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A Study of the Occurrence and Distribution of Bdellovibrios in Estuarine Sediment over an Annual Cycle

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Abstract. The recovery of bdellovibrios from estuarine sediments over an annual cycle was studied. Greater numbers of the predators were recovered in sediment than in the water column. Increases in the number of bdellovibrios recovered from sediment over various periods of time suggest that multiplication of the predators occurred. Sediment was observed to be an important ecosystem for the survival of bdellovibrios in the winter months. As has been observed in water, the number of bdellovibrios in sediment fluctuated, with seasonal and temperature changes declining to very low numbers during the winter months. In the colder months, low numbers of the predators appeared to winter-over in sediment, with greater numbers of the organisms being recovered from deeper sediment. As the water temperature warmed in the spring, increases in the number of bdellovibrios occurred first in sediment and subsequently in water. This increase of bdellovibrios in sediment may have resulted in the shedding of the organisms into the water column where their numbers subsequently increased. Population fluctuations of bdellovibrios were similar in both water and sediment. Although the temperature may account for much of the observed fluctuation in the number of bdellovibrios, other factors, including salinity and the number of host bacteria, may also play a major role. The number of bdellovibrios recovered from sediment correlated positively with the water temperature, and negatively with the water salinity and the number of bacterial colony-forming units from sediment. The results of this study revealed the significance of sediment to the seasonal cycle, survival, and growth of the bdellovibrios in an estuarine environment.

Introduction

Most studies on bdellovibrios have been concerned with the physiology of the predators and their interactions with host cells. With the exception of a relatively small number of reports [1, 4, 5, 8, 13–15], the ecology of the bdellovibrios has been a neglected area of study. Of the few ecological studies reported, most have primarily focused on the ecology of bdellovibrios in aquatic environments and in particular, the water column [5, 10, 13–15]. Although bdellovibrios have been recovered from the water column of many diverse bodies of water, in

many cases they are found in relatively low numbers, less than 10 per ml [10, 13, 15]. A previous report revealed that even lower numbers of bdellovibrios are recovered during the winter months when the temperature is below 5°C [14]. Their numbers appeared to increase to a peak during the warm months [14].

Data from several studies [2, 12] indicate there is some question as to whether the number of host bacteria in the water column is sufficient to support large populations of the parasitic, predatory bdellovibrios. These studies have reported that bdellovibrios require from 10^5–10^6 host cells per ml for a stable existence. In a recent study, bdellovibrios appeared to survive with a host concentration of 10^4 organisms per ml [9]. Data on the number of bacteria recovered from samples collected from the water column of various bodies of water [12] indicate that in order for bdellovibrios to persist, nearly every bacterium present would have to be susceptible. This appears unlikely since many bdellovibrio strains studied have shown some degree of host specificity [3, 8].

Based on current knowledge, it appears that aquatic habitats other than the water column may be better suited for the propagation and survival of the bdellovibrios [7]; however, there has been little study on the presence of these organisms in such habitats [1]. One environment that may be suitable for bdellovibrios is the sediment. It is more densely populated with microorganisms than the water column, with sediment having as many as 10^{11} bacteria per g [6]. Also, the number of bdellovibrio-susceptible organisms in sediment is presumed to be more numerous. As suggested in a previous report [14], sediment may serve as a primary habitat for the bdellovibrios to winter-over since in the winter months the number of the predators in the water column frequently decreases to undetectable levels. Because of the potential importance of the benthos to the multiplication, persistence, and seasonal cycle of the bdellovibrios, the occurrence and distribution of these predators in sediment over an annual cycle was studied. The results are presented in this report.

