ROLE OF SUSPENDED SOLIDS IN THE SURVIVAL AND TRANSPORT OF ENTERIC VIRUSES IN THE ESTUARINE ENVIRONMENT

FINAL REPORT

Project No. R/ER-17
USM Nos. 0221701183-283
January 1987 to December 1988
Extension: January to June 1989

Submitted to

Mississippi-Alabama Sea Grant Consortium
Caylor Building, Gulf Coast Research Laboratory
Ocean Springs, MS
Dr. James I. Jones, Executive Director

by

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Abstract

This investigation was performed to determine the levels of solids-associated enteric viruses in a polluted Mississippi estuary. One hundred gallon quantities of estuarine water were filtered to remove the suspended solids fraction from which viruses were isolated and quantitated. The Back Bay of Biloxi, Mississippi was selected for the study, and six sites within the bay were sampled continuously during a nineteen month period. A variety of physical, chemical, biologic and geologic factors were also measured to assist in the understanding of virus persistence and transport in the estuary.

Virus numbers associated with suspended solids from 100 gallons of estuarine water ranged from 0 to 35 for any one site on a given month. The majority of analyses (76.3%) contained between 0 and 10 plaque forming units; only 5% of the samples contained greater than or equal to 21 plaque forming units per 100 gallons. This demonstrates that the estuary receives a constant but low dose of viral contamination from a variety of sources, and that viral distribution in the estuary is evenly disseminated.

Virus levels in the estuary either did not correlate or showed a weak positive correlation with salinity, turbidity, pH, CEC, fecal coliform level, % organic matter, % carbonate carbon, % smectite, % kaolinite, % illite, and mean particle diameter. A similar correlation profile was observed when each of these parameters was measured against the fecal coliform number.

This lack of correlation between three measurements (virus numbers, fecal coliform counts and mean particle diameter), and physical/chemical/geologic parameters suggested an examination of the relationship of other factors such as the amount of rainfall. Virus numbers did not correlate with rainfall with the exception of one sampling site. Fecal coliforms correlated with the amount of rainfall at two of the sites tested. Mean particle diameter did not correlate with the amount of rainfall. Other comparisons did not reveal any relationship between virus levels and temperature or the date of sampling.

The study suggests that viruses and fecal coliform numbers in Back Bay are highly variable. The random isolation pattern of these organisms imply a pattern of water movement which is strongly influenced by disturbances such as tidal patterns, motorboat activity, and rainfall. Thus, viral isolations are random, and it is difficult to identify the source of virus input. The water flow toward the mouth of the bay is along the Bay's channel and virus associated with suspended solids are able to reach this area in a short period of time (1 to 3 hours) when the correct conditions are available.

Table of Contents

Title: Role of suspended solids in the survival and transport of enteric viruses in the estuarine environment

I. Research Hypothesis:

Enteric human viruses are shed in excrement and are found in sewage. When raw or treated sewage enters an estuarine environment, the majority of viruses are solids-associated or rapidly associate with suspended solids in the water column. This relationship accentuates virus persistence in the estuarine or marine ecosystem and is thought to be responsible for the hydrotransportation of virus particles to unpolluted waters. The size and composition of suspended solids in a given locality, as well as the chemical/physical aspects of the system, determine the extent of virus movement.

II. Introduction:

The contamination of coastal waters by animal wastes is associated with a variety of sources of pollution including farms (61,79), septic tanks (10,33), sewage effluents of cities and small towns (60), sewage sludges (58,73), and storm runoff (17,68). Enteric viruses, as part of the microbial flora of sewage, must contend with a myriad of soluble and particulate, organic and inorganic fractions which serve either to destroy or protect the infectious nature of the virus. More than 113 different virus types are found in human sewage (22,77), but other animal viruses, originating from domestic and wild animal excreta (25,78), and viruses of fish, plants, and microorganisms (51,52) are part of the flora which enters this environment. Not surprisingly, the greatest research effort has been toward an understanding of the environmental biology of human enteric viruses (2,3,26,29). This research focused on the quantitative and qualitative aspects of suspended solids and human enteric viruses in estuarine waters, a physical/chemical characterization of the suspended particulate matter, and the possible role of flocculation in the removal of suspended solids associated virus from the water column.

III. Review of Pertinent Literature:

A. Estuarine Turbidity: Estimates indicate that approximately 250×10^{14} grams of sediments are transported annually to the ocean and is deposited in marine and mixed (continental-marine) depositional environments (24). These sediments consist of both dissolved and particulate materials derived from land by the chemical and mechanical weathering of rocks and the weathering and/or reworking of older sediments. Approximately 80% of this load is particulate. The sediments are carried to the sea primarily by rivers, however, wind and glaciers are also important locally. Most sediment is deposited in near shore or coastal deposi-

tional environments such as estuaries and deltas, beaches and offshore basins. This deposition, as well as transportation, may also be influenced, if not controlled by human activities. Human activities may contribute to both the amount and type of particulate and dissolved sediments due to accelerated erosion of land and the introduction of both chemical and organic waste products into the depositional system.

The deposition of particulate sediment is dependent upon the nature of the sedimentary particle and the chemistry, biology and energy of the depositional environment (14,55). Estuarine sediments consists primarily of silt and clay with subordinant sand, and are deposited in a generally low-energy environment. Sediment size is defined in terms of the Wentworth scale, given here in phi units (a geometric scale), where sand is -1 to 4.0; silt is 4.0 to 8.0, and clay is finer than 8.0. The deposition or settling of sand and silt size particles in still water is controlled by Stokes's Law which relates settling rate of a given size particle to the specific gravity of the particle and the viscosity of the water. Sands settle out most rapidly and are deposited in the upper reaches of the estuary whereas the finer silts and clays are carried further toward the mouth of the estuary. Although the energy of the estuary is low, it is not still, and particles may remain in suspension due to the action of waves and currents.

The deposition of the clay-size particles is also controlled by Stoke's Law, but it is influenced by the mineralogy of the clay and the chemistry and biological activity of the water. Clays are highly reactive particles which possess electrically charged surfaces and which are either attached to or repulsed from one another by electrical forces. When repulsive forces are greater than attractive forces, the clay particles remain separated and are dispersed; when the opposite is true, the particles aggregate or flocculate forming, in effect, larger size particles which would settle out faster than those particles which are dispersed. Generally, dispersion for a clay mineral is controlled by the water chemistry, including organic constituents. Specifically, dispersion is enhanced when the chemical composition of the water is such that sufficient thickness of "oriented" water may build up around the clay particle and, thus, overcome the influences of the attractive forces. In most instances, potassium and sodium are dispersing ions whereas calcium, magnesium, aluminum, and hydrogen are flocculating ones. However, chemical concentration may be equally important as ionic composition; for example, high concentrations of "dispersing" ions can cause flocculation by the common ion effect. Finally, a dispersing ion for one clay mineral may not necessarily be dispersing for another.

Flocculation may begin when freshwater is mixed with saltwater even in estuaries where the salinity is relatively low (2 - 5 ppt). Flocculation may occur through ingestion of clays by planktonic and bentonic fauna. Those clays which are not flocculated may be transported along the coast or into the ocean (18). Previously deposited, fine-grained sediment may also be resuspended by waves, currents, or storm activity to be carried further out to sea or

along the coast (19). Ordinarily, most fine-grained sediment transport occurs between upper tidal limits and depths of 15 m.

Clay minerals are fine-grained, platey, hydrous alumino-silicates belonging to the phyllosilicate class (41). They are classified primarily on the basis of their internal crystallographic structure and secondarily on the basis of their chemistry. Because of the details of their crystal chemistry, these minerals exhibit unique physical and chemical properties such as ion sorption, ion exchange, sorption of water, shrinkage, swelling, and dispersability (31). They may also adsorb organic matter and other colloidal material. These reactive properties are dependent on clay mineral type and the details of the crystal structure of the clay mineral. The more common clay types include kaolinite, illite, chlorite and smectite. Kaolinite is a two-layered, dioctahedral clay; it is usually coarser in size, exhibits the least ion exchange and water sorption, and is generally less reactive. Illite and chlorite have different structures but exhibit similar size and reactivities which are intermediate between those of kaolinite and smectite. The smectite group (montmorrillonite is the most common species) is a small highly reactive three-layer, di- or trioctanhedral clay exhibiting essential sodium, calcium or magnesium. Most of these clay minerals have polymorphs characterized by differing cationic substitutions within the lattice.

The clay mineral suites associated with most coastal waters are similar to those of the rivers emptying into these waters although differential flocculation may effect clay mineral percentages within the estuary and out to sea. For example, estuaries and marine environments along the northeastern Atlantic coast of the United States exhibit clay mineral suites consisting of chlorite and smectite. The difference is due to the presence of kaolinite and smectite in the Coastal Plain sediments in the southeast. On the west coast, the clay mineral suites are highly variable and consist of varying amounts of kaolinite, illite, chlorite, and smectite. Along the Gulf of Mexico between Texas and Florida, clay mineral composition of coastal waters consists of kaolinite, illite and smectite; kaolinite predominates in the eastern Gulf whereas smectite predominates in the western Gulf.

The composition of suspended solids in estuarine waters is variable, and consists of diatom frustules, dinoflagellates, organic aggregates, mineral grains, opaque particles thought to be of anthropomorphic origin, and particles of iron hydrous oxide (35,66).

Estuarine sediments exhibit considerable variation in the content of organic carbon. Bottom sediments composed mostly of sand usually contain <= 1% organic carbon, in contrast to silts and clays (<= 5%)(63). Anaerobic bottom waters containing abundant vegetal matter or raw sewage may experience organic carbon values of as high as 10 - 20%. Thus, organic matter levels increase in estuarine waters polluted with sewage, and as a result of photo-synthesis (72). Surface waters carry only a small portion of the organic matter; the majority is biodegraded and bioconverted in

sediments. The non-living organic fraction found in water is assumed to be fecal pellets, minute plankton remains, and platelike aggregates possibly formed when air bubbles act as nuclei for adsorption of dissolved organic and inorganic compounds (45). This process, initiated by air and the polymeric excretions of bacteria and plankton, could account for the adsorption of virus to suspended estuarine particles and their eventual disappearance from the water column.

B. Viral Persistence in Estuaries

Enteric viruses naturally bind to cells, tissues, absorptive flocs, and other materials. We can logically assume that this characteristic will be exhibited when virus enters the estuarine environment. Long term persistence of virus in this ecosystem appears to be synonymous with adsorption to sediment. Smith et. al. (70) found that enteric viruses survive from 1.5 to 5 times longer in sediment than in estuarine water. Levels of viruses in sediments are generally greater than the concentration found in overlying waters (5,44).

Virus entering the marine environment via sewage may exist as single or aggregate particles, or as virus adsorbed to sewage solids. Solids-associated enteroviruses in sewage have been observed by several investigators (28,76). Schaub and Sorber (64) and Smith et al. (70) have suggested that viruses from sewage adsorb to estuarine particulates, and are protected from inactivation. Of the types of particulates found in estuaries, suspended solids were more often associated with virus isolation than compact or fluffy sediments (59). It is probable that variations in the levels of free versus adsorbed virus occur constantly as a result of chemical equilibria, changes in salinity, conductivity and pH, physical mixing by tidal and wind activity, as well as natural aggregation properties of suspended matter.

Viral inactivation in seawater (50) can be classified as chemical, physical or biological phenomena. The presence of sodium chloride is considered destructive to free virus in seawater (56), but salinity alone cannot account for the decrease in virus number. Other cations are known to inactivate viruses (for example, rotavirus SA-11 heated to 50C in 2M MgCl2), but have an opposite effect on related species (reovirus type 1) under identical conditions (23).

The protective effect of clay may also be due to adsorption of antiviral enzymes (15,30,34), increased viral protein stability (27), inactivation of ribonucleases (69), adsorption of trace metals by clays (6), or the insertion of virus into clay particle complexes. Protection also appears to be a result of virus morphology, and the placement of the virus on the surface of the suspended particles. For example, tailed bacteriophage which attach to clay via tail proteins are more susceptible to the effects of chlorine (11).

The role of organic matter (6) in the protection of viruses in the estuarine environment is not clear, but estuarine waters are

known to contain soluble animal and plant proteins and polysaccharides, mucilaginous slimes, microbial proteins, detritus, the feces and exudates of marine animals, and seafood processing wastes (9).

