 3 (at approximately 49° 35′ N, 123° 15′ W) within the basin to the north of the sill (Fig. 1) at depths of 1, 5, 10, 20, 40, 75, 150, and 250 m by using a 5-liter Van Dorn sampler. The depth of 1% of the surface light was usually at 30 m; hence the 0 to 30 m water column was considered to be the euphotic zone. Sampling days were as follows: 1, 13, and 27 March (days 1, 13, and 27, respectively), 3 and 19 April (days 34 and 50), 7 and 23 May (days 67 and 84), 10 and 24 July (days 132 and 146), 8 and 21 August (days 162 and 174), 4 September (day 188), 3 and 15 October (days 217 and 229), and 19 December (day 294), 1984; 21 January (day 327), 5 and 19 February (days 342 and 356), 5 March (day 370), 24 April (day 420), and 15 May (day 441), 1985.

Silt concentrations were determined by filtering 1-liter quantities of water through precombusted (500°C) and tared 47-mm-diameter Whatman GF/C glass fiber filters. Each filter was then combusted at 500°C for 24 h and reweighed. The weight difference was considered to be silt.

Salinities were determined with the use of a Yellow Springs Instruments model S-C-T 33 salinometer.

Water samples (usually 1 liter) to be analyzed for chl *a*

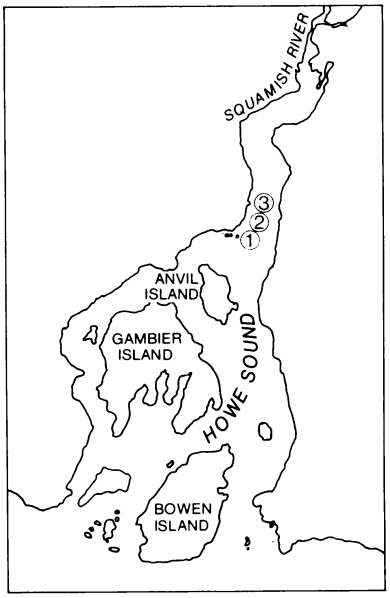

FIG. 1. Chart of Howe Sound showing sampling stations 1, 2, and 3.

were filtered through 0.45-μm nominal pore size cellulose nitrate filters (Millipore Corp.) or Whatman GF/C glass fiber filters (47-mm diameter). After filtration each filter was wrapped in aluminum foil and frozen at −15°C until assayed (within 1 week) by the spectrophotometric assay of Strickland and Parsons (25) after acetone extraction.

Primary productivities were determined by a modified technique of Steemann Nielsen (23). Water samples were removed from depths of 1, 5, 10, 20, and 30 m (usually) and placed in 300-ml bottles (two light and two dark for each sample). Each of the bottle contents was then treated with 60 μCi of $Na_2{}^{14}CO_3$ (specific activity, 8.4 Ci M mol^{-1}), mixed, and stoppered. The bottles were then replaced at the depth from which the water was removed for 3 to 4 h of incubation between 9:00 a.m. and 3:00 p.m. Reactions were stopped by filtering the contents of each bottle through a 0.45-μm nominal pore size cellulose nitrate filter. The radioactivity retained on each filter was then determined by using a Beckman LS8000 scintillation spectrometer and the external standards ratio method for quench correction.

Free bacterial numbers were determined by filtering a portion of the water sample previously preserved at the time of sampling by adding 3.7% (final concentration) formaldehyde through a 2-μm pore size polycarbonate (Nuclepore) 25-mm diameter filter. The bacteria in the filtrate were considered to be free and unattached. These were stained with acridine orange, filtered onto 0.2-μm pore size Nuclepore filters and counted by using epifluorescent microscopy (15). Total bacteria were determined by treating a portion of the formaldehyde-preserved water sample with 0.002 M (final concentration) $Na_4P_2O_7$ for 20 min, and then sonicating it for 20 s at 100 W. This technique deflocculates and disperses attached bacteria (i.e., cells which cannot pass through a 2-μm pore) evenly through the suspension medium (Velji, M.Sc. thesis, Simon Fraser University, 1984). The dispersed bacteria were then stained and counted as described above. Attached bacteria were determined as the difference between the total and free bacterial counts.

Unialgal cultures of *Prorocentrum minimum* (Pavillard) Schiller, *Dunaliella tertiolecta* (Butcher), and *Skeletonema costatum* (Greville) Cleve were obtained from the North East Pacific Culture Collection, University of British Columbia. These cultures were maintained at 15°C and a light intensity of 300 microeinsteins per square meter per day and a regime of 18 h light and 6 h dark. Batch cultures were prepared by inoculating 1 liter of a defined growth medium (14) contained in a 2-liter Fernbach flask with 10 ml of a 5-day culture. Each culture was then grown at a shaking rate of 30 strokes per min at the temperature and light conditions described above.