Materials and Methods

Sediment and water samples were collected near the mouth of the Patuxent River, a subestuary of the Chesapeake Bay. Twice each month over a 12 month period, four water samples and five sediment samples were collected off the pier at the Chesapeake Biological Laboratory. This protocol was designed to allow for the collection of as many sediment samples as possible, a large number of samples being desirable for statistical analysis. Although a study of the annual distribution of bdellovibrios in water at this site has been reported [14], water was sampled in this study as it was desirable to observe how the annual distribution pattern of bdellovibrios in sediment would correspond to that in water. However, the primary focus in the study was sediment, and only four water samples (collected twice each month) could be managed. The water depth at the site of collection ranged from 1.5–2 m. Prior to the collection of samples, temperature and salinity were measured as described elsewhere [13]).

Two surface and bottom water samples were collected from the same area in which the sediment samples were collected. Surface samples were obtained by submerging sterile glass bottles approximately 10 cm below the water surface. Bottom water was collected approximately 10 cm above the sediment surface using a J-Z water sampler [16]. Upon retrieval of the J-Z sampler, the bottom water sample was transferred to two sterile collection bottles yielding two subsamples.

The five sediment samples were always obtained from the same sites, all of which were located

within an area of approximately 1 m^2. Sediment cores were obtained with a sediment collector crafted in this laboratory consisting of a metal, cylindrical hollow tube, 0.5 in length and 2 cm in diameter, attached to a 2 m wooden handle. Holes located in the side walls of the metal tube approximately 10 cm from the bottom permitted water overlying the sediment core to escape before removal of the core so as to prevent the mixing of water with the sediment sample. To obtain the core, the collector was passed vertically through the water column onto the surface of the sediment and was manually rotated while applying pressure, forcing the open end of the tube approximately 8–10 cm into the sediment. The collector was then withdrawn from the sediment and brought to the surface and onto the pier. Approximately 30 sec was allowed for the water overlying the sediment core to escape through the holes in the walls of the collector. With some shaking and agitation of the collector, a uniform sediment core passed from the tube of the collector onto a sterile wire screen mounted in an aluminum pipet basket. Samples from various depths of the sediment core were collected to study the distribution of bdellovibrios with increasing sediment depth. From the surface end, the sediment core was divided into three portions, each approximately 2.5 cm in length. From each of these respective portions, an amount of sample material was added to 20 ml of seawater in a 50 ml conical centrifuge tube to increase the total volume by 5 ml (yielding a sediment sample of 5 ml) to 25 ml. This procedure is a modification of a technique described in the 1978 Marine Microbiology Cruise Manual, published by the Microbiology Department, University of Maryland College Park. This protocol was repeated for each of the five samples. Following the collection of each sample, the hollow tube of the sediment collector was cleaned with a test tube brush soaked with 70% alcohol. The top of the tube was flamed or time was allowed for the alcohol to evaporate before collecting the next sample.

Immediately following collection, all samples were placed in an ice chest cooled with frozen ice packs (Ice Pac, Inc., Stanbel, Springfield, Massachusetts) and transported to the laboratory for processing. In the laboratory, each of the tubes containing the sediment samples was agitated at maximum speed for 2 min on a mechanical mixer (Vortex, model S8223, Scientific Products, Evanston, Illinois) to elute the bacteria from the sediment. The tubes were than placed in an upright position for up to 1 hour until the sediment settled. The supernatant fluid was removed and placed into sterile tubes. One milliliter of the supernatant fluid was 10-fold serially diluted in sterile, diluted (70%) seawater. One-tenth milliliter of each dilution was spread-plated in duplicate onto plates of estuarine agar (EA) [13] for determining the total number of colony-forming units (cfu) of bacteria. This data was needed to measure any correlation that may exist between the number of cfu and the number of bdellovibrios. The EA plates were incubated at 25°C for 10 days.The number of colonies observed on the plates was recorded and the number of cfu per ml of sediment was calculated.

Culturing for the enumeration of bdellovibrio plaque-forming units (pfu) was done as previously described by Williams and Falkler [15]. Five milliliters of the sediment supernatant fluid was added to a top agar tube containing 3.3 ml of melted polypeptone 20 (Pp 20) agar previously inoculated with 0.5 ml of a 10^8 suspension of host cells (*V. parahaemolyticus*, P-5). The top agar mixture was overlayed onto Pp 20 bottom agar in a 150 × 15 mm petri dish. Duplicate plates were made of each sample. Time was allowed for the agar to solidify and the agar plates were incubated at 25°C for 10 days.