The most prominent physical factors which contribute to virus inactivation in the estuarine environment are temperature and solar radiation (7,37). Temperature may be the more important and has received considerable attention (21,46). Colwell and Kaper (16) showed that human enteroviruses were stable and detectable after 46 weeks at 4 C, and over a salinity range of 10-34 ppt. At 25 C virus was rarely detected after 8 weeks. Direct exposure to sunlight, particularly the uv and blue wavelengths, is detrimental to viruses. The presence of clay and algae in marine waters retards sunlight penetration and protects viruses from inactivation.

The presence of certain microorganisms adversely influences the persistence of viruses in the estuarine environment (67); the role of marine vibrios has been examined with respect to virus loss in seawater (49). A possible role of algae and protozoa in the viricidal activity of seawater has been proposed, but the extent of their influence remains unclear (37).

C. Nature of the adsorptive/protective process

Viruses appear to readily adsorb to sand, clay minerals, natural clay mixtures, and other particulate matter found in the estuarine environment (8,12,27,32,36,42,57,63,65). Viruses as hydrophilic colloids, depend on the presence of cations, the pH of the suspending medium (80), the virus isoelectric point, the size and shape of the virus particle, the presence or absence of an envelope, and the charge density on the virion for adsorption. The electrical charge of colloidal particles in natural waters, with reference to clay, is influenced by pH, salinity, concentration of cations, and the concentration and type of clay mineral. Carlson et al. (12) speculated that clay, organic matter, and other suspended particulates in estuarine water retain a net electronegative charge at the pH of the natural water; thus, particles in suspension repel one another. A lowering of the pH and the addition of metal cations to the mixture lowers the electronegativity of the particles wherein the cation is able to bridge the gap between virus and clay particle. Adsorption of virus to solids may also proceed in a fashion similar to the process of solids coagulation where the presence of cations reduces the energy of the particle interaction (48). Interaction energy is the net value of the columbic electrostatic repulsive energy and van der Walls or other energies considered together (53). The metal cation is thought to reduce the repulsive forces on solids and virus allowing shorter range forces such as hydrogen bonding or van der Walls interactions to support binding (71).

Differences in ion concentration occur between river and sea waters and have a direct effect on virus adsorption. Those cations which significantly effect adsorption include Na, K, Ca, Mg; the affect on adsorption of the minor ions are poorly understood. The

net electronegative charge of estuarine particulates results from the dissociation of ionizable groups on the surface of viruses, and the unequal charge distribution on clay minerals. A double cation layer develops on the electronegative particle, the fixed or Stern layer, and the outer, diffuse Gouy layer. In the Gouy layer, the ions move freely, producing a decrease in electrical potential as the distance from the surface increases. A compression of the double layer may occur by an increase in the molar cation concentration or an increase in ion valency. This compression allows suspended particulates to move closer, and permits other bonding interactions (43). By extension, compression of the double layer should occur as sewage effluent or river water particulates are mixed with salt water allowing greater virus-suspended solids interactions.

Lipson and Stotzky (47,48) have stressed the interrelationships of virus adsorption and the cation exchange capacity of a clay mineral. They demonstrated that adsorption occurs mainly to negative sites on montmorillonite and kaolinite, even though the virus is also negatively charged. Adsorption was observed in distilled and saline water, and was not related to the surface area of the clay. They speculate (48) that the pH of the colloidal clay surface is 3-4 units below the pH of the suspension, and that the acidity results in the protonation of the virus particle followed by cation exchange at the clay-virus interface.

Recently, Wait and Sobsey (75) have concluded that in addition to electrostatic interactions, hydrophobic residues may be involved in the adsorption of viruses to particulate matter. This theory was supported by the demonstration of non-polar residues on the picornavirus surface, and by a dramatic increase in the elution of virus from sediments by chaotrophic agents. Studies by Johnson et al. (36) have also noted a similar increase in virus elution from sediment using an isoelectric casein mixture supplemented with phosphatidyl choline.

D. Viral Transport in the marine environment:

From a public health standpoint, viral transport in the marine environment is a critical problem. Adsorption is probably a prerequisite for transport, but both factors need additional evaluation. Several studies have noted the significant public health problem posed by viral transport to bacteriologically approved estuarine waters. Metcalf et al. (54) examined the numbers and types of viruses in wastewater treatment plant effluents flowing into the Houston Ship Channel, at downstream sites on the Channel, and in Galveston Bay. Virus numbers were shown to decrease in direct proportion to the distance of the sampling location downstream from the plants. Virus survival appeared quantitative, resulting in greater isolations of the viruses which had been found in high numbers in plant effluents. Although an approximately 10 fold reduction in virus numbers occurred during transport (3.5 - 23.1 pfu/gal in discharge; 0.3 - 2.5 pfu/gal in channel waters), viable virus was found 8 miles downstream in channel water. Virus

was not detected in water from an upper Galveston Bay site 21 miles from the nearest discharge point. Although no virus was found in Galveston Bay water volumes of 105 gal, and no indication of a public health concern was obvious from fecal coliform counts of this water, virus was found in the shellfish collected at the same site. Virus was also isolated from bottom mud of the ship channel, and from the upper Galveston Bay sampling site, indicating that virus adsorption to natural particulates was responsible, at least in part, for removal of virus from surface water.

Viral transport is not unique to coastal waters, and has been examined in other ecosystems. Reports by Schaiberger et al. (62) and Dahling and Safferman (20) illustrate this point. In the former study, performed at Miami Beach, raw sewage was discharged from a deep marine outfall 3.6 km (2.3 mi) offshore, and at a depth of 44 m. Enteric viruses were not isolated from the water beyond 200 meters of the outfall, but were found in beach sediments. As expected, virus numbers in sediments declined over this distance (78 to 112 pfu/L at the outfall, and 0 to 30 pfu/L at the beach). Bacterial indicators were not found in beach sediments demonstrating the importance of sediment analysis for virus as an integral part of public health sampling regimes.

The latter study resulted in the demonstration of virus in the subartic Tanana River 317 km from a source of domestic pollution. The degree to which virus was solids-associated was not determined in this investigation, but the data indicate that virus transport over long distances is possible when conditions are present which favor virus survival.

Transport of viruses has been difficult to analyze under field conditions. Problems arise from the low numbers of viruses in estuarine waters, the dilution of virus numbers at sampling sites away from a point source of pollution, the rate and direction of suspended solids movement, and the fate of virus at a site of public health significance following transport. Drs. Thomas and Julia Lytle (Gulf Coast Research Laboratory, Ocean Springs, MS; personal communication) investigated the movement of hydrocarbons in estuary models, and have shown that movement is restricted to a 3 to 4 mile distance from the source of contamination. One mechanism of hydrocarbon transport is adsorption to suspended solids. These studies indicate that virus movement may also be limited by the sedimentation processes in estuaries.

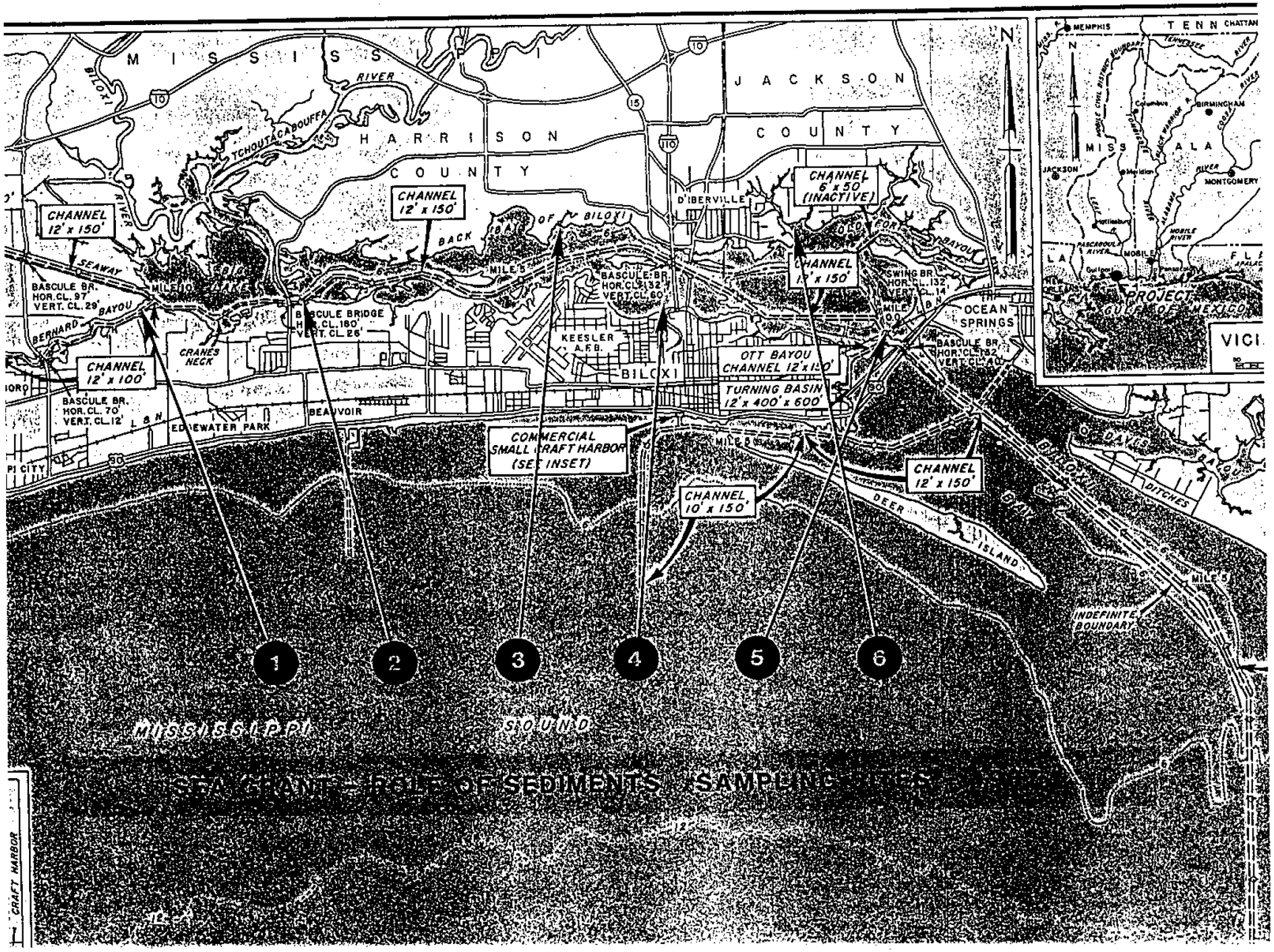
Methods and Procedures:

A. Site Selection and Sample Collection:

Site selection was accomplished by reviewing fecal coliform data for all major estuaries along the Mississippi coast. This information was provided by representatives of the Mississippi Department of Shellfish Sanitation. Estuaries having consistently high fecal coliform loads during 1985-1986 were selected for further evaluation. Preliminary virus samplings were conducted at 5 sites in Biloxi Bay and Bayou Heron during March and April, 1987. These data revealed that virus isolations were more consistent in Biloxi Bay and this site was selected for the overall study. The selection of this site was fortuitous for several reasons. First, low level virus contamination occurred constantly throughout the system allowing an adequate viral profile of the estuary. Second, water change in the estuary was sufficient to follow the movement of virus and other microorganisms. Third, the estuary is of prime importance to state and national agencies governing oyster production and we were privy to information which would not have been available had we worked in another ecosystem.

Back Bay has been closed to shellfishing for over two decades. This closure was the direct result of excessive levels of fecal coliforms entering the estuary at a time when domestic waste water treatment and control of non-point pollution sources were non-existent, or when they became inadequate for the rapidly growing population of this highly desirable urban area.

Back Bay of Biloxi (Figure 1) sampling sites were selected on the basis of the type of discharge entering the system, proximity to other sites, and the incidence of fecal coliforms and viruses at a particular site. Five locations within the estuary were originally selected for detailed sampling; a sixth site was added in June 1987 to evaluate viral input from another portion of the watershed. Site 1 was located at the upper reaches of the estuary in Bayou Benard. This site is surrounded by marsh and is probably influenced by residential areas to the north, south and west and by several camps on the bayou. Site 2 was near the effluent of the West Biloxi sewage treatment plant and on the southern edge of the estuary channel. Site 3 was located in a small inlet across from Keesler Air Force Base which served as a drainage area for a small ditch. North of this ditch was a residential area containing septic tanks. Sampling site 4 was near the East Biloxi sewage treatment plant effluent (Keegan Bayou). Site 5 was located under the railway bridge at the mouth of the bay. The water depth at this site was approximately 30 feet and the water was generally clearer than that observed at other sites. A site which reflected the level of pollution associated with the D'Iberville (septic tank) area on the northern side of the estuary was also sampled (site 6).