RESULTS

The mean silt content of the water at stations 1, 2, and 3 in Howe Sound tended to be quite variable from one sampling trip to the next. Nevertheless, imposed on this short-term variability was a discernible annual cycle in silt concentrations (Fig. 2). In most cases silt concentrations were relatively uniform throughout the water column for any one sampling period. Silt levels were quite high in October 1984, probably due to Squamish River flow. This peak in silt content coincided with a marked decrease in primary production and rise in percent attached bacteria (see Fig. 5 and 6). The water column silt levels then greatly decreased through late October and November and increased again in December 1984 (Fig. 2). The December increase in silt levels was not associated with an increase in percent attached bacteria. Water column silt levels again gradually increased from January 1985 through to the Squamish River freshet of May through June 1985. During this time a variable portion of the bacteria was attached. Silt concentrations in both the euphotic and aphotic (30- to 250-m) zones (Fig. 2) showed correlation coefficients with percent attached bacteria (see Fig. 6) of 0.60 (where $\alpha = 0.05$ and $n = 13$) and 0.22 (where $\alpha > 0.9$ and $n = 13$), respectively.

The mean salinities of water removed from 150- and 250-m depths at the three stations in Howe Sound over the 24 sampling trips varied over a rather narrow range of 27.5 to 31.3‰ whereas water removed from the euphotic zone ranged in salinity from 4.5 to 28.3‰. The lowest surface water salinities measured at these stations (Fig. 3) coincided with high Squamish River flows of early summer and fall. There was no significant correlation between euphotic and aphotic water salinity (Fig. 3) and percent attached bacteria (see Fig. 6) (r values of 0.28 and −0.37, respectively).

Mean chl *a* concentrations within the euphotic zone were low in late fall and early winter. Beginning in January, chl *a* concentrations increased to between ca. 2.0 and 6.1 mg m^{-3} through late summer (Fig. 4). There was a negative correlation between these euphotic zone mean chl *a* concentrations and the percent attached bacteria (Fig. 4) ($r = -0.60$, where $\alpha = 0.01$ and $n = 19$).

Primary production occurred during most of the year in the euphotic zone at stations 1, 2, and 3 (Fig. 5). This production was low during October through December 1984, but the phytoplankton again became quite active in January 1985. In 1984 and 1985 a vernal phytoplankton bloom occurred between January and March (Fig. 4 and 5). Phytoplankton production then decreased in April and May (perhaps due to nutrient limitation). The highest primary production values were noted each year during the summer months of June through August (Fig. 5). Primary production decreased suddenly in mid-October 1984 (perhaps due to water turnover).

A significant portion of the euphotic zone bacteria became attached at approximately the same time that primary production increased in January 1985. Most of the aphotic zone

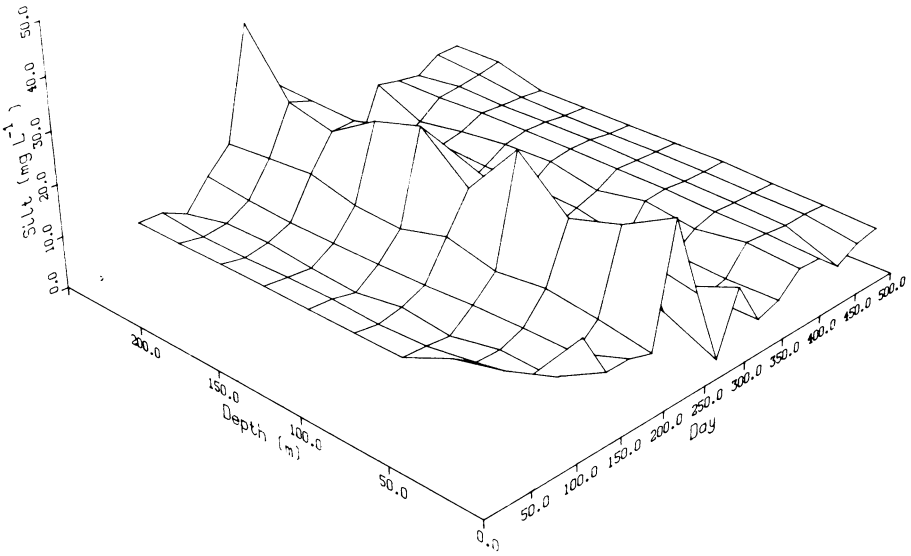

FIG. 2. Mean silt concentrations through the 250-m-deep water column at stations 1, 2, and 3 in Howe Sound as sampled at various times. For dates corresponding to day numbers, see Materials and Methods.

bacteria remained unattached (Fig. 5 and 6). During February 1985 and March of each year the proportion of the bacteria which were attached gradually increased throughout the water column. In late spring after the decrease in the vernal phytoplankton blooms the proportions of the bacteria which were attached also initially increased (Fig. 5 and 6). During the most productive period of each year (June through August) the portion of bacteria which were attached was low. The marked and sudden decrease in phytoplankton production which occurred in early fall was immediately followed by a very high portion of the bacteria becoming attached, particularly in the euphotic zone. With time this high proportion of attached bacteria occurred throughout the 250-m water column. By late winter there was only significant attachment of bacteria in water removed from the euphotic zone (Fig. 5 and 6).