Estuarine agar and Pp 20 agar plates were examined daily for the presence of cfu and pfu, respectively. Colonies and plaques were marked as they appeared. Plaques observed on the Pp 20 plates were randomly selected and confirmed for the presence of bdellovibrios via phase contrast microscopy, as previously described [13]. The total number of marked bacterial cfu/pfu were recorded after the 10 day incubation period.

The number of bdellovibrio pfu and bacterial cfu recorded was analyzed by the Kruskal-Wallis one-way analysis of variance test (ANOVA) to determine significant differences in the number of pfu and cfu recovered from sediment and water collected at various depths. The number of bdellovibrios recovered from both sediment and water samples in cold months (based on water temperature), December thru April, was compared with the number recovered in the warm months (May through November) using the Mann-Whitney statistical test. The number of bdellovibrios recovered from sediment samples collected in each of the two sampling periods in March (March 4 and March 24, respectively) was analyzed for significant differences using the Kruskal-Wallis

test, as the number recovered on March 24 was observed to have increased. The Pearson Product Moment Correlation Test was done to measure correlations between the number of cfu and pfu recovered from water and sediment. Correlations were also determined between the number of cfu and pfu, respectively, and temperature and salinity.

Following compilation and analyses of the data on the distribution of bdellovibrios in sediment it was desirable to determine the oxidation-reduction (redox) potential of the sediment at the site from which samples were collected. Redox potentials in millivolts were measured using an Orion Research analog pH meter (model 301) with either a combination redox electrode (Orion 977800) or a custom manufactured platinum electrode and a calomel reference electrode (Orion 900600). Five sediment samples were collected within a month using a sediment collector (Wildco, Saginew, MI; no. 2422-H70) with holes 1 cm in diameter spaced 2.5 cm apart along the longitudinal axis of the bottom half of the collector. Prior to the collection of samples, duct tape was placed over the holes. Upon retrieval, the sediment sample was secured by placing a rubber stopper in the bottom end of the collector. Holes were made in the tape and the redox electrode was placed through the hole into the sediment.

Results

Bdellovibrios lytic against *V. parahaemolyticus* were recovered, with one exception, from both sediment and water samples in each of the 12 months during which samples were collected and in February of the following year (Table 1). The mean number of bdellovibrios recovered per ml of sediment in each of the 12 months was always greater than the number recovered from the same volume of water. For both sediment and water samples, the number of bdellovibrios recovered on duplicate agar plates inoculated from the same sample showed wide variation, as reflected in the relatively high standard deviation values. This has also been observed in previous quantitative studies of bdellovibrios [15]. Over the annual cycle, no significant difference was found in the number of the bdellovibrio pfu (Table 2) recovered from the three depths of sediment collected. However, it was observed that the greater numbers of bdellovibrios were more frequently recovered from the deeper sediment samples (obtained at depths below 2.5 cm from the sediment surface) than from samples collected within the top 2.5 cm. This was especially pronounced during the months of January through March when the mean water temperature was 5.4°C. Of the 29 sediment samples collected during this period, only in two were the greatest number of bdellovibrio pfu recovered from the top 2.5 cm. In the remaining 27 samples, the greatest number of pfu were recovered from between 2.6 and 7.5 cm of the sediment core. In the warm months, the number of bdellovibrios recovered from sediment was more uniformly distributed. During the period from July to August, the greatest number of bdellovibrios were recovered from the uppermost sediment samples in seven of 30 samples collected. During this time, the mean water temperature was 26.5°C. A significant difference was found in the number of cfu recovered at various depths of sediment. However, the meaning of the differences was not discernible. The range of redox potential measurements in mV taken subsequent to the conclusion of the cultural studies was as follows: top 2.5 cm, +240 to −40; 2.6 to 5.0 cm, −180 to −360; and 5.1 to 7.5, −180 to −380.