MISSISSIPPI
RIVER
BILOXI
TCHOUTACABOUFFA
HARRISON
COUNTY
JACKSON
COUNTY
CHANNEL 12' x 150'
CHANNEL 12' x 150'
CHANNEL 6' x 50' (INACTIVE)
CHANNEL 12' x 150'
CHANNEL 12' x 100'
CHANNEL 12' x 150'
CHANNEL 10' x 150'
CHANNEL 12' x 150'
SEAWAY
BACK BAY OF BILOXI
D'IBERVILLE
BASCULE BR. HOR. CL. 97' VERT. CL. 29'
BASCULE BRIDGE HOR. CL. 180' VERT. CL. 26'
BASCULE BR. HOR. CL. 132' VERT. CL. 60'
SWING BR. HOR. CL. 132' VERT. CL. 14'
BASCULE BR. HOR. CL. 70' VERT. CL. 12'
BASCULE BR. HOR. CL. 132' VERT. CL. 40'
BERNARD BAYOU
CRANES NECK
KEESLER A.F.B.
BILOXI
OCEAN SPRINGS
OTT BAYOU CHANNEL 12' x 150'
TURNING BASIN 12' x 400' x 600'
BEAUVOIR
EDGEWATER PARK
COMMERCIAL SMALL CRAFT HARBOR (SEE INSET)
DEER ISLAND
GULF
INTRACOASTAL WATERWAY
DITCHES
INDEFINITE BOUNDARY
MILE 0.0
MILE 5
MILE 6
MILE 10
MEMPHIS
TENN
CHATTAN
TENNESSEE RIVER
BIRMINGHAM
MONTGOMERY
JACKSON
MISS
ALA
MOBILE
PROJECT
GULF OF MEXICO
1
2
3
4
5
6
MISSISSIPPI SOUND
SEA GRANT - ROLE OF SEDIMENTS - SAMPLING SITES
CRAFT HARBOR

Sampling was conducted at sites 1 - 5 on a monthly basis from April 1987 to December 1988. Sampling at site 6 began in June 1987 and continued to December 1988.

Standard Methods measurements conducted at each site included pH, temperature, depth, turbidity, salinity, time of sampling, and status of the tide. A standard fecal coliform count (A1 procedure) was also performed. Rainfall data was requested from Keesler Air Force Base personnel; data included the amount of rainfall (inches) for the day of sampling and the three preceding days.

One hundred gallons of estuarine water was pumped through a pre-filter and a sterile 0.2 micron bag type filter (Cole-Parmer, N-02913-30); following the filtration procedure the filters were cut into small strips, on site, and the strips placed into 300 ml of 3% beef extract in 0.05 M glycine, pH 9.5. The bottles (media plus strips) were kept on ice and processed upon return to the laboratory. This procedure was used to collect suspended solids for virus isolation.

The quantity of suspended solids necessary for geological and geochemical analysis required that the fluffy layer above the compact sediments be sampled. This was accomplished by removing the filtration equipment from the pumping system and connecting the pump to a sediment sampling device. This device consisted of a length of PVC pipe mounted to a container with a screened opening and sufficient openings to allow water to enter at rapid rate. Twenty five liters of fluffy sediment/water mixture yielded sufficient material to complete the following analyses: cation exchange capacity, % organic carbon, % carbonate carbon, % illite, % smectite, % kaolinite, and size of sediment particles.

B. Sample Processing:

Virus Elution and Quantitation:

Figure 2 demonstrates the steps of elution of virus from the filters used to collect suspended solids (38). Figure 3 lists the steps of concentrate treatment on the day of plaque assay. Figure 4 contains the actual plaque assay procedure. These procedures were highly effective at removing virus from suspended solids and produced sample concentrates which were rarely toxic to cell cultures. All plaque assays were conducted in MA-104 cells (1), passages 43 to 57; counts were made three days after the assay and continued until no new plaques appeared for 2 consecutive days. Plaques in an individual assay were picked and used to inoculate MA-104 cells; isolates demonstrating cytopathic effect were confirmed as the virus count per 100 gallons.

Clay mineral analysis, organic and carbonate carbon analysis, cation exchange capacity, and particle size analysis:

Figure 2

Elution of Virus from suspended solids (100 gal estuarine water)

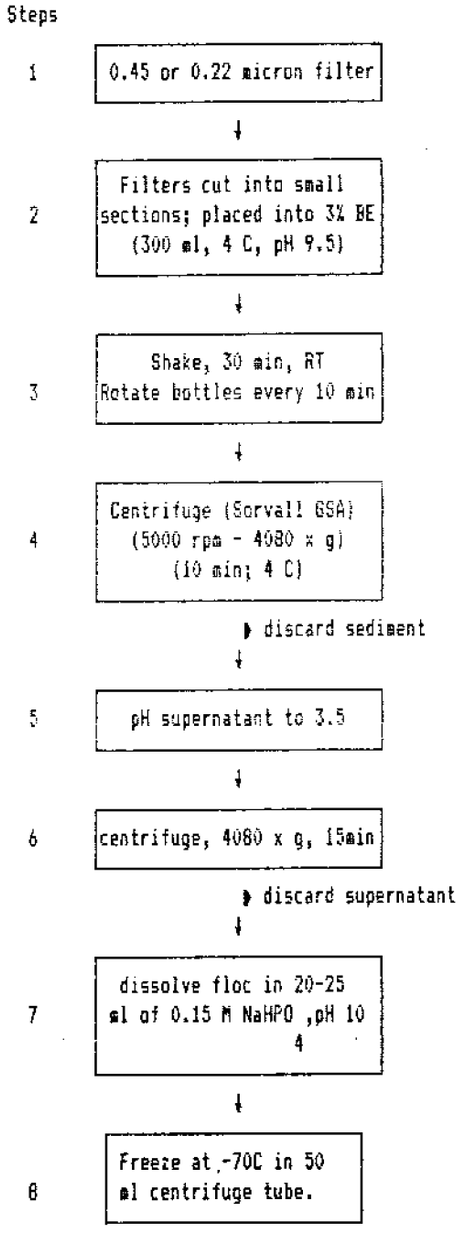


Figure 3 Sample processing on day of Plaque Assay

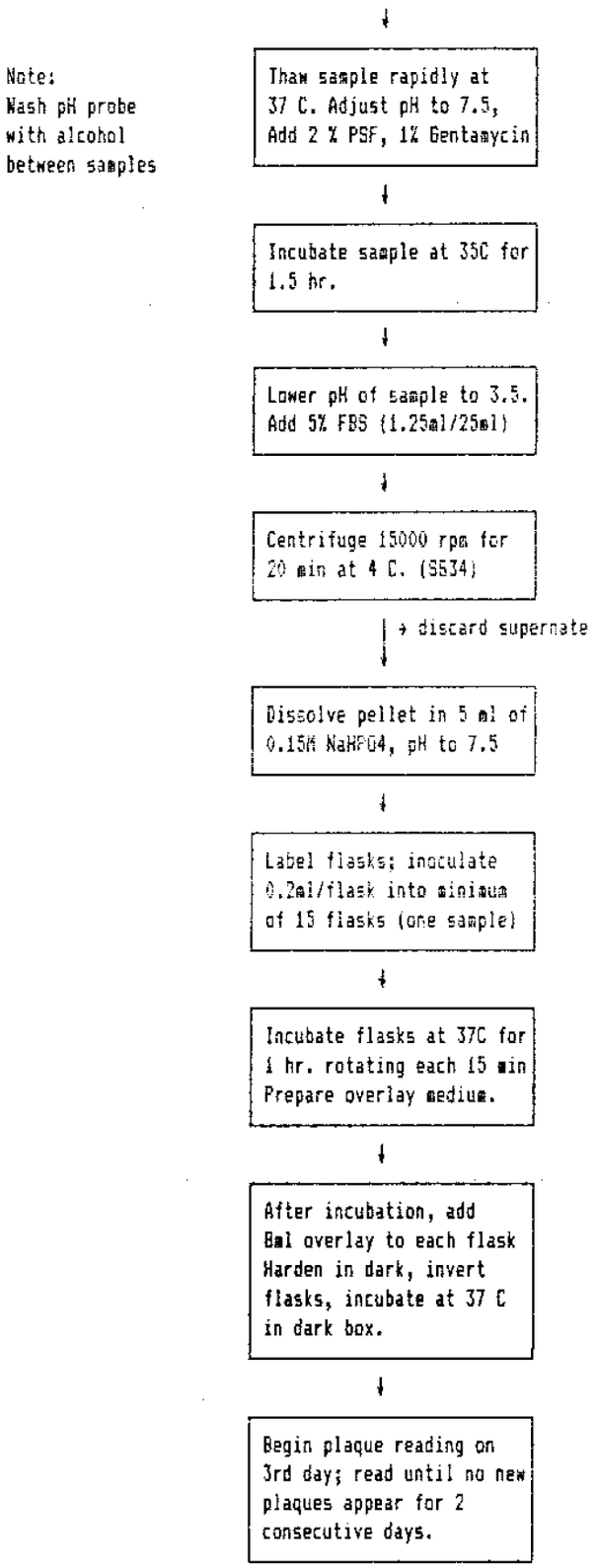


Figure 4. Plaque Assay Methods - Role of Sediments

Needed: 25 sq.cm. confluent MA104 monolayers; 6 days old; note the passage

Sterile 2X autoclavable MEM
Sterile 2% purified agar
100X PSF; 10 ug gentamycin solution
100mM L-glutamine
Fetal Bovine Serum
Neutral Red 1:300
7.5% Sodium bicarbonate

Calculation: The # of flasks times 8 gives the acutal ml of final overlay medium. Always make more than needed.

Procedure: (to make 500 ml)

1. Prepare 2X MEM (yellow); after autoclaving cool to 45C if used on that day or store in refrigerator
2. Prepare 2X agar (need 5 gr in 250 ml); autoclave 20 min.; place in water bath at 50 - 54 C.
3. To 200 ml of sterile (cool) 2X MEM add: 10 ml of 100X PSF, 1 ml gentamycin, 5 ml of L-glutamine, 10 ml FBS, 15 ml 7.5% sodium bicarbonate. Stir the 200 ml volume between each addition.
4. Before you mix the 2X mixture to the 2X agar, add 15 ml of neutral red; then add sufficient yellow 2X MEM to q.s. to 250 ml.
5. Pour the 2X MEM plus supplements mixture into 250 ml of 2X purified agar. Mix well. Store at 50 - 54 C.

Plaque Assay Method:

1. Number all flasks.
2. Assign numbered flasks to individual samples.
3. Pour off media of a group of flasks designated for a particular sample.
4. Inoculate each 25 sq.cm. flask with 0.2 ml sample being carefull not to place the inoculum directly onto the monolayer.
5. Collect all inoculum on one edge of the flask and distribute it across the monolayer.
6. Place the flask aside (box)
7. Repeat steps 3 - 7 for each sample tested.
8. Tighten all caps, check notebook for accuracy, rotate all flasks to redistribute inoculum, place all in box and at 35-35C for 1 hr, redistributing the inoculum each 15 min.

Overlay:

1. After the hour of incubation, bring all flasks to the laminar flow hood. Rinse the overlay bottle with 70% ethanol and place on wad of paper.
2. Carefully and aseptically put 8 ml of overlay first into the control flasks (remember no bubbles) and then into the inoculated flasks. Let agar solidify in the hood with the lights off.
3. When all flasks are hard, invert each flask and place in light tight box.
4. Incubate at 35 - 37 C.
5. Do not distrub for 3 days. Count plaques until the 10th day.

Clay mineral analysis was conducted by X-ray diffraction. Clay minerals were identified and distinguished on the basis of their overall crystallographic structure and interplanar spacings within their lattices (**14**). Quantitative XRD analysis (**4,13,40,74**) permitted the determination of the relative amounts of the various clay minerals present in a sample.

Cation exchange capacity was determined by the methods of Kearns (**39**). Carbon compounds found in association with estuarine sediments were analyzed using a LECO Induction furnace equipped with an automatic sulfur titrator. The method which was used was developed by John Hunt at Wood's Hole Oceanographic Institute (personal communication).