The observed associations of the alga *D. tertiolecta* with bacteria in the culture were as follows. (i) During growth of this phytoplankter, bacteria were present as both free and attached cells. These attached bacteria were not adhered to the algal cells, but appeared to be enmeshed in a sheetlike organic matrix (Fig. 7). (ii) When this algal culture entered

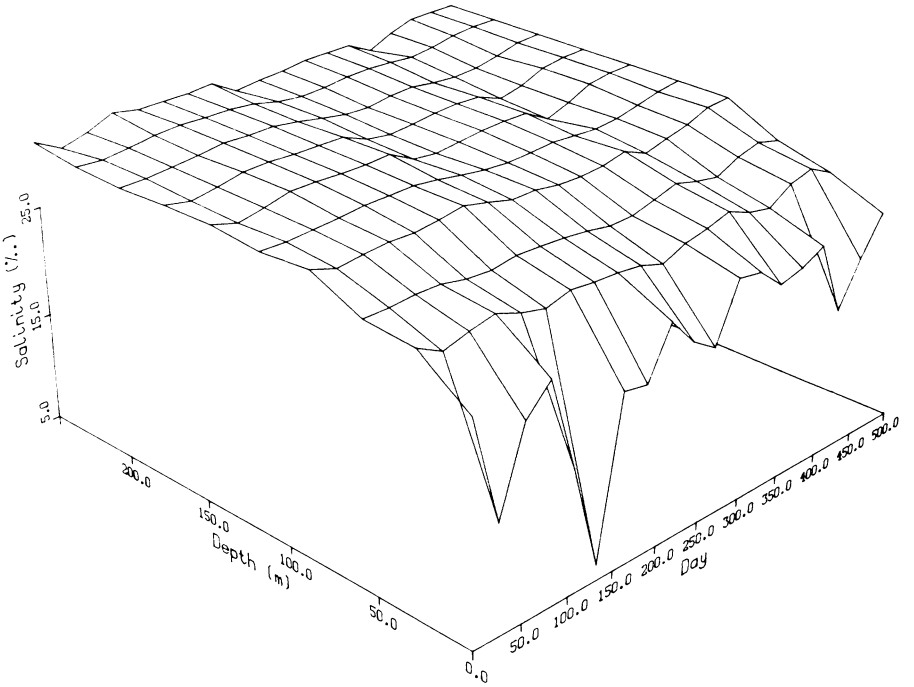

FIG. 3. Mean salinities through the 250-m-deep water column at stations 1, 2, and 3 in Howe Sound as sampled at various times. For dates corresponding to day numbers, see Materials and Methods.

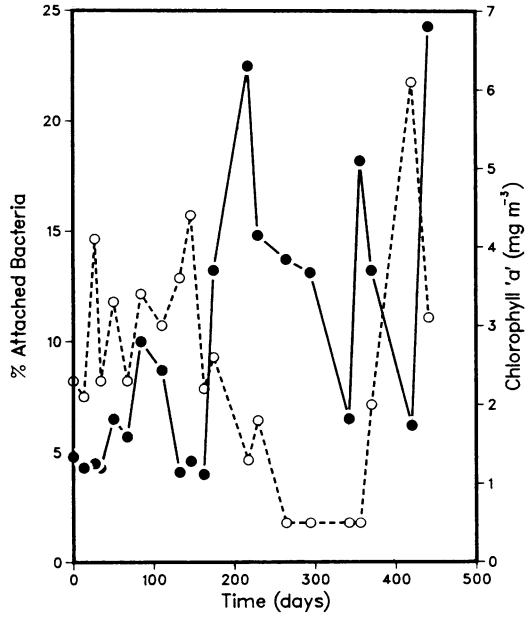

FIG. 4. Mean percent attached bacterial (●) and chl *a* (○) concentrations in the euphotic (0- to 30-m) zone sampled at stations 1, 2, and 3 in Howe Sound versus day of sampling. For dates corresponding to day numbers, see Materials and Methods.

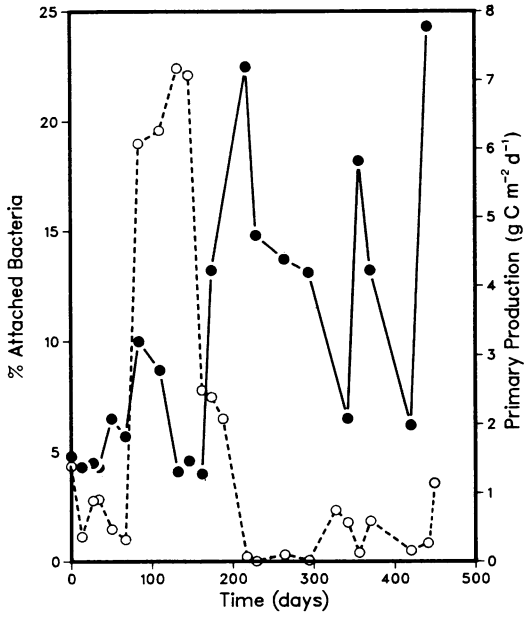

FIG. 5. Mean values of primary production (integrated over the 0- to 30-m water column) (○) and euphotic zone percent attached bacteria (●) at stations 1, 2, and 3 in Howe Sound as assayed over the period of 1 March 1984 to 14 June 1985. For dates corresponding to day numbers, see Materials and Methods.