A significant difference was found in the number of bdellovibrios recovered from top and bottom water samples with more bdellovibrios recovered from

Table 1. The number of bdellovibrio plaque-forming units (pfu) and bacteria colony-forming units (cfu) recovered per ml of estuarine sediment and water

	Bdellovibrios			
Months	N[a]	Mean pfu + SD[b]/ml of sediment	N[c]	Mean pfu + SD/ml of water
Grp 01	59	14.93 ± 41.47	15	0.02 ± 0.09
02	57	5.59 ± 20.9	11	0.0
03	60	1.33 ± 1.66	12	0.03 ± 0.07
04	59	4.03 ± 13.8	16	0.28 ± 0.37
05	60	43.76 ± 131.54	14	0.41 ± 0.26
06	60	25.61 ± 39.06	14	0.22 ± 0.29
07	59	61.23 ± 118.26	14	2.50 ± 2.20
08	59	39.27 ± 61.82	14	3.30 ± 2.01
09	60	9.41 ± 12.44	16	2.02 ± 2.54
10	29	94.00 ± 90.62	8	4.40 ± 2.43
11	58	130.50 ± 221.82	16	0.55 ± 0.50
12	29	49.0 ± 60.90	8	1.65 ± 1.06
01	—[d]	—	—	—
02	25	0.6 ± 1.2	6	0.33 ± 0.74
	Bacteria			
Months	N[a]	Mean cfu × 10^5 + SD[b]/ml of sediment	N[c]	Mean cfu × 10^3 + SD/ml of water
01	50	30.4 ± 27.1	16	6.3 ± 5.1
02	49	25.8 ± 18.9	12	2.4 ± 1.9
03	59	9.1 ± 7.8	12	57.1 ± 39.8
04	55	33.4 ± 27.2	12	13.5 ± 12.0
05	53	34.0 ± 24.3	13	77.8 ± 12.0
06	23	12.1 ± 10.2	14	35.5 ± 3.5
07	28	26.1 ± 13.1	13	68.9 ± 61.0
08	59	22.1 ± 11.6	15	86.6 ± 40.7
09	60	11.9 ± 11.7	16	13.8 ± 11.8
10	30	7.3 ± 3.9	8	2.4 ± 0.4
11	40	18.4 ± 14.6	16	8.3 ± 2.4
12	16	10.1 ± 7.0	—	—
01	—[d]	—	—	—
02	28	6.5 ± 5.6	2	22.5 ± 33.5

[a] Number of sediment sample counts used to calculate mean and standard deviation (SD)
[b] SD
[c] Number of water samples used to calculate mean and SD
[d] No sample collected or data not available

the top water samples (Table 2). The mean number of pfu recovered from top and bottom water samples, respectively, was 1.9 ± 2.0 pfu/ml and 1.4 ± 1.6 pfu/ml. No significant differences were observed in the number of bacterial cfu recovered from top and bottom water samples.

In the months when the water temperature was lower (December through April), significantly smaller bdellovibrio counts were observed in sediment and in water than in the other months of the year (Mann-Whitney U = 71,603,

Table 2. Significant differences as analyzed by Analysis of Variance Test

Variable	Number of pfu		Number of cfu	
	Chi-square[a]	Probability	Chi-square	Probability
Water depth[b]	4.604	<0.05	0.000	NS[c]
Sediment depth[d]	4.513	NS[c]	17.854	<0.0001
Number of pfu recovered on Mar. 24 vs Mar. 4	8.528	<0.01		

[a] Chi-square corrected for *ties*
[b] Two depths included: 10 cm below air-water interface and 10 cm above the sediment surface
[c] Not significant
[d] Three depths included: surface to 2.5 cm; 2.5–5.0 cm; and 5.1–7.5 cm

Table 3. Correlation of bacterial counts vs temperature, salinity and plaque-forming units (pfu) and colony-forming units (cfu)