Particle size analysis was accomplished using a model 5000ET Sedigraph, X-ray particle analyzer which has an accuracy to 0.1 microns.

Results

A. Suitability of the Sampling Sites and Methods:

The initial evaluation of possible sampling sites along the Mississippi coast was a valuable contribution to the overall effort in that the final sites selected contained low to moderate levels of fecal coliforms and solids-associated viral isolates during twenty-one months of continuous sampling. The selection of Back Bay sampling sites (Figure 1) was also fortunate since it was a major estuary selected for a comprehensive sanitary survey by the Northeast Technical Services Unit, Shellfish Sanitation Branch, Food and Drug Administration (June 8-18, 1987). We were able to coordinate the virus sampling effort (June 10 & 17, 1987) with this comprehensive effort and take advantage of their results. Of particular interest to this study was the dye release investigation which demonstrated the pattern of water movement in the estuary during several tidal conditions.

The techniques described in Methods and Procedures performed well and indicated that continuous sampling of estuarine waters for solids-associated virus could be commonplace. The 100 gal water samples were filtered at each site in an average of 30 minutes; during that period, the other parameters were measured and the fecal coliform test was performed. The entire 6 sites could be tested in roughly 4.5 hours, including boat time. The actual fecal coliform procedure was modified for these trials and consisted of the following: water samples were collected at each site and immediately inoculated into A-1 dilutions. Tubes were placed in an ice chest and held at approximately 10C until they reached the laboratory (generally 6 to 6.5 hours after the first sample was taken). At the laboratory, each rack was placed into a 44.5C circulating water bath and held for the remainder of its 24 hour incubation . All FC samples were treated in the same manner.

The Corning bag filters (0.20 micron) worked exceptionally well; in no instance was clogging a problem. They were readily cut in the field into strips which were stored in 300 ml 3% BE, pH 9.5, and held on ice until they reached the laboratory where they were immediately processed (steps 3-8). The plaque assay procedure previously described was occasionally susceptible to contamination by fungi or experienced toxicity problems. When they did appear, such problems were usually manifest by the sixth or seventh day of plaque counting and did not significantly interfere with virus counts.

B. Summary of all data collected during the project:

A summary of all raw data (by date of collection) is presented in Table 1. The data is not uniform during the early phase of the investigation. The first three months of the study (January to March, 1987) were used for site selection and equipment purchases and installation. Following selection of Back Bay as

the primary estuary, trial runs were performed during April and May 1987 to field test the sampling protocol. During this period it was determined that suspended solids samples were not able to be obtained in sufficient quantity for geologic analysis (30 gallons of estuarine water yielded <1 gm suspended solids), and an alternate protocol was adopted (collection of 20 liters of fluffy layer yielding 25 to 30 gm). Beginning with the June 10, 1987 sampling, additional changes had been made, including the addition of the fecal coliform count at all sites, and the addition of an additional site (#6) to document the pollution level in another section of the estuary. Note that two sampling trials were performed during June 1987 to coincide with the FDA sanitary survey mentioned earlier. Missing data includes the geologic information from Nov '87 and Jan and Feb '88. An accident occurred in Dr. Isphording's laboratory and the results were lost. Pump failure occurred during the May 1988 sampling and only 20 gallons could be collected per site. Also note that no sediment profiles are available for site 5 during the study. Site 5 was located in the middle of the channel at the mouth of the estuary and had a depth of approximately 30 feet. The constant flow at this site scoured the bottom and no sediment could be collected. The rainfall data collected by Keesler Air Force Base personnel was taken near site 3, but the data is presented as representing all sites.

A computer sort of Table 1, to present all data by Site, is shown in Table 2. This sort procedure was used to arrange the data for ease of statistical analysis as presented in the tables below.

C. Input from the Sanitary Survey:

A comprehensive sanitary survey of the Back Bay of Biloxi was conducted June 8-18, 1987. Of particular interest to this investigation was the data on the efficiency of waste water treatment facilities, tidal flow and dye movement which were collected during this period. The following is an excerpt from that document:

"Overall this comprehensive survey showed Biloxi Bay and Back Bay to be receiving extremely large volumes of pollution from a variety of point and nonpoint sources and the overall estuarine system was experiencing considerable environmental stress. From a public health point of view, two extremely hazardous pollution conditions were observed, the high bacterial MPNs in Back Bay which should preclude this area's use for swimming and water contact sports and the use of tributyl tin boat bottom paints by the large fleet of fishing boats."

Certain facts from this survey which effect the data collected during our investigation include:

1. The wastewater treatment systems often produce erratic treatment results by overloading the plants with seafood processing wastes. Under certain conditions (seasonal) chlorination was ineffective due to the high chlorine demand of the organic matter.

2. The rapid growth of residential and commercial development

Table 1. page 1

DATE	SITE	Gal	Sal	INCH	pH	T(C)	Tide	Filter
42987	1	100	10		7.5	21	I	0.45
42987	2	100	9		7.4	21	I	0.45
42987	4	100	14		7.5	21	I	0.45
42987	5	100	24		7.9	22	I	0.45
51887	1	92	0		6.7	20	N	0.2
51887	2	100	2		7.0	21.5	I	0.2
51887	3	100	5		7.1	23.5	I	0.2
51887	4	100	6		7.3	26	I	0.2
51887	5	100	10		8.1	22	I	0.2
61087	1	100	0	28	7.1	26	I	0.2
61087	2	100	0.5	18	7.0	24	I	0.2
61087	3	100	6	18	7.1	24	I	0.2
61087	4	100	8	19	7.3	24	I	0.2
61087	5	100	16	36	8.3	24.5	I	0.2
61087	6	100	8	19	7.6	25	I	0.2
61787	1	100	0	22	7.1	24.5	I	0.45
61787	2	100	2	21	7.2	24.5	I	0.45
61787	3	100	3	16	7.1	24	I	0.45
61787	4	100	2	15	7.2	24	I	0.45
61787	5	50	7	24	7.8	25	I	0.45
61787	6	100	2	16	7.1	24	I	0.45
72087	1	100	1	26	7.6	27	I	0.45
72087	2	100	6	24	7.5	25	I	0.45
72087	3	100	7	26	7.3	25	N	0.45
72087	4	100	8	25	6.7	25	N	0.45
72087	5	100	14	28	6.3	25	0	0.45
72087	6	100	8	24	7.1	25	0	0.45
81787	1	100	0	16	6.7	23	0	0.22
81787	2	100	0	13	6.5	21	0	0.22
81787	3	100	0	13	6.5	24	0	0.22
81787	4	100	0	12	6.6	22	0	0.22
81787	5	100	0	14	6.5	22	0	0.22
81787	6	100	0	9	6.7	23	0	0.22
92887	1	100	6.5	18	7.9	22	I	0.22
92887	2	100	7	16	7.5	22	I	0.22
92887	3	100	12	16	7.5	22	I	0.22
92887	4	100	12	25	7.8	22.5	I	0.22
92887	5	100	15	27	7.9	23	I	0.22
92887	6	100	15	15	7.7	23	I	0.22
102687	1	100	11	24	7.8	20	0	0.22
102687	2	100	11.5	25	8.4	20	0	0.22
102687	3	100	17.5	19	8.6	20	0	0.22
102687	4	100	15.5	26	8.7	20	0	0.22
102687	5	100	21	32	8.8	20	0	0.22
102687	6	100	20	20	8.6	20	0	0.22
110987	1	100	14	30	7.8	21	0	0.22
110987	2	100	10	30	7.8	21	0	0.22
110987	3	100	19	20	8	20	0	0.22
110987	4	100	18.5	31	7.8	20	0	0.22
110987	5	100	23	32	8.3	20	0	0.22
110987	6	120	21	18	7	20	0	0.22
120987	1	100	15	24	7.3	18	0	0.22
120987	2	100	15	22	7.5	18	0	0.22
121487	1	100	9	31	7.3	21	I	0.22
121487	2	100	14	31	7.3	20	I	0.22
121487	3	100	18	31	7.4	19	I	0.22
121487	4	100	18	30	7.7	19	I	0.22
121487	5	100	25	34	8.5	18	I	0.22
121487	6	100	20	30	8.3	19	I	0.22
012888	1	100	1	24	6.85	10	0	0.22
12888	2	100	2	30	6.9	10	0	0.22
12888	3	100	9	36	7.11	10	0	0.22
12888	4	100	10	32	7.44	10	0	0.22
12888	5	100	18	40	7.88	10	0	0.22
12888	6	100	8	36	8.01	10	0	0.22
022588	1	100	NT	28	6.8	12	0	0.22
22588	2	100	NT	28	6.9	13	0	0.22
22588	3	100	NT	20	6.9	13	0	0.22
22588	4	100	NT	24	7.4	14	0	0.22
22588	5	100	NT	32	7.9	13	0	0.22
22588	6	100	NT	18	7.9	14	0	0.22
031788	1	100	6	28	7.12	15	0	0.22
31788	2	100	8	30	7.43	14	0	0.22
31788	3	100	10	24	7.45	14.5	0	0.22

Table 1. page 2

Time	Depth	CEC	Viruses	FC/100ml	rain-3	rain-2	rain-1	rain 0
700	3		6		0	0	0	0
830	2.5		5		0	0	0	0
930	1.5		5		0	0	0	0
1030	5		0		0	0	0	0
630	2	17.41	3		0.03	0	1.24	0.7
730	2.5	17.95	1		0.03	0	1.24	0.7
1006	1.5	8.7	1		0.03	0	1.24	0.7
1130	1.5	15.78	4		0.03	0	1.24	0.7
1230	5		0		0.03	0	1.24	0.7
655	4.2	21.88	2	2	0	0	0	0
752	3.5	20.04	2	110	0	0	0	0
903	1	16.83	5	800	0	0	0	0
1012	1.5	24.41	1	1700	0	0	0	0
1210	5		2	2	0	0	0	0
1125	2	13.46	1	300	0	0	0	0
730	3.7	10.2	4	300	4	0.21	0.08	2.23
815	2	23.56	5	50	4	0.21	0.08	2.23
905	0.5	15.3	0	90	4	0.21	0.08	2.23
947	2.5	22.2	1	1700	4	0.21	0.08	2.23
1115	5		0	90	4	0.21	0.08	2.23
1036	1.2	12.31	35	13000	4	0.21	0.08	2.23
545	4	10.2	3	170	0	0	0.03	0.01
630	3	17.53	4	800	0	0	0.03	0.01
730	1.5	17	2	280	0	0	0.03	0.01
830	2.5	21.76	3	8000	0	0	0.03	0.01
1015	6		2	30	0	0	0.03	0.01
930	2	31.65	3	1300	0	0	0.03	0.01
612	1	15.15	4	1100	1.33	0.94	1.51	0
650	0.7	21.67	6	1700	1.33	0.94	1.51	0
739	1	18.05	1	90000	1.33	0.94	1.51	0
815	2	22.22	5	800	1.33	0.94	1.51	0
923	5		3	17000	1.33	0.94	1.51	0
847	1	17.98	1	800	1.33	0.94	1.51	0
925	5		33	300	0	0	0	0
1003	6		10	220	0	0	0	0
1045	0.5		8	9400	0	0	0	0
1120	3.5		20	110	0	0	0	0
1238	8		10	75	0	0	0	0
1200	2		7	4900	0	0	0	0
925	1	16.5	22	1800	0	0.05	0	0.12
1030	0.7	14	9	33	0	0.05	0	0.12
1130	1	11.6	0	470	0	0.05	0	0.12
1215	2	12.1	6	3300	0	0.05	0	0.12
200	5		0	0	0	0.05	0	0.12
105	1	16.9	2	2300	0	0.05	0	0.12
1000	1	15.53	13	790	0	0.35	0.05	0
1050	0.7	12.12	0	24000	0	0.35	0.05	0
1130	1	12.9	11	180	0	0.35	0.05	0
1206	2	11.96	3	22000	0	0.35	0.05	0
130	5		1	22	0	0.35	0.05	0
1245	1	33.9	5	490	0		0.05	0
1000	1	7.5	1	220	0	0.68	0.02	0
1050	0.7	20.3	3	22	0	0.68	0.02	0
900	3.7	17.2	0	490	0	0.03	0.03	0.02
945	2	21.2	0	490	0	0.03	0.03	0.02
1040	0.5	17.8	7	43	0	0.03	0.03	0.02
1130	2.5	30.3	0	79	0	0.03	0.03	0.02
115	5		0	49	0	0.03	0.03	0.02
1220	1	32.7	3	790	0	0.03	0.03	0.02
1100	1	3.6	2	79	0	0	0	0
1145	0.7	9.3	8	130	0	0	0	0
1230	1	12.3	3	49	0	0	0	0
110	2	4.4	3	33	0	0	0	0
300	5		11	17	0	0	0	0
200	1	24.1	9	33	0	0	0	0
945	1	14.6	1	23	0	0.07	0.01	0
1030	0.7	16.5	2	26	0	0.07	0.01	0
1115	1	9.6	3	23	0	0.07	0.01	0
1150	2	17.6	5	4	0	0.07	0.01	0
100	5		0	2	0	0.07	0.01	0
1220	1	15.7	5	33	0	0.07	0.01	0
930	1	16.9	3	49	0	0	0	0.05
1010	0.7	13.1	12	110	0	0	0	0.05
1050	1	22.2	23	13	0	0	0	0.05