the stationary phase, the bacterial numbers within the culture increased; many accumulated near, but not on, the algal cells as aggregated masses of cells within what appeared to be a translucent mesh (in general, bacteria did not adhere to all three marine algae while the phytoplankton were viable) (Fig. 7). (iii) Shortly after *D. tertiolecta* entered the stationary phase, some bacteria attached to the decaying algae; most of the bacteria remained as unattached cells (Fig. 7). (iv) Approximately 40 days after these algae entered the stationary phase (60 days from the date of inoculation) few cells remained recognizable as *D. tertiolecta*; most of the culture was composed of a mass of amorphous detrituslike particles containing bacteria (Fig. 7). This sequence of events also occurred in both the *P. minimum* and *S. costatum* cultures.

Filtration of the three algal cultures through Nuclepore membranes of different pore sizes was used for fractionating and counting free and attached bacteria. During the early growth phase of each alga a significant portion of the bacteria became enmeshed in what appeared to be sheets of a translucent organic matrix and attached to detrituslike particles, but not adhered to the algae. During the middle and late growth phases of each alga the percent attached bacteria remained relatively constant in the *P. minimum* culture, decreased in the *D. tertiolecta* culture, and slightly increased in the *S. costatum* culture (Fig. 8A, B, and C, respectively). Shortly after *P. minimum* and *D. tertiolecta* entered the stationary phase a second peak in numbers of attached bacteria occurred (Fig. 8A and B). In the *P. minimum* culture that portion of bacteria which was attached gradually decreased with time until >99% of the bacteria were free living by day 90. At that time no viable algae remained (Fig. 8A). The portion of the bacteria which was attached in the *D. tertiolecta* culture also increased and then decreased with time after cessation of growth of this alga (Fig. 8B). A greater portion of the bacteria were also attached before and after the second growth cycle (from days 34 to 38 approximately) of *S. costatum* (Fig. 8C).

DISCUSSION

Bell and Albright (2) earlier identified particulate concentrations and salinites as being significantly correlated with numbers of attached bacteria in 44 diverse aquatic environments. This subsequent and intensive study of one of these environments (i.e., Howe Sound at stations 1, 2, and 3 [Fig. 1]) has now given us greater insight into the influence of these factors upon attachment of bacteria.

There were several occasions when high concentrations of silt coincided with a high proportion of attached bacteria throughout the water column. These periods most notably occurred during the 18 June and the 3 and 19 October 1984 sampling trips. The greatest silt concentrations occurred during the 3 October 1984 trip at a time when the percent attached bacterial cells were also greatest. However, the percent attached bacterial numbers remained high in November 1984 at a time when the silt concentrations had dropped to a quite low mean value of ca. 3.6 mg liter^{-1}, whereas the percent attached bacteria in the aphotic zone had greatly decreased. The correlation of percent attached bacteria with silt was low (r = 0.22) for samples removed from the aphotic zone, whereas the correlation between these two was markedly greater (r = 0.60) for samples removed from the euphotic zone. Clearly, there was no consistent pattern of percent attached bacteria associated with the silt concentrations, and it would appear that factors other than silt are more important in regulating the percent attached bacteria in the euphotic zone (see below).

The percent attached bacteria in the aphotic zone varied considerably with time, although the salinities changed very little (Fig. 3). And there was no consistent pattern of percent attached bacteria varying with the salinity in the euphotic zone (see Results). It appears to us that, in this water,

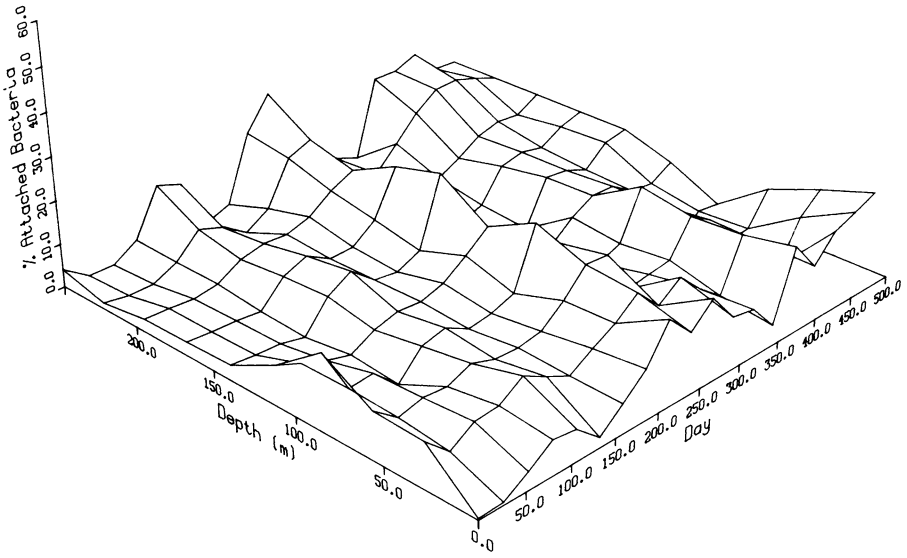

FIG. 6. Mean percent attached bacteria versus sampling time and depth at stations 1, 2, and 3 in Howe Sound. For dates corresponding to day numbers, see Materials and Methods.

salinity does not greatly influence the attachment of bacterial cells.