Correlation between	Temperature	Salinity	Water cfu	Sediment cfu
Water cfu	(0.1590; 0.024)[a]	(0.1161; 0.075)	—[b]	—
Sediment cfu	(−0.0166; 0.336)	(0.1248; 0.001)	—	—
Water pfu	(0.4441; 0.0001)	(−0.2612; 0.001)	(0.0592; 0.232)	—
Sediment pfu	(0.3022; 0.0001)	(−0.2354; 0.001)	—	(−0.1474; 0.0001)

[a] (r value; probability value); $P < 0.05$ taken as level of significance
[b] Not analyzed

$z = 9.78$: $P < 0.0001$ for sediment and $U = 4{,}420$, $z = -6.59$; $P < 0.001$ for water).

The number of bdellovibrio pfu recovered from both water and sediment, respectively, correlated positively with temperature (Table 3). Although the correlation between the bdellovibrio count in water and temperature ($r = .44$) was higher than between the bdellovibrio count in sediment and temperature ($r = .30$), no significant differences were found between the two correlations. A negative correlation was found between the numbers of bdellovibrios from both water and sediment samples and salinity.

A significant correlation ($P < 0.05$) was found between the total number of cfu recovered from water and the temperature of the water. A correlation was not found in water between the number of cfu and salinity measurements; to the contrary, in sediment, the cfu correlated significantly with the water salinity but not with the water temperature (Table 3).

A negative correlation was found between the number of pfu and the number of cfu in sediment; however, no correlation was found in the water samples.

The relationship between the monthly mean number of pfu recovered from both sediment and water samples (Table 1) and temperature, salinity, and the number of bacterial cfu recovered is shown in Fig. 1.

Although in Fig. 1 it appears that the first increase in the number of bdel-

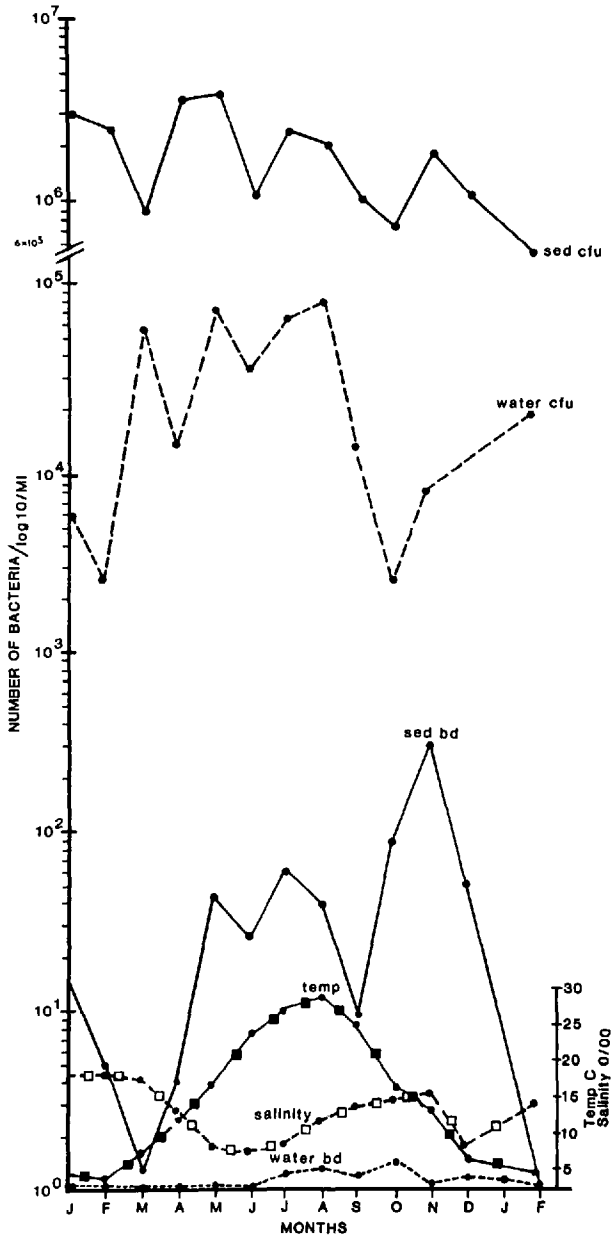

Fig. 1. The relationship over an annual cycle between the number of bdellovibrio plaque-forming units (*bd*) recovered from sediment (*sd*) and (1) the number of bd recovered from water; (2) the number of bacteria (*cfu*) recovered from both sediment and water; (3) water temperature (*temp*); and (4) water salinity.