Table 1. page 3

% Organic C	% co3 C	% Smectite	% Illite	% Kaolinite	Median d (micron)	Mean d (micron)
1.29	0.26	15	0	85	42.25	17.90
0.86	0.41	20	0	80	64.73	11.37
0.77	0.09	36	8	56	6.46	4.31
1.34	0.18	40	10	50	27.51	13.42
0.00	0.00	0	0	0	0.00	0.00
0.92	0.31	32	5	63	3.25	3.86
1.11	0.13	0	0	100	50.55	26.33
1.26	0.07	1	0	99	16.69	10.88
1.42	0.33	32	16	52	17.99	8.65
1.04	0.41	45	0	55	2.29	3.42
0.00	0.00	0	0	0	0.00	0.00
0.67	0.09	50	9	41	3.96	4.94
1.26	0.22	0.5	0.5	99	48.22	27.76
0.00	0.00	0	0	0	0.00	0.00
1.18	0.51	38	0	62	5.95	5.83
0.81	0.37	40	10	50	2.48	3.30
0.00	0.00	0	0	0	0.00	0.00
0.93	0.16	55	0	45	7.09	5.3
1.02	0.44	52	5	43	1.615	3.59
0.83	0.87	59	nd	41	1.611	3
0	0	0	0	0	0	0
1.87	0.69	46	7	47	1.3779	5.91
1.41	0.28	36	10	54	1.107	1.46
0.99	0.24	29	8	63	61.803	13.35
0.63	0.91	44	7	49	14.19	7.19
0	0	0	0	0	0	0
2.61	1.02	52	6	42	1.697	3.882
1.63	0.72	42	12	46	1.217	3.198
1.01	0.51	51	nd	49	13.881	4.253
2.33	0.55	58	nd	42	2.371	5.002
3.04	0.67	42	5	53	20.518	8.524
0	0	0	0	0	0	0
1.03	0.08	44	8	48	20.268	8.679
0.77	0.11	53	nd	47	15.161	9.096
1.09	0.28	41	Trace	59	11.845	7.882
0.84	1.07	32	nd	68	15.2	7.924
2.22	0.81	39	Trace	61	28.112	11.498
0	0	0	0	0	0	0
1.26	1.04	45	nd	55	25.075	8.75
1.63	1.31	21	3	76	78.73	32.848
0.85	1.81	56	3	41	40.37	20.596
1.31	1.22	56	9	35	41.32	12.441

Table 1. page 4

StDv	Skewness	Kurtosis
3.12	0.753	0.733
4.06	0.602	0.616
3.35	0.140	0.528
2.83	0.741	0.763
0.00	0.000	0.000
2.59	-0.138	0.538
2.91	1.234	1.219
3.20	0.184	0.647
3.09	0.458	0.605
2.56	-0.280	0.578
0.00	0.000	0.000
2.20	-0.437	0.709
2.84	1.540	1.511
0.00	0.000	0.000
2.75	-0.001	0.567
3.16	-0.159	0.561
0.00	0.000	0.000
2.41	-0.412	0.775
3.33	-0.345	0.302
4.48	-0.2	0.219
0	0	0
4.42	-0.104	0.222
0.565	-0.07	0.186
3.15	0.649	1.176
3.85	0.225	0.185
0	0	0
3.52	-0.339	0.246
3.9	-0.357	0.199
5.43	0.314	0.197
2.89	-0.373	0.488
2.88	0.44	0.261
0	0	0
2.794	0.438	0.27
2.69	0.273	0.296
2.69	0.244	0.243
2.73	0.344	0.259
0	0.424	0.255
2.81	0	0
	5.41	0.269
2.99	0.634	1.291
2.84	0.441	0.85
3.11	0.557	0.464

Table 1. page 5

31788	4	100	12	30	7.75	14.5	0	0.22
31788	5	100	18	32	8.83	14.5	0	0.22
31788	6	100	14	20	7.5	15	0	0.22
42688	1	100	4	12	7.5	23	I	0.22
42688	2	100	5	12	7.7	24	I	0.22
42688	3	100	6.5	14	7.8	25	I	0.22
42688	4	100	10	12	7.7	25	I	0.22
42688	5	100	12	24	8	26	I	0.22
42688	6	100	10	13	7.6	25	I	0.22
52388	1	20	9	30	7.6	27	0	0.22
52388	2	20	10	34	7.8	24	0	0.22
52388	3	20	12	36	7.3	25	0	0.22
52388	4	20	12	40	8.1	25	0	0.22
52388	6	20	13	30	7.4	26	0	0.22
61588	1	100	12	25	8.3	27	I	0.22
61588	2	100	12	27	8.1	26	I	0.22
61588	3	100	17	34	8.6	28	I	0.22
61588	4	100	15	22	8.3	27	I	0.22
61588	5	100	21	30	8.4	27	I	0.22
61588	6	100	16	23	8.5	27	I	0.22
71288	1	100	12	16	8.5	30	I	0.22
71288	2	100	14	18	7.9	28	I	0.22
71288	3	100	15	16	8.3	28	I	0.22
71288	4	100	16	12	8	28	I	0.22
71288	5	100	21	20	7.9	29	I	0.22
71288	6	100	19	18	8.4	29	I	0.22
80488	1	100	9	20	8.2	31	I	0.22
80488	2	100	11	24	7.7	31	I	0.22
80488	3	100	12	20	8.4	31	I	0.22
80488	4	100	13	28	8.5	31	I	0.22
80488	5	100	15	32	8.4	29	I	0.22
80488	6	100	15	24	8	30	I	0.22
92388	1	100	2	12	7.86	31	I	0.22
92388	2	100	3	14	7.76	30	I	0.22
92388	3	100	3	12	7.68	31	I	0.22
92388	4	100	4	18	7.75	30	I	0.22
92388	5	100	6	28	7.72	30	I	0.22
92388	6	100	5	20	7.45	30	I	0.22
100688	1	100	2	18	7.19	21	I	0.22
100688	2	100	2	20	7.29	21	I	0.22
100688	3	100	2	18	7.81	22	I	0.22
100688	4	100	2	12	7.97	19	I	0.22
100688	5	100	10	30	8.3	19	I	0.22
100688	6	100	6	14	7.77	20	I	0.22
111188	1	100	10	16	8.56	21	0	0.22
111188	2	100	10	30	8.03	22	0	0.22
111188	3	100	12	23	8.4	22	0	0.22
111188	4	100	13	18	8.22	22	0	0.22
111188	5	100	15	24	8.37	20	0	0.22
111188	6	100	14	12	8.3	19	0	0.22
120988	1	100	8	24		15	0	0.22
120988	2	100	7	20	5.9	16	0	0.22
120988	3	100	10	30	8.4	15	0	0.22
120988	4	100	10	24	8.3	15	0	0.22
120988	5	100	15	36	8.6	15	0	0.22
120988	6	100	14	12	8.4	15	0	0.22

Table 1. page 6

1125	2	27.1	5	5	0	0	0	0.05
110	5		4	4	0	0	0	0.05
1200	1	17.6	12	49	0	0	0	0.05
1000	5	10.1	1	130	0	0	0	0
1045	6	18.9	26	2400	0	0	0	0
1130	0.5	25.7	4	540	0	0	0	0
1215	3.5	31.9	18	1400	0	0	0	0
200	8		26	23	0	0	0	0
100	2	42.1	3	190	0	0	0	0
945	1	27.4	3	220	0	0.6	0.15	0.02
1050	0.7	17.6	2	23	0	0.6	0.15	0.02
1130	1	18	12	70	0	0.6	0.15	0.02
1230	2	7.6	0	2200	0	0.6	0.15	0.02
130	1	64.1	1	79	0	0.6	0.15	0.02
1220	5	24.1	3	23	0	0	0	0
1140	6	27.7	5	23	0	0	0	0
1106	2	27	6	33	0	0	0	0
1030	3.5	11.3	8	23	0	0	0	0
910	9		3	49	0	0	0	0
950	2	61.7	5	170	0	0	0	0
1120	3	31.8	4	230	0	0.4	0	0
1045	3	42.1	9	79	0	0.4	0	0
1005	2	22.8	8	33	0	0.4	0	0
935	4	11.3	9	79	0	0.4	0	0
835	6		3	49	0	0.4	0	0
900	2	25.7	12	46	0	0.4	0	0
100	3	25.2	12	130	0	0	0	0
1215	3	34.2	7	180	0	0	0	0
1130	2	23.4	11	23	0	0	0	0
1105	4	35.8	7	70	0	0	0	0
945	6		12	23	0	0	0	0
1020	2	61.7	13	130	0	0	0	0
215	3	56.7	16	1700	0	0	0	0
145	3	27.4	11	110	0	0	0	0
100	2	48.3	9	220	0	0	0	0
1230	4	7.4	1	350	0	0	0	0
1050	6		8	43	0	0	0	0
1120	2	44.1	14	180	0	0	0	0
230	3	36.4	13	23	0	0	0	0
145	3	27.4	12	2200	0	0	0	0
115	2	50.5	6	790	0	0	0	0
1230	4	15.9	5	2200	0	0	0	0
1100	6		3	49	0	0	0	0
1145	2	47.7	0	3300	0	0	0	0
150	1	26.8	3	110	0	0	0	0
1230	0.7	18.5	3	33	0	0	0	0
1200	1	57.5	3	23	0	0	0	0
1115	2	16	1	49	0	0	0	0
945	5		0	2	0	0	0	0
1015	1	66.8	0	1600	0	0	0	0
1245	1	24.4	0	94	0	0	0.12	0.01
1215	0.7	28.7	12	79	0	0	0.12	0.01
1130	1	103	23	70	0	0	0.12	0.01
1100	4	39	2	79	0	0	0.12	0.01
930	6		0	17	0	0	0.12	0.01
1000	1	121	0	280	0	0	0.12	0.01

Table 1. page 7

0.12	1.01	50	8	42	164.57	43.677
1.77	0.98	56	3	41	4.11	4.497
1.2	1.34	42	6	52	105.35	39.574
2.4	1.64	49	5	46	36.58	22.11
0.43	1.62	50	7	43	30.83	11.845
0.63	1.12	60	5	35	16.21	13.424
0.8	1.92	52	4	43	15.44	9.337
0.98	1.79	41	5	54	20.75	14.248
0.41	1.9	53	5	42	104.94	41.277
0.98	0.65	48	7	45	161.39	43.099
1.38	1.95	46	5	49	11.16	7.855
2.46	1.15	39	5	56	69.59	37.1
2.85	1.39	41	11	48	27.63	13.372
0.37	1.72	54	7	39	64.32	26.109
0.47	1.42	55	7	38	95.83	65.027
1.18	3.15	43	7	50	13.28	7.932
1.4	1.53	52	8	40	0.7	1.1
1.92	1.11	38	10	52	8.84	7.65
1.6	1.12	39	trace	61	30.84	16.01
0.3	1.47	48	trace	52	0.1	0.2
2.4	2.58	38	15	47	0.74	1.59
1.61	0.76	39	10	51	8.3	2.7
1.04	1.66	46	5	49	2.09	2.49
0.37	1.63	40	11	49	8.96	7.23
0.29	6.03	51	7	42	28.62	19.41
2.59	2.07	40	8	52	24.66	12.24
1.32	2.49	60	12	28	1.8	0.9
0.21	4.36	46	14	40	31.74	16.61
1.82	1.08	48	12	40	31.66	19.2
0.18	1.2	46	6	48	28.37	5.23
1.04	2.11	40	12	48	1.39	1.96
1.45	3.01	39	trace	61	26.3	19.1
0.55	1.43	58	16	24	126.44	71.91
2.92	2.02	40	10	50	22.88	10.63
0.03	8.02	35	10	55	43.21	29.22
1.81	1.48	54	10	36	34.13	20.81
2.08	1.37	47	12	41	37.2	19.2
0.7	1.04	54	10	36	37.66	19.8
1.88	2.2	50	7	43	22.48	12.47
0.79	1.83	44	trace	56	28.3	12.03
2.51	1.49	36	10	54	3.39	3.92
1.74	1.61	58	15	27	9.92	3.5
0.89	1.09	42		58	25.65	11.18
2.04	3.71	47	5	48	1.96	2.01
1.8	0.97	39		61	8.96	7.23
1.27	2.22	35	9	56	9.57	10.78