The significant negative correlation between chl *a* concentrations and percent attached bacteria ($r = -0.60$) was puzzling. However, phytoplankton are the major organic substrate for the heterotrophic bacteria in this ecosystem; this observation thus merited further attention. We therefore investigated whether it is the quality of the phytoplankton (of which chl *a* is a major constituent) rather than the quantity which is inducing bacterial attachment. One way of approaching this problem was to consider the physiological state (i.e., whether the cells were growing) of the algae vis-a-vis bacterial attachment. We used both laboratory cultures of several algae which were typical members of the phytoplankton community of this fjord-embayment as well as doing an extensive field study of primary production and percent attached bacteria in Howe Sound.

The results of these studies suggest to us that the physiological state of the phytoplankton has a major influence upon the attachment of the heterotrophic bacteria of this water column. There was a similarity in the times at which the bacteria became attached during (i) the growth cycles of the three cultured phytoplankton strains (Fig. 7 and 8) and (ii) the annual cycle in production in the euphotic zone of the natural phytoplankton assemblage in Howe Sound (Fig. 5). In both the laboratory cultures and this fjord-embayment the portion of the bacterial cells which were attached increased at approximately the same time that phytoplankton growth started (in Howe Sound this was late winter). While the algae were growing that portion of the bacteria which was attached decreased. After cessation of growth of both the laboratory strains and the natural assemblage of phytoplankton in the euphotic zone of Howe Sound a much greater percentage of the bacteria again became attached. We suggest that this attachment pattern may be indicative of a close coupling between the physiological state of these microalgae and their associated bacteria.

During the normal growth cycle of phytoplankton there are several types of interactions which may occur between these cell types and their associated bacteria. One of these is an antibiosis effect. Several authors have demonstrated that many phytoplankton synthesize antibioticlike substances which can inhibit the growth of heterotrophic bacteria (4, 8, 16, 21). In the case of *S. costatum* these are believed to include fatty acids (8). Cooper et al. (8) have also shown that these bacterial growth inhibitors occur in particularly high concentrations in *S. costatum* cells during the middle to late exponential growth of this alga. During the course of the study we looked for, but rarely observed, bacteria attached to phytoplankton which were actively growing in both Howe Sound and in the laboratory cultures. It was only after the phytoplankton cultures had become senescent that we observed a significant number of cells to have an attached bacterial flora (Fig. 7). The few algae in Howe Sound which we did find to have attached bacteria tended to occur after the active growth period (October) in this natural water. Our data suggest that while a natural assemblage of phytoplankton is actively growing the portion of bacterial cells attached is low, perhaps due in part to algal antibiotic production.

Shortly after phytoplankton growth ceased, the portion of the bacteria which was attached increased in both the laboratory cultures and in the natural assemblage in Howe Sound. The photomicrographs (Fig. 7) indicate that it is at this time that the bacteria became enmeshed in a matrix of amorphous detrituslike material which at times also enclosed some phytoplankton cells. The bacteria were not observed attached to the algae at this time. One possible explanation may be that the newly senescent algae released organic nutrients which are quickly scavenged by the heterotrophic bacteria. In the laboratory phytoplankton cultures it is at this time that the bacteria underwent their greatest growth rates. Several investigators have shown that growing bacteria synthesize glycocalyx (6, 9, 18). It is the synthesis of this material by the actively growing bacteria which may aid these cells in producing an attachment matrix (Fig. 7B).

After the marked increase in percent attached bacteria which occurred at the time of early senescence of the laboratory cultures (ca. day 21 to day 31) and at the time (October) when phytoplankton productivities greatly decreased in Howe Sound, the portion of the bacteria which was attached decreased with time (Fig. 6 and 8). One plausible reason for this may be that these aged phytoplank-