lovibrios occurred in April, an analysis of the two sampling periods in March revealed that the first actual increase occurred in samples collected on March 24 (Fig. 2). The number of bdellovibrio pfu recovered on March 24 increased significantly over the number recovered on March 4, as determined by the Kruskal-Wallis test (Table 2). No such increase was observed in the previous month. Increases in the number of bdellovibrios between the first and second

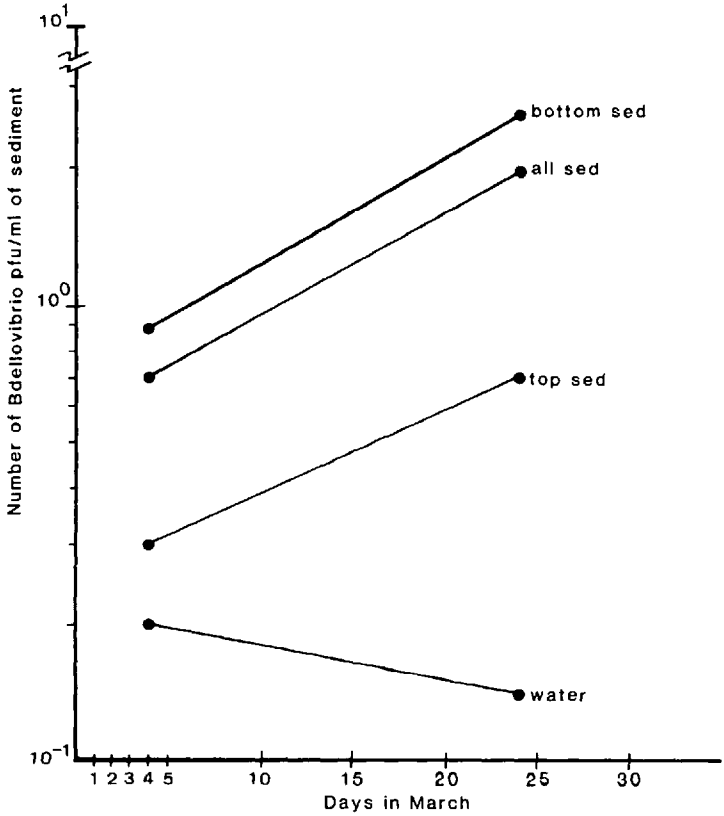

Fig. 2. The number of bdellovibrios recovered from sediment (*sed*) and *water* samples collected on March 4 and on March 24. *Top sed* samples were collected from the top 2.5 cm of sed. *Bottom sed* samples were collected from a depth ranging from 2.6–7.5 cm. *All sed* represents the mean number of bdellovibrios recovered from both top and bottom sed samples.

sampling periods in March were observed in both top and bottom sediment samples (Fig. 2). The number of bdellovibrios recovered from the three depths of sediment sampled on March 4 was not significantly different, although the mean number recovered from the bottom sediment samples was greater. However, the number of bdellovibrios recovered from the bottom sediment samples collected on March 24 (2.6–5.0 cm and 5.1–7.5, respectively) was significantly greater ($F = 4.628$; $P < 0.0187$) than the number recovered from top (sediment surface to 2.5 cm) sediment samples. No significant difference was found between the number of pfu recovered from sediment depth 2.6–5.0 cm and from 5.1–7.5 cm. Therefore, in Fig. 2 the counts from these two sediment portions were treated as equal and were pooled as the bottom sediment pfu count.