Table 1. page 8

2.62	0.731	1.162
2.55	-0.107	0.694
2.35	0.602	0.828
2.6	0.364	0.893
2.83	0.424	0.771
3.04	0.059	0.724
2.44	0.229	0.705
2.8	0.149	0.72
2.68	0.563	1.477
3	0.657	1.559
2.75	0.185	0.329
2.37	0.496	1.545
2.66	0.135	0.903
2.24	0.603	1.296
2.03	0.276	1.339
2.65	0.28	0.311
3.6	0.26	0.55
2.56	0.14	0.54
2.2	0.71	1.21
5.57	0.19	0.53
3.37	0.87	0.81
4.52	0.48	0.58
3.08	0.03	0.49
3.05	1.77	1.31
2.44	0.07	0.42
2.16	0.62	0.74
4.66	0.51	0.58
1.97		
2.63		
5.6	1.06	0.52
1.52	1.34	1.41
2.77		
1.92		
2.23	0.97	0.68
5.01		
2.83	0.56	0.53
2.43	0.69	1.3
2.64	0.86	0.78
1.71	0.62	0.6
1.82	0.72	0.65
2.71	0.14	0.48
3.71	0.56	0.57
2.5	0.65	0.74
2.17	1.42	1.37
3.05	0.16	0.52
2.76	0.06	0.47

Table 2. page 1

DATE	SITE	Sal	Sal	INCH	pH	T(C)	Tide	Filter
42987	1	100	10		7.5	21	I	0.45
51887	1	92	0		6.7	20	N	0.2
61087	1	100	0	28	7.1	26	I	0.2
61787	1	100	0	22	7.1	24.5	I	0.45
72087	1	100	1	26	7.6	27	I	0.45
81787	1	100	0	16	6.7	23	O	0.22
92887	1	100	6.5	18	7.9	22	I	0.22
102687	1	100	11	24	7.8	20	O	0.22
110987	1	100	14	30	7.8	21	O	0.22
120987	1	100	15	24	7.3	18	O	0.22
121487	1	100	9	31	7.3	21	I	0.22
42987	2	100	9		7.4	21	I	0.45
51887	2	100	2		7.0	21.5	I	0.2
61087	2	100	0.5	18	7.0	24	I	0.2
61787	2	100	2	21	7.2	24.5	I	0.45
72087	2	100	6	24	7.5	25	I	0.45
81787	2	100	0	13	6.5	21	O	0.22
92887	2	100	7	16	7.5	22	I	0.22
102687	2	100	11.5	25	8.4	20	O	0.22
110987	2	100	10	30	7.8	21	O	0.22
120987	2	100	15	22	7.5	18	O	0.22
121487	2	100	14	31	7.3	20	I	0.22
51887	3	100	5		7.1	23.5	I	0.2
61087	3	100	6	18	7.1	24	I	0.2
61787	3	100	3	16	7.1	24	I	0.45
72087	3	100	7	26	7.3	25	N	0.45
81787	3	100	0	13	6.5	24	O	0.22
92887	3	100	12	16	7.5	22	I	0.22
102687	3	100	17.5	19	8.6	20	O	0.22
110987	3	100	19	20	8	20	O	0.22
121487	3	100	18	31	7.4	19	I	0.22
42987	4	100	14		7.5	21	I	0.45
51887	4	100	6		7.3	26	I	0.2
61087	4	100	8	19	7.3	24	I	0.2
61787	4	100	2	15	7.2	24	I	0.45
72087	4	100	8	25	6.7	25	N	0.45
81787	4	100	0	12	6.6	22	O	0.22
92887	4	100	12	25	7.8	22.5	I	0.22
102687	4	100	15.5	26	8.7	20	O	0.22
110987	4	100	18.5	31	7.8	20	O	0.22
121487	4	100	18	30	7.7	19	I	0.22
42987	5	100	24		7.9	22	N	0.45
51887	5	100	10		8.1	22	I	0.2
61087	5	100	16	36	8.3	24.5	I	0.2
61787	5	50	7	24	7.8	25	I	0.45
72087	5	100	14	28	8.3	25	O	0.45
81787	5	100	0	14	6.5	22	O	0.22
92887	5	100	15	27	7.9	23	I	0.22
102687	5	100	21	32	8.8	20	O	0.22
110987	5	100	23	32	8.3	20	O	0.22
121487	5	100	25	34	8.5	18	I	0.22
61087	6	100	8	19	7.6	25	I	0.2
61787	6	100	2	16	7.1	24	I	0.45
72087	6	100	8	24	7.1	25	O	0.45
81787	6	100	0	9	6.7	23	O	0.22
92887	6	100	15	15	7.7	23	I	0.22
102687	6	100	20	20	8.6	20	O	0.22
110987	6	120	21	18	7	20	O	0.22
121487	6	100	20	30	8.3	19	I	0.22
012888	1	100	1	24	6.85	10	O	0.22
022588	1	100	NT	28	6.8	12	O	0.22
031788	1	100	NT	28	7.12	15	O	0.22
42588	1	100	4	12	7.5	23	I	0.22
52388	1	20	9	30	7.6	27	O	0.22
61588	1	100	12	25	8.3	27	I	0.22
71288	1	100	12	16	8.5	30	I	0.22
80488	1	100	9	20	8.2	31	I	0.22
92388	1	100	2	12	7.86	31	I	0.22
100688	1	100	2	18	7.19	21	I	0.22
111188	1	100	10	18	8.56	21	O	0.22
120988	1	100	8	24		15	O	0.22
12888	2	100	2	30	6.9	10	O	0.22
22588	2	100	NT	28	6.9	13	O	0.22
31788	2	100	NT	30	7.43	14	O	0.22

Table 2. page 2

Time	Depth	CEC	Viruses	FC/100ml	rain-3	rain-2	rain-1	rain 0
700	5		6		0	0	0	0
630	2	17.41	3		0.03	0	1.24	0.7
655	4.2	21.88	2	2	0	0	0	0
730	3.7	10.2	4	300	4	0.21	0.08	2.23
545	4	10.2	3	170	0	0	0.03	0.01
612	1	15.15	4	1100	1.33	0.94	1.51	0
925	5		33	300	0	0	0	0
925	1	16.5	22	1800	0	0.05	0	0.12
1000	1	15.53	13	790	0	0.35	0.05	0
1000	1	7.5	1	220	0	0.68	0.02	0
900	3.7	17.2	0	490	0	0.03	0.03	0.02
830	2.5		5		0	0	0	0
730	2.5	17.95	1		0.03	0	1.24	0.7
752	3.5	20.04	2	110	0	0	0	0
815	2	23.56	5	50	4	0.21	0.08	2.23
630	3	17.53	4	800	0	0	0.03	0.01
650	0.7	21.67	6	1700	1.33	0.94	1.51	0
1003	6		10	220	0	0	0	0
1030	0.7	14	9	33	0	0.05	0	0.12
1050	0.7	12.12	0	24000	0	0.35	0.05	0
1050	0.7	20.3	3	22	0	0.68	0.02	0
945	2	21.2	0	490	0	0.03	0.03	0.02
1006	1.5	8.7	1		0.03	0	1.24	0.7
903	1	16.83	5	800	0	0	0	0
905	0.5	15.3	0	90	4	0.21	0.08	2.23
730	1.5	17	2	280	0	0	0.03	0.01
739	1	18.05	1	90000	1.33	0.94	1.51	0
1045	0.5		8	9400	0	0	0	0
1130	1	11.6	0	470	0	0.05	0	0.12
1130	1	12.9	11	180	0	0.35	0.05	0
1040	0.5	17.8	7	43	0	0.03	0.03	0.02
930	1.5		5		0	0	0	0
1130	1.5	15.78	4		0.03	0	1.24	0.7
1012	1.5	24.41	1	1700	0	0	0	0
947	2.5	22.2	1	1700	4	0.21	0.08	2.23
830	2.5	21.76	3	8000	0	0	0.03	0.01
815	2	22.22	5	800	1.33	0.94	1.51	0
1120	3.5		20	110	0	0	0	0
1215	2	12.1	6	3300	0	0.05	0	0.12
1206	2	11.96	3	22000	0	0.35	0.05	0
1130	2.5	30.3	0	79	0	0.03	0.03	0.02
1030	5		0		0	0	0	0
1230	5		0		0.03	0	1.24	0.7
1210	5		2	2	0	0	0	0
1115	5		0	90	4	0.21	0.08	2.23
1015	6		2	30	0	0	0.03	0.01
923	5		3	17000	1.33	0.94	1.51	0
1238	8		10	75	0	0	0	0
200	5		0	0	0	0.05	0	0.12
130	5		1	22	0	0.35	0.05	0
115	5		0	49	0	0.03	0.03	0.02
1125	2	13.46	1	300	0	0	0	0
1036	1.2	12.31	35	15000	4	0.21	0.08	2.23
930	2	31.65	3	1300	0	0	0.03	0.01
847	1	17.98	1	800	1.33	0.94	1.51	0
1200	2		7	4900	0	0	0	0
105	1	16.9	2	2300	0	0.05	0	0.12
1245	1	33.9	5	490	0		0.05	0
1220	1	32.7	3	790	0	0.03	0.03	0.02
1100	1	3.6	2	79	0	0	0	0
945	1	14.6	1	23	0	0.07	0.01	0
930	1	16.9	3	49	0	0	0	0.05
1000	5	10.1	1	130	0	0	0	0
945	1	27.4	3	220	0	0.6	0.15	0.02
1220	5	24.1	3	23	0	0	0	0
1120	3	31.8	4	230	0	0.4	0	0
100	3	25.2	12	130	0	0	0	0
215	3	56.7	16	1700	0	0	0	0
230	3	36.4	13	23	0	0	0	0
150	1	26.8	3	110	0	0	0	0
1245	1	24.4	0	94	0	0	0.12	0.01
1145	0.7	9.3	8	130	0	0	0	0
1030	0.7	16.5	2	26	0	0.07	0.01	0
1010	0.7	13.1	12	110	0	0	0	0.05

Table 2. page 3

% Organic C	% co3 C	% Smectite	% Illite	% Kaolinite	Median d (micron)	Mean d (micron)
1.29	0.26	15	0	85	42.25	17.90
1.11	0.13	0	0	100	50.55	26.33
1.26	0.22	0.5	0.5	99	48.22	27.76
1.63	0.72	42	12	46	1.217	3.198
0.77	0.11	53	nd	47	15.161	9.096
0.86	0.41	20	0	80	64.73	11.37
1.26	0.07	1	0	99	16.69	10.88
0.00	0.00	0	0	0	0.00	0.00
1.41	0.28	36	10	54	1.107	1.46
1.01	0.51	51	nd	49	13.881	4.253
1.09	0.28	41	Trace	59	11.845	7.882
0.77	0.09	36	8	56	6.46	4.31
1.42	0.33	32	16	52	17.99	8.65
1.18	0.51	38	0	62	5.95	5.83
1.02	0.44	52	5	43	1.615	3.59
0.93	0.24	29	8	63	61.803	13.35
2.33	0.55	58	nd	42	2.371	5.002
0.84	1.07	32	nd	68	15.2	7.924
1.34	0.18	40	10	50	27.51	13.42
1.04	0.41	45	0	55	2.29	3.42
0.81	0.37	40	10	50	2.48	3.30
0.83	0.87	59	nd	41	1.611	3
0.63	0.91	44	7	49	14.19	7.19
3.04	0.67	42	5	53	20.518	8.524
2.22	0.81	39	Trace	61	28.112	11.498
0	0	0	0	0	0	0
0.92	0.31	32	5	63	3.25	3.86
0.67	0.09	50	9	41	3.96	4.94
0.93	0.16	55	0	45	7.09	5.3
1.87	0.69	46	7	47	1.3779	5.91
2.61	1.02	52	6	42	1.697	3.882
1.03	0.08	44	8	48	20.268	8.679
1.26	1.04	45	nd	55	25.075	8.75
1.63	1.31	21	3	76	78.73	32.848
1.2	1.34	42	6	52	105.35	39.574
0.98	1.79	41	5	54	20.75	14.248
2.46	1.15	39	5	56	69.59	37.1
1.4	1.53	52	8	40	0.7	1.1
1.61	0.76	39	10	51	8.3	2.7
1.32	2.49	60	12	28	1.8	0.9
1.45	3.01	39	trace	61	26.3	19.1
2.08	1.37	47	12	41	37.2	19.2
1.74	1.61	58	15	27	9.92	3.5
0.85	1.81	56	3	41	40.37	20.596