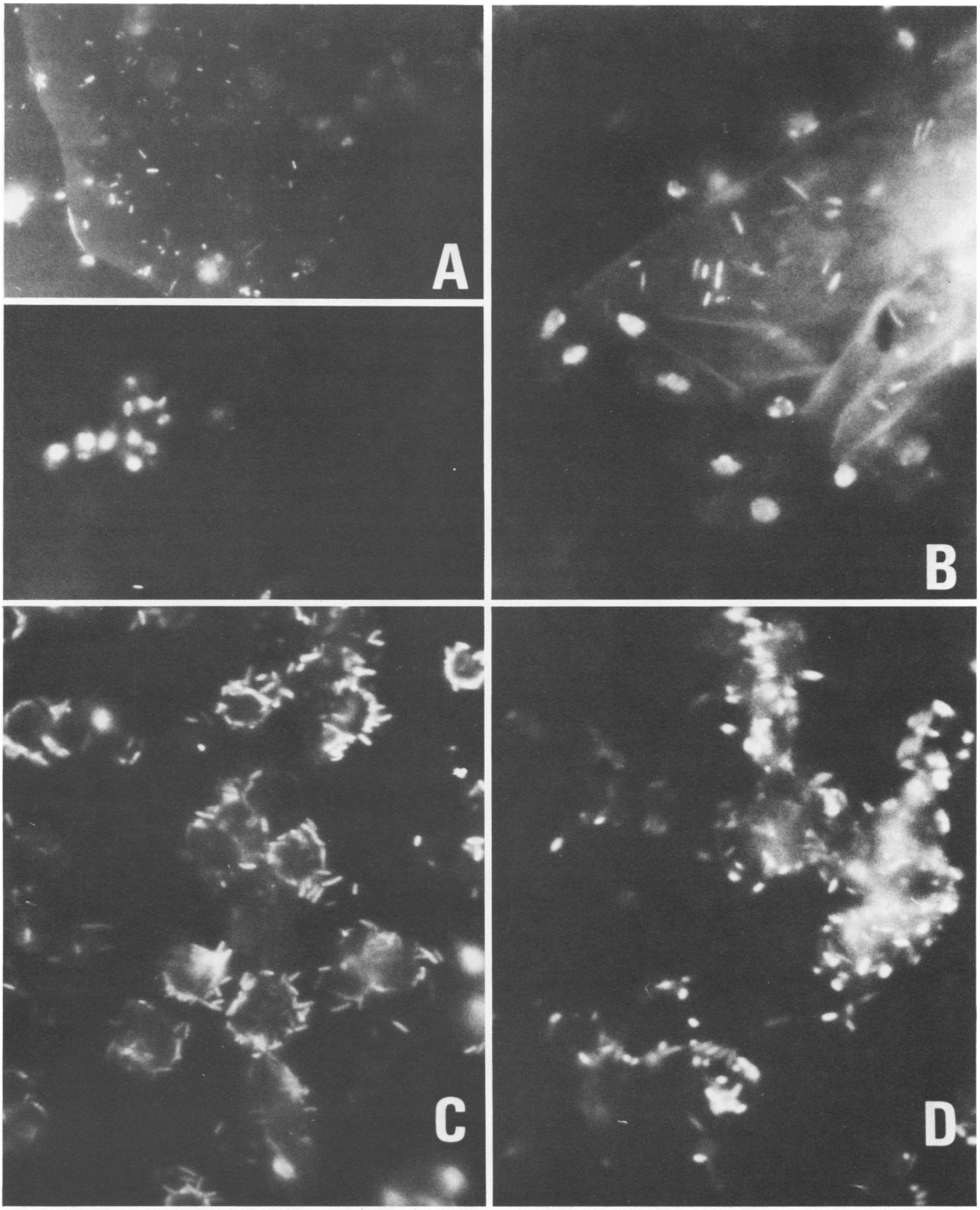

FIG. 7. Epifluorescent micrographs of *D. tertiolecta*-bacterial associations during the growth and stationary phases of this alga: (A) day 29, (B) day 37, (C) day 44, and (D) day 66 after inoculation into algal growth medium.