Discussion

The results of this study clearly reveal for the first time the nature of the association of bdellovibrios with sediment in the aquatic environment. The

number of bdellovibrios recovered from sediment samples was consistently higher than the number recovered from samples of the overlaying subsurface waters. The relatively low number of bdellovibrios recovered from water samples adds support to the opinion that in the aquatic environment bdellovibrio activity is minimal or nonexistent in the water column and occurs primarily in other selected habitats [7]. Earlier reports [2, 11] suggested that bdellovibrios could not sustain their population without a host concentration of 10^5–10^6 cells per ml. More recently, Varon [9] has observed that bdellovibrios were capable of sustaining themselves in equilibrium with their host at a host concentration of 10^4 organisms per ml. This concentration of host cells is similar to the number of bdellovibrio suspects (bacteria susceptible to bdellovibrios) in Chesapeake Bay waters. This is supported by studies currently under way in our laboratory. Although the report by Varon [9] indicates that bdellovibrios may be capable of some activity at low prey density, as is observed in the water column, the higher number of cultivable bacteria and bdellovibrios observed in sediments in this study suggests that the benthos was a more favorable habitat for the predators.

The number of bdellovibrios recovered from both sediment and water samples exhibited seasonal fluctuations. Significantly lower numbers were recovered during the colder months than during the warmer months. However, the decrease in the number of predators was much greater in water than in sediment. In water samples, bdellovibrios were frequently not detected, the mean number recovered from January through April being less than 0.1 pfu per ml. By comparison, the number of bdellovibrios recovered from sediment during the winter months was higher by several orders of magnitude. The fluctuations observed in the numbers of bdellovibrios recovered from both sediment and water followed a similar pattern over the annual cycle of the study. Increases or decreases in the number of bdellovibrios recovered from sediment frequently occurred with corresponding changes in the number of the predators in the water column. However, in contrast to the abrupt and sharp increases of bdellovibrios observed in sediment samples, increases in the number of the organisms recovered from water samples were considerably lower. The first increase in the number of bdellovibrios in sediment following the winter months was observed in samples collected on March 24. The observed increase was statistically significant. The number of bdellovibrios was increased at all sediment depths sampled, but the greatest increase was in the bottom sediment samples. The observed increase in the number of bdellovibrios recovered in the month of March when the water temperature was between 8.0 and 10.6°C revealed for the first time that, in nature, the predators are capable of multiplication at low temperatures. In laboratory studies, Marbach et al. [3] observed no multiplication of bdellovibrio strains below 10°C. Since the number of bdellovibrios from sediment samples generally continued to increase following the initial increase observed on March 24, it would appear that the predator count of March 24 represented the initial growth of bdellovibrios in sediment. An increase in the number of bdellovibrios was not observed in the water column until 3 months later. These results represent the strongest evidence to date that bdellovibrios multiply primarily in aquatic habitats other than the water column.

In both water and sediment the number of the predators peaked in the fall season followed by a rapid decline which continued through the winter months of the following year. It is apparent from these observations that bdellovibrios survive the winter months in sediment and repopulate the water column only after substantial multiplication has occurred in sediments. Based on these observations, it can be hypothesized that bdellovibrios are primarily benthic organisms and their presence in the water column results primarily from the organisms shedding from the sediment and other habitats. A subject for further study is to determine whether the increase in bdellovibrios in the water column is due solely to shedding from such habitats.

The winter survival of bdellovibrios in sediment suggests that this habitat provides not only a large number of host bacteria, but a protective environment shielding the bdellovibrios from colder temperatures and other extreme environmental parameters. Of interest was the observation that a greater number of bdellovibrios was frequently recovered from the deeper areas (2.6–7.5 cm) of sediment than the top 2.5 cm. Statistical analysis of the number of bdellovibrios recovered from various depths over the annual cycle revealed an approximately even distribution of the organisms from the surface of the sediment core to a depth of 7.5 cm. This result was unexpected since bdellovibrios have been characterized as being strict aerobes, although the predators have been recovered from areas lacking dissolved oxygen [15]. Previously, it was reported that bdellovibrios were not recovered in sediment below 5 cm [1].