Table 2. page 4

StDv	Skewness	Kurtosis
3.12	0.753	0.733
2.91	1.234	1.219
2.84	1.540	1.511
3.9	-0.357	0.199
2.69	0.273	0.296
4.06	0.602	0.616
3.20	0.184	0.647
0.00	0.000	0.000
0.565	-0.07	0.186
5.43	0.314	0.197
2.69	0.244	0.243
3.35	0.140	0.528
3.09	0.458	0.605
2.75	-0.001	0.567
3.33	-0.345	0.302
3.15	0.649	1.176
2.89	-0.373	0.488
2.73	0.344	0.239
2.83	0.741	0.763
2.56	-0.260	0.578
3.16	-0.159	0.561
4.48	-0.2	0.219
3.85	0.225	0.185
2.88	0.44	0.261
0	0.424	0.255
2.81	0	0
2.59	-0.138	0.538
2.20	-0.437	0.709
2.41	-0.412	0.775
4.42	-0.104	0.222
3.52	-0.339	0.246
2.794	0.438	0.27
	5.41	0.289
2.99	0.634	1.291
2.35	0.602	0.828
2.8	0.149	0.72
2.37	0.496	1.545
3.6	0.26	0.55
4.52	0.48	0.58
4.66	0.51	0.58
2.77		
2.43	0.69	1.3
3.71	0.56	0.57
2.84	0.441	0.85

Table 2. page 5

42688	2	100	5	12	7.7	24	I	0.22
52388	2	20	10	34	7.8	24	0	0.22
61588	2	100	12	27	8.1	26	I	0.22
71288	2	100	14	18	7.9	28	I	0.22
80488	2	100	11	24	7.7	31	I	0.22
92388	2	100	3	14	7.75	30	I	0.22
100688	2	100	2	20	7.29	21	I	0.22
111188	2	100	10	30	8.03	22	0	0.22
120988	2	100	7	20	5.9	16	0	0.22
12888	3	100	9	36	7.11	10	0	0.22
22588	3	100	NT	20	6.9	13	0	0.22
31788	3	100	NT	24	7.45	14.5	0	0.22
42688	3	100	6.5	14	7.8	25	I	0.22
52388	3	20	12	36	7.3	25	0	0.22
61588	3	100	17	34	8.6	28	I	0.22
71288	3	100	15	16	8.3	28	I	0.22
80488	3	100	12	20	8.4	31	I	0.22
92388	3	100	3	12	7.68	31	I	0.22
100688	3	100	2	18	7.81	22	I	0.22
111188	3	100	12	23	8.4	22	0	0.22
120988	3	100	10	30	8.4	15	0	0.22
12888	4	100	10	32	7.44	10	0	0.22
22588	4	100	NT	24	7.4	14	0	0.22
31788	4	100	NT	30	7.75	14.5	0	0.22
42688	4	100	10	12	7.7	25	I	0.22
52388	4	20	12	40	8.1	25	0	0.22
61588	4	100	15	22	8.3	27	I	0.22
71288	4	100	16	12	8	28	I	0.22
80488	4	100	13	28	8.5	31	I	0.22
92388	4	100	4	19	7.75	30	I	0.22
100688	4	100	2	12	7.97	19	I	0.22
111188	4	100	13	18	8.22	22	0	0.22
120988	4	100	10	24	8.3	15	0	0.22
12888	5	100	18	40	7.88	10	0	0.22
22588	5	100	NT	32	7.9	13	0	0.22
31788	5	100	NT	32	8.83	14.5	0	0.22
42688	5	100	12	24	8	26	I	0.22
61588	5	100	21	30	8.4	27	I	0.22
71288	5	100	21	20	7.9	29	I	0.22
80488	5	100	15	32	8.4	29	I	0.22
92388	5	100	6	28	7.72	30	I	0.22
100688	5	100	10	30	8.3	19	I	0.22
111188	5	100	15	24	8.37	20	0	0.22
120988	5	100	15	36	8.6	15	0	0.22
12888	6	100	8	36	8.01	10	0	0.22
22588	6	100	NT	18	7.9	14	0	0.22
31788	6	100	NT	20	7.5	15	0	0.22
42688	6	100	10	13	7.6	25	I	0.22
52388	6	20	13	30	7.4	26	0	0.22
61588	6	100	16	23	8.5	27	I	0.22
71288	6	100	19	18	8.4	29	I	0.22
80488	6	100	15	24	8	30	I	0.22
92388	6	100	3	20	7.45	30	I	0.22
100688	6	100	6	14	7.77	20	I	0.22
111188	6	100	14	12	8.3	19	0	0.22
120988	6	100	14	12	8.4	15	0	0.22

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1045	6	19.9	26	2400	0	0	0	0
1050	0.7	17.6	2	23	0	0.6	0.15	0.02
1140	6	27.7	5	23	0	0	0	0
1045	3	42.1	9	79	0	0.4	0	0
1215	3	34.2	7	180	0			
145	3	27.4	11	110	0	0	0	0
145	3	27.4	12	2200	0	0	0	0
1230	0.7	18.5	3	33	0	0	0	0
1215	0.7	28.7	12	79	0	0	0.12	0.01
1230	1	12.3	3	49	0	0	0	0
1115	1	9.6	3	23	0	0.07	0.01	0
1050	1	22.2	23	13	0	0	0	0.05
1130	0.5	25.7	4	540	0	0	0	0
1130	1	18	12	70	0	0.6	0.15	0.02
1106	2	27	6	33	0	0	0	0
1005	2	22.8	8	33	0	0.4	0	0
1130	2	23.4	11	23	0			
100	2	48.3	9	220	0	0	0	0
115	2	50.5	6	790	0	0	0	0
1200	1	57.5	3	23	0	0	0	0
1130	1	103	23	70	0	0	0.12	0.01
110	2	4.4	3	33	0	0	0	0
1150	2	17.6	5	4	0	0.07	0.01	0
1125	2	27.1	5	5	0	0	0	0.05
1215	3.5	31.9	18	1400	0	0	0	0
1230	2	7.6	0	2200	0	0.6	0.15	0.02
1030	3.5	11.3	8	23	0	0	0	0
935	4	11.3	9	79	0	0.4	0	0
1105	4	35.8	7	70	0			
1230	4	7.4	1	350	0	0	0	0
1230	4	15.9	5	2200	0	0	0	0
1115	2	16	1	49	0	0	0	0
1100	4	39	2	79	0	0	0.12	0.01
300	5		11	17	0	0	0	0
100	5		0	2	0	0.07	0.01	0
110	5		4	4	0	0	0	0.05
200	8		26	23	0	0	0	0
910	9		3	49	0	0	0	0
835	6		3	49	0	0.4	0	0
945	6		12	23	0			
1050	6		8	43	0	0	0	0
1100	6		3	49	0	0	0	0
945	5		0	2	0	0	0	0
930	6		0	17	0	0	0.12	0.01
200	1	24.1	9	33	0	0	0	0
1220	1	15.7	5	33	0	0.07	0.01	0
1200	1	17.6	12	49	0	0	0	0.05
100	2	42.1	3	190	0	0	0	0
130	1	64.1	1	79	0	0.6	0.15	0.02
950	2	61.7	5	170	0	0	0	0
900	2	25.7	12	46	0	0.4	0	0
1020	2	61.7	13	130	0			
1120	2	44.1	14	180	0	0	0	0
1145	2	47.7	0	3300	0	0	0	0
1015	1	66.8	0	1600	0	0	0	0
1000	1	121	0	280	0	0	0.12	0.01

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2.4	1.64	49	5	46	36.58	22.11
0.41	1.9	53	5	42	104.94	41.277
2.85	1.39	41	11	48	27.63	13.372
1.92	1.11	38	10	52	8.84	7.65
1.04	1.66	46	5	49	2.09	2.49
0.21	4.36	46	14	40	31.74	16.61
0.55	1.43	58	16	24	126.44	71.91
0.7	1.04	54	10	36	37.66	19.8
0.89	1.09	42		58	25.65	11.18
1.31	1.22	56	9	35	41.32	12.441
0.43	1.62	50	7	43	30.83	11.845
0.37	1.72	54	7	39	64.32	26.109
1.6	1.12	39	trace	61	30.84	16.01
0.37	1.63	40	11	49	8.96	7.23
1.82	1.08	48	12	40	31.66	19.2
2.92	2.02	40	10	50	22.88	10.63
1.88	2.2	50	7	43	22.48	12.47
2.04	3.71	47	5	48	1.96	2.01
0.12	1.01	50	8	42	164.57	43.677
0.63	1.12	60	5	35	16.21	13.424
0.98	0.65	48	7	45	161.39	43.099
0.47	1.42	55	7	38	95.83	65.027
0.3	1.47	48	trace	52	0.1	0.2
0.29	6.03	51	7	42	28.62	19.41
0.18	1.2	46	6	48	28.37	3.23
0.03	8.02	35	10	55	43.21	29.22
0.79	1.83	44	trace	56	28.3	12.03
1.8	0.97	39		61	8.96	7.23
1.77	0.98	56	3	41	4.11	4.497
0.8	1.92	52	4	43	15.44	9.337
1.38	1.95	46	5	49	11.16	7.855
1.18	3.15	43	7	50	13.28	7.932
2.4	2.68	38	15	47	0.74	1.59
2.59	2.07	40	8	52	24.66	12.24
1.04	2.11	40	12	48	1.39	1.96
1.81	1.48	54	10	36	34.13	20.81
2.51	1.49	36	10	54	3.39	3.92
1.27	2.22	35	9	56	9.57	10.78

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2.6	0.364	0.893
2.68	0.563	1.477
2.66	0.135	0.903
2.56	0.14	0.54
3.08	0.03	0.49
1.97		
1.92		
2.64	0.86	0.78
2.5	0.65	0.74
3.11	0.557	0.464
2.83	0.424	0.771
2.24	0.603	1.296
2.2	0.71	1.21
3.05	1.77	1.31
2.63		
2.23	0.97	0.68
1.71	0.62	0.6
2.17	1.42	1.37
2.62	0.731	1.162
3.04	0.059	0.724
3	0.657	1.559
2.03	0.276	1.339
5.57	0.19	0.53
2.44	0.07	0.42
5.6	1.06	0.52
5.01		
1.82	0.72	0.65
3.05	0.16	0.52

2.55	-0.107	0.694
2.44	0.229	0.705
2.75	0.185	0.329
2.65	0.28	0.311
3.37	0.87	0.81
2.16	0.62	0.74
1.52	1.34	1.41
2.83	0.56	0.53
2.71	0.14	0.48
2.76	0.06	0.47

Table 3A. Virus Numbers Recorded Per Site for Each Month Sampled during 1987 and 1988

1987

Site	4/29	5/18	6/17	7/20	8/17	9/28	10/26	11/09	12/14
1	6	3	4	3	4	33	22	13	0
2	5	1	5	4	6	10	9	0	0
3	-	1	0	2	1	8	0	11	7
4	5	4	1	3	5	20	6	3	0
5	0	0	0	2	3	10	0	1	0
6	-	-	35	3	1	7	2	5	3

1988

	1/28	2/25	3/17	4/26	5/23	6/15	7/12	8/04	9/23	10/06	11/11	12/09
1	2	1	3	1	3	3	4	12	16	13	3	0
2	8	2	12	26	2	5	9	7	11	12	3	12
3	3	3	23	4	12	6	8	11	9	6	3	23
4	3	5	5	18	0	8	9	7	1	5	1	2
5	11	0	4	26	-	3	3	12	8	3	0	0
6	9	5	12	3	1	5	12	13	14	0	0	0

Table 3B. Number of Analyzes containing specific plaque levels.
(Total no. assays: 114)

No. plaques/analysis	No. Analyzes	%
0-10	87	76.3
11-20	19	16.7
21-30	5	4.4
>30	2	1.7
not available	1	.8

Table 3C. Number viruses per site by year

	June-December 1987		January-December 1988	
Site #	# Viruses	(Ave/month)	# Viruses	(Ave/month)
1	79	11.3	61	5.1
2	34	4.8	109	9.1
3	29	4.1	111	9.3
4	38	5.4	64	5.3
5	16	2.3	70	6.4
6	56	8.0	74	6.2

along the Bay was overwhelming the treatment systems.