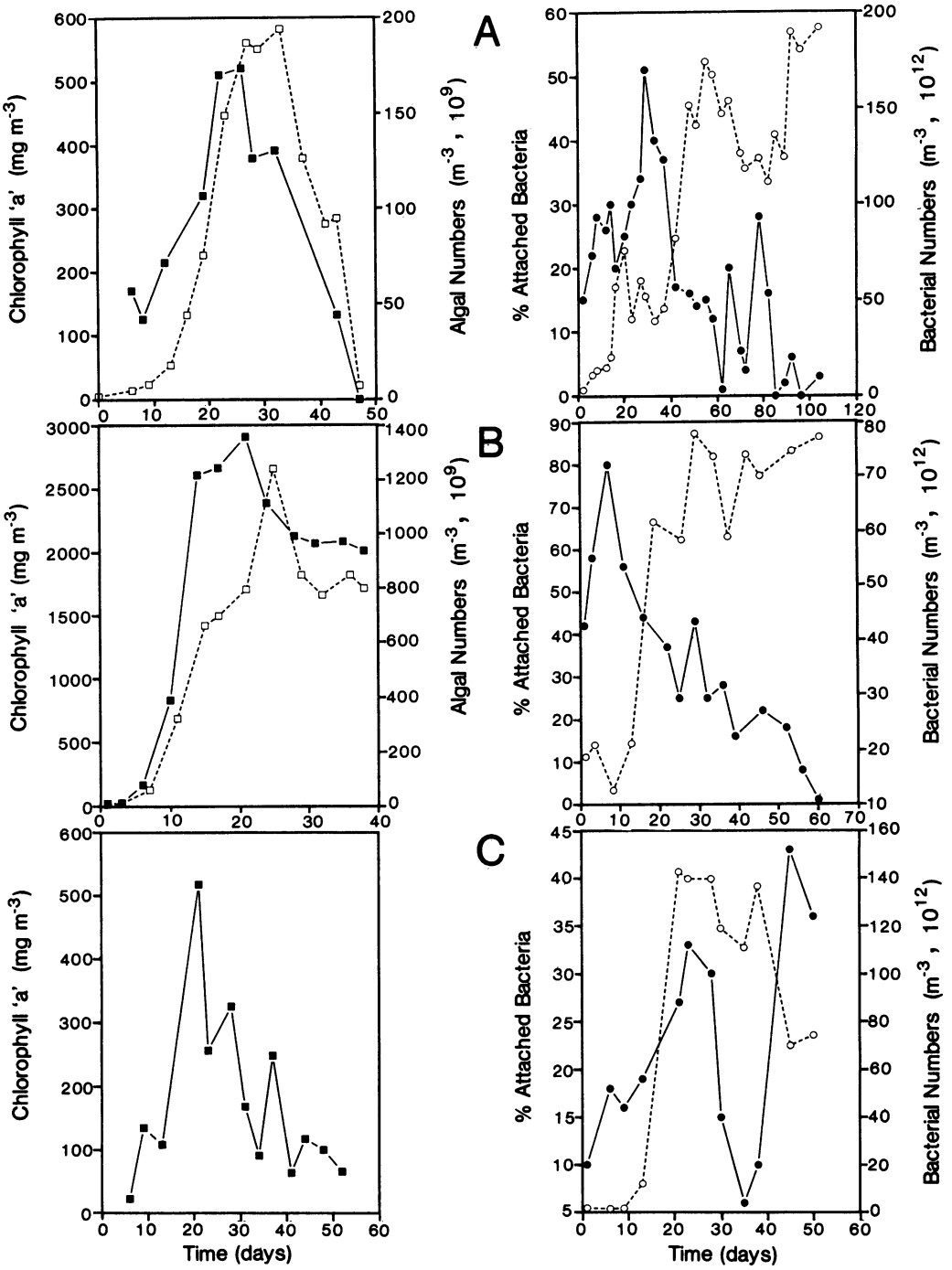

FIG. 8. Percent attached bacteria (●), bacterial concentrations (○), chl *a* concentrations (■), and algal cell concentrations (□) versus time of incubation for (A) *P. minimum*, (B) *D. tertiolecta*, and (C) *S. costatum* cultures.

ton have leached their more labile nutrients and as a result were less nutritious for the heterotrophic bacteria.

A significant portion of the *S. costatum* and *D. tertiolecta* cells possessed what appeared to be firmly attached bacteria during the late senescent period (Fig. 7C). It is possible that at this time many of these algae have leached their cellular contents to the medium and the heterotrophic bacteria were using the cell wall and other less soluble materials as substrates.

We suggest that in a phytoplankton culture or a natural assemblage of phytoplankton the associated heterotrophic bacteria become attached when the phytoplankton (i) initiate growth and (ii) cease growing. The mechanism by which this happens is unclear; however, we suggest that in the first instance there may be a release of some of the newly synthesized organic nutrients by the phytoplankton. In the latter case, the release of nutrients by senescent phytoplankton may cause a stimulation in bacterial activities which

causes bacterial clumping and attachment. Biddanda (6) has shown that growth of heterotrophic marine bacteria on dissolved organic matter results in the formation of aggregates heavily colonized with bacteria. A major type of bridging molecule binding the biotic and abiotic components of these aggregates was probably bacterial glycocalyx. Bacteria with a high glycocalyx content tend to readily attach to each other as well as to both inorganic and organic particulates (9, 10, 17). Such a phenomenon may have occurred in both the laboratory cultures and the natural phytoplankton assemblages in Howe Sound. Attachment of bacteria to growing phytoplankton cells did not occur because of production of cell-attached bacterial growth inhibitors by the phytoplankton (8, 16).

If this explanation is correct, one can then predict that when phytoplankton cells become newly active and newly senescent a relatively high proportion of the bacteria become attached. The latter of these two predictions is easier to test in a natural water such as that at stations 1, 2, and 3 in Howe Sound. In the aphotic zone, where dead and senescent phytoplankton are falling to the sediment, there should be a greater portion of the bacteria which are attached as compared to the bacteria in the euphotic zone. In 11 of the 21 sampling times, this was indeed the case (Fig. 6). It was during these times that primary productivities in the euphotic zone were relatively high (Fig. 5). During the remaining 10 sampling times when the percent attached bacteria in the euphotic zone was greater or equal to that in the aphotic zone the primary productivities were low or declining from the previous sampling period. One would therefore have expected the percent attached bacteria in the euphotic zone to be relatively high. These data were consistent with the observation that maximal percent bacterial attachment occurs when phytoplankton initiate growth and when they show declining growth rates and become senescent, but not while they are active and growing.