Although in this study the number of bdellovibrios recovered from sediment was always greater than the number in water, some exceptions to this pattern have been observed in other bodies of water. These exceptions may result from differences in the physical nature and the chemical characteristics of the sediments. All sediments may not support the growth or distribution of bdellovibrios to the extent observed in this study.

The difference observed between the number of bdellovibrios recovered from top water samples as compared to bottom water samples is in contrast to data previously reported [14]. This difference may be due to two factors. First, the previous study was conducted in a different subestuary of the Bay and reflected samples collected over a 24 hour period. Secondly, the top water samples were collected at a depth of 0.5 m. In contrast, the results of this study were obtained from data collected over a 1 year period and from top water samples collected 10 cm below the water surface.

The number of bdellovibrios recovered from both sediment and water correlated positively with temperature changes indicating that their distribution may be influenced to a large extent by temperature. This is consistent with previously reported results on the seasonal distribution of bdellovibrios from water obtained from the same site used in this study [5].

The negative correlation found between the number of bdellovibrios and the total number of cfu in sediment suggests that bdellovibrios may impact on the population dynamics of the microbial population in some natural ecosystems. In this study, as the number of bdellovibrios increased in sediment, the microbial population decreased and vice versa. This is in contrast to previously reported results by Fry and Staples [1] in which a correlation was not found

between the number of bdellovibrios and heterotrophs. However, the results reported here are based on data from far more samples than used by Fry and Staples resulting in increased confidence in the findings. Nevertheless, further studies are suggested, including quantitation using the acridine orange direct count method in addition to total plate counts to confirm a negative correlation between the number of bdellovibrios and bacterial cfu in sediment. A similar correlation, as found in sediment between the predator and the bacterial population, was not observed in the water samples collected. This may be explained by the fact that the susceptible bacterial cell density in the water column is so low that any change in their population caused by bdellovibrios is too small to detect with the methods used in this study.

A negative correlation between the number of bdellovibrios and salinity suggests that within the range of salinity measurements (7.0–18.4 ppt) in this study, the lower the salinity the greater the number of bdellovibrios and vice versa. It has been previously reported that the greater number of bdellovibrios in the Chesapeake Bay were recovered in areas of moderate salinity (8–12 ppt) as opposed to areas of low (<5 ppt) and high (<25 ppt) salinities [14]. However, one should be mindful that there are complex interrelationships between salinity, temperature, and other environmental factors, all of which may affect the population dynamics of the bdellovibrios. Any conclusions based on the results of the correlation tests can be only tentative until studies that include measurements of other environmental parameters are conducted.

In another study [1], the occurrence of bdellovibrios in river water and sediment was largely attributed to the input of sewage, and the predators were thought to be allochthonous in some rivers. Although sewage and run-off from farmland may be sources of terrestrial or freshwater bdellovibrios, it is doubtful whether halophilic bdellovibrios, which require moderate concentrations of salt, occur in these sources which normally contain relatively low salt concentrations. Although identifying the source of bdellovibrios in water and sediment was not an objective in this study, no such sources were obvious. Further, the results suggested that the population of bdellovibrios was capable of sustaining itself by growth and winter-over cycles in sediment and that the organisms were indigenous to the water and benthic region at the site studied. Studies under way in our laboratory suggest this is true of other estuarine habitats within the salinity range required of the bdellovibrios.

In conclusion, the association of bdellovibrios with sediment and the importance of this habitat to the seasonal cycle, survival, and growth of the predators in an estuarine environment has been established. Studies on the ecology of bdellovibrios in sediments and other aquatic ecosystems where the organisms occur in high numbers may reveal more about the nature of these unique bacterial predators than studies in the water column where most of the previous ecological work has been conducted.

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