ACKNOWLEDGMENTS

We appreciate the efforts of Jack Littlepage (vessel coordinator) and Don Horn (master) in arranging for use of MSSV John Strickland for this research.

This research was supported by a Natural Sciences and Engineering Research Council of Canada Operating Grant (to L.J.A) and a Core Grant for operation of MSSV John Strickland (to the University of Victoria).

LITERATURE CITED

1. **Azam, F., and R. E. Hodson.** 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. **22**:492–501.
2. **Bell, C. R., and L. J. Albright.** 1982. Attached and free-floating bacteria in a diverse selection of water bodies. Appl. Environ. Microbiol. **43**:1227–1237.
3. **Bent, E. J., and R. Goulder.** 1981. Planktonic bacteria in the Humber Estuary: seasonal variation in population density and heterotrophic activity. Mar. Biol. **62**:35–45.
4. **Berland, B. R., D. J. Bonin, A. L. Cornu, S. Y. Maestrini, and J.-P. Marino.** 1972. The antibacterial substances of the marine alga *Stichochrysis immobilis* (Chrysophyta). Phycology **8**:383–392.
5. **Berman, T., and M. Stiller.** 1977. Simultaneous measurement of phosphorus and carbon uptake in Lake Kinneret by multiple isotopic labelling and differential filtration. Microb. Ecol. **3**:279–288.
6. **Biddanda, B. A.** 1985. Microbial synthesis of macroparticulate matter. Mar. Ecol. Prog. Ser. **20**:241–251.
7. **Cammen, L. M., and J. A. Walker.** 1982. Distribution and activity of attached and free-living suspended bacteria in the Bay of Fundy. Can. J. Fish. Aquat. Sci. **39**:1655–1663.
8. **Cooper, S., A. Battat, P. Marsot, and M. Sylvestre.** 1983. Production of antibacterial activities by two *Bacillariophyceae* grown in dialysis culture. Can. J. Microbiol. **29**:338–341.
9. **Costerton, J. W., R. T. Irwin, and K.-J. Cheng.** 1981. The bacterial glycocalyx in nature and disease. Annu. Rev. Microbiol. **35**:299–324.
10. **Fenchel, T., and B. B. Jorgensen.** 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. p. 158. *In* M. Alexander (ed.), Advances in microbial ecology, vol. 1. Plenum Publishing Corp., New York.
11. **Ferguson, R. L., and P. Rublee.** 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. **21**:141–144.
12. **Geesey, G. G., and J. M. Costerton.** 1979. Microbiology of a northern river: bacterial distribution and relationship to suspended sediment and organic carbon. Can. J. Microbiol. **25**:1058–1062.
13. **Goulder, R.** 1976. Relationships between suspended solids and standing crops and activities of bacteria in an estuary during a Neap-Spring tidal cycle. Oceologia **24**:83–90.
14. **Harrison, P. J., R. E. Waters, and F. J. R. Taylor.** 1980. Broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J. Phycol. **16**:28–35.
15. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
16. **Niewolak, S.** 1971. The influence of alcohol extracts of some algae (*Chlorella* and *Scenedesmus*) on aquatic microorganisms. Pol. Arch. Hydrobiol. **18**:31–42.
17. **Paerl, H. W.** 1973. Detritus in Lake Tahoe: structural modification by attached microflora. Science **180**:496–498.
18. **Paerl, H. W.** 1974. Bacterial uptake of dissolved organic matter in relation to detrital aggregation in marine and freshwater systems. Limnol. Oceanogr. **19**:966–972.
19. **Pedros-Alio, C., and T. D. Brock.** 1983. The importance of attachment to particles for planktonic bacteria. Arch. Hydrobiol. **98**:354–379.
20. **Pickard, G. L.** 1975. Annual and longer term variations of deepwater properties in the coastal waters of southern British Columbia. J. Fish. Res. Board Can. **32**:1561–1587.
21. **Sieburth, J. M.** 1964. Antibacterial substances produced by marine algae. Dev. Ind. Microbiol. **5**:124–134.
22. **Sieburth, J. M., R. D. Brooks, R. V. Gessner, C. D. Thomas, and J. L. Tootle.** 1974. Microbial colonization of marine plant surfaces as observed by scanning electron microscopy. p. 418–432. *In* R. R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
23. **Steeman Nielsen, E.** 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. Conseil Perm. Intl. Explor. Mer **18**:117–140.
24. **Stockner, J. G., D. D. Cliff, and D. B. Buchanan.** 1977. Phytoplankton production and distribution in Howe Sound, British Columbia: a coastal marine embayment-fjord under stress. J. Fish. Res. Board Can. **34**:907–917.
25. **Strickland, J. D. H., and T. R. Parsons.** 1972. A practical handbook of seawater analysis, 2nd ed. Bull. Res. Board Can. Bull. no. 167.
26. **Wiebe, W. J., and L. R. Pomeroy.** 1972. Microorganisms and their association with aggregates and detritus in the sea: a microscopic study. Mem. Ist. Ital. Idrobiol. Dott Marco de Marchi Pallanza Italy **29**(Suppl.):325–352.