3. The Bay is an out of balance estuarine system due to the amount of waste that enters the system and the relatively shallow and slow-to-flush tidal action.

4. The dye tracer study showed that treated wastewater was fairly evenly distributed throughout Back Bay.

D. Examination of Virus Levels Associated with Suspended Particulates:

Although many investigators have shown that virus persistence in the estuarine environment is enhanced by an association of the virus particles with suspended particulate matter, few studies have analyzed the number of solids-associated viruses or attempted to relate this association to the geochemistry of the particles. Such an evaluation was the principal objective of this investigation.

As noted earlier, Back Bay was chosen for study because it was found to contain consistent levels of viruses (not necessarily the highest levels of fecal coliforms) when compared to other estuaries along the Mississippi coast.

Table 3A presents the number of virus particles found associated with the suspended solids fraction from 100 gallons of estuarine water at each of the sites during each month of the investigation. Virus numbers ranged from a low of 0 to a high of 35 for any one site on a given month. Of a possible 114 viral counts (19 months X 6 sites), 87 counts contained 0-10 plaques, 19 contained 11-20 plaques, 5 contained 21-30 plaques, 2 contained >30 plaques, and 1 sample was lost and could not be analyzed (Table 3B). Thus, the majority of samples tested (76.3%) contained from 0-10 plaques; 16.7% of the samples contained from 11-20 plaques. Only approximately 5% of the samples contained greater than 21 plaques. This indicates that the estuary receives a constant but low level of viral contamination from a variety of sources, and probably that the flow of water in the estuary is such that virus distribution is uniform in the system.

Table 3C shows the number of viruses by site for the 19 months of sampling. During the sampling of June 87 to December 87, the highest number of viruses, 79 (11.3%;average per month) was isolated from site 1 followed by 56 (8%) at site 6. The lowest number of viral isolates per site was 16 (2.3%) which was associated with site 5. The average for all sites during this period was 42. This average increased during the sampling period January 88 to December 88 to 81, probably due to the 5 additional months of sampling. During this period, the highest number of viruses per site was 111 (9.3%), followed by site 2, 109 (9.1%). The lowest number of isolates per site was found at site 1, 61 (5.1%), as compared to site 5 which was lowest during the previous seven month period. These data again suggest that the virus input into the estuary is variable but continuous. An

examination of virus correlations with chemical and physical data also collected follows.

E. Virus and Bacterial Indicator Levels vs. Physical, Chemical, and Geologic Parameters:

Table 4 describes the correlational statistics for all virus counts vs. physical, chemical, and geologic measurements made during the 19 continuous months of sampling (June 87 to December 88). The data demonstrates that virus isolations from suspended solids of 100 gallon volumes rarely correlates with the measured parameters. Weak positive correlations were found for virus vs. CEC at site 3, FC levels at site 1, and carbonate carbon levels at site 3. A stronger positive correlation was found for virus levels vs. FC at site 6. Other relationships were either weaker or negative.

Table 5, the correlational values for fecal coliforms vs. physical, chemical and geologic parameters, can be interpreted in basically the same manner as Table 4. The majority of correlations are very weak or negative; only four weakly positive values were found between the FC count and pH at site 3, % smectite at site 4 and between salinity, turbidity and pH at site 5.

An examination of correlations between mean particle diameter and the physical, chemical and geologic parameters measured (Table 6) also demonstrates that as a general rule, positive values are not consistent. This table indicates that the largest number of positive correlations occurs between MPD and the three clay types; MPD correlated with the % Smectite at sites 2,3, and 6, with the % Illite at sites 2,3, and 4, and with the % Kaolinite at sites 1,3, and 6.

These results prompted us to examine other possible relationships to account for the variability of virus and FC counts in the estuary.

F. Other Statistical Relationships:

The lack of strong, positive correlations between virus and bacterial numbers and a variety of physical, chemical and geological measurements during this study suggests that the variability in the estuary is the general rule or that other measurements would be appropriate to predict the fluctuations of these microorganisms. One such parameter is rainfall. During this study, rainfall data was obtained from Keesler Air Force Base, from a point approximately 0.5 miles southeast of sampling Site 3. Basically, if rain occurs at site 3, it will also occur at other sites since the estuary is relatively small. Rain on the day of sampling (designated in Table 7 as R0) can produce both a dilution of estuarine water and an influx of sediment and waste products into the estuary. If rainfall occurs on days preceding the day of sampling, its residual effects may also affect the levels of microorganisms as well as the other

Table 4. Correlational Statistics: Viruses vs. Physical, Chemical, and Geologic Parameters at each Site

Site	Salinity	Turbidity	pH	CEC	FC	%Org	%CC	%Sme	%Ill	%Kao	MPD
1	.153	.262	.274	.002	.465	.170	.001	.102	.046	.257	.376
2	.219	.046	.047	.074	.228	.429	.393	.404	.274	.203	.266
3	.230	.259	.245	.469	.223	.035	.458	.062	.093	.059	.000
4	.110	.295	.090	.107	.140	.153	.080	.269	.270	.045	.030
5	.060	.057	.040	-	.070	-	-	-	-	-	-
6	.230	.064	.240	.321	.680	.093	.130	.060	.220	.056	.280

Table 5. Correlational Statistics: Fecal Coliforms vs. Physical, Chemical, and Geologic Parameters at each Site

Site	Salinity	Turbidity	pH	CEC	%Org	%CC	%Sme	%Ill	%Kao	MPD
1	.047	.209	.138	.299	.115	.003	.132	.267	.224	.417
2	.099	.158	.118	.208	.245	.220	.185	.185	.366	.119
3	.379	.314	.484	.120	.014	.164	.198	.010	.074	.150
4	.277	.234	.122	.129	.119	.176	.459	.045	.403	.215
5	.482	.610	.458	-	-	-	-	-	-	-
6	.280	.240	.250	.361	.000	.333	.298	.207	.070	.080

Critical Value for r (n-2) = .456 (.05); .575 (.01)

CEC, Cation Exchange Capacity; FC, Fecal Coliforms/100 ml; % Org, % Organic Carbon; %CC, % Carbonate Carbon; %Sme, % Smectite; %Ill, % Illite; %Kao, %Kaolinite; MPD, Mean Particle Diameter

parameters measured.

Table 7 presents the correlational statistics of virus, FC and mean particle diameter values for each sampling site using all data from the 19 months of sampling. The values are presented cumulatively, asking whether rain on the day of sampling was associated with these 3 parameters, whether rain on the day of sampling plus any rain on the preceding day affected the 3 parameters, etc., back to the third day preceding the day of sampling. Rainfall correlated very strongly with virus levels at site 6, but not at the other sites tested. Correlation values increased as rainfall accumulated over the four day period at this site. A weak, positive relationship was also observed between virus numbers and rainfall over the 4 day period at site 5. Other values were negative. The distance between sites probably is insignificant in terms of the amount of rainfall, therefore, rainfall is probably not a good predictor of changes in virus numbers at a given site as suggested by the results at site 6. It is difficult to evaluate the reason for the strong correlations at site 6, whose physical nature is not altogether different from sites 1 or 3 (small bayous in the marsh). As noted above, rainfall could possibly decrease the level of virus in the water by dilution effects, but could also be a factor which increases the virus level by washing surrounding contaminated materials from the land (for example, septic tank effluent) into the estuary. Perhaps site 6 is somehow different from the other sites in a way that was not measured during this project.

Correlations between fecal coliforms and rainfall were not positive at site 6 but showed strong correlations at sites 3 and 5. These positive values did not occur on the day of sampling but were associated with the previous 3 day periods. It appears that rainfall on the previous days encourages fecal coliform levels to either increase or decrease; these organisms can survive in estuarine water for several days and can be protected by organic matter in the water. In addition, rainfall may bring nutrients into the water which stimulate the fecal coliform population to grow (a consideration which cannot be associated with virus numbers). Rainfall can affect salinity, turbidity and pH values, and these parameters demonstrated positive though weak correlation with fecal coliforms at site 5 only. The reason for these discrepancies are not understood, but underscore the need for additional research to search for other appropriate parameters of comparison.

An examination of the relationship between rainfall and mean particle diameter at each site is also included in Table 7. Obviously, rainfall and any associated weather disturbance would mix the waters and sediments of a shallow estuary such as Back Bay. The addition of rain water into the estuary may be associated with particle disassociation, leading to changes in particle size. The correlation values do not reflect these hypothetical considerations. Only one weakly positive value was noted at site 3 and considering all of the days of possible

Table 6. Correlational Statistics: Mean Particle Diameter vs. Physical, Chemical, and Geologic Parameters by Site

Site	Salinity	Turbidity	pH	CEC	%Org	%CC	%Sme	%Ill	%Kao
1	.067	.006	.159	.097	.548	.232	.073	.095	.738
2	.092	.009	.103	.198	.071	.435	.588	.588	.130
3	.124	.207	.409	.106	.284	.332	.615	.494	.510
4	.215	.230	.389	.006	.085	.281	.406	.556	.175
5	-	-	-	-	-	-	-	-	-
6	.050	.163	.191	.372	.350	.346	.560	.262	.460

Table 7. Correlational Statistics: Viruses, Fecal Coliforms and MPD vs. Rainfall at each sampling site

		Site					
		1	2	3	4	5	6
Virus vs.	R0	.079	.099	.268	.198	.193	.821
	R0,R-1	.153	.140	.331	.212	.220	.834
	R0,R-1,R-2	.163	.354	.339	.253	.285	.835
	R0,R-1,R-2,R-3	.172	.356	.427	.378	.497	.871
Fecal Coliforms vs.	R0	.020	.081	.068	.025	.061	.063
	R0,R-1	.289	.082	.985	.051	.996	.137
	R0,R-1,R-2	.291	.362	.987	.367	.996	.269
	R0,R-1,R-2,R-3	.459	.368	.990	.367	.995	.271
MPD vs.	R0	.238	.034	.004	.140	-	.126
	R0,R-1	.318	.164	.228	.201	-	.208
	R0,R-1,R-2	.376	.206	.315	.207	-	.210
	R0,R-1,R-2,R-3	.377	.208	.488	.255	-	.259

R0, Rainfall on day of Sampling; R0,R-1, Rainfall on the day of sampling and on the previous day; R0,R-1,R-2, Rainfall on the day of sampling and the two preceeding days; R0,R-1,R-2,R-3, Rainfall on the day of sampling and on the three previous days.

Critical Values for r (n-1) = .456 (.05); .575 (.01)

rainfall. Therefore, it cannot be said from this study that any relationship exists between rainfall and mean particle diameter.

The use of statistics to compare all of the data collected during the study could but did not yield correlations which indicated strong positive trends. The question was then raised whether individual sampling dates would correlate with all fecal coliform, virus and mean particle diameter data at all sites. These results are shown in Table 8. Fecal coliforms produced 6 of 19 positive values; viruses, 7 of 19, and MPD, 6 of 16 (3 samples lost during the investigation). Therefore, there is no significant trend to suggest that the individual dates of sampling were correlated during the study with these three parameters.

Temperature as well does not appear to have a significant influence on virus, fecal coliform or MPD values (Table 9). With the exception of MPD:site 3, all correlations were extremely low.

6. Viral persistence and transport:

Viral input into Back Bay appears to be constant, but the low numbers of isolates in our sampling effort may not reflect the true numbers of viruses in the estuary. There are no estimates of the volume of water which moves through Back Bay daily, but the volume is considerable. Although viruses were found consistently at each site, only 2 of the sites were situated near defined sewage outfalls which would be easily recognized as a potential source of viruses. Since viruses do reach site 5, with no apparent source of virus other than other sampling sites in the estuary, viral transport to this point implies viral movement of at least .5 to 1 mile. Sediment flocculation should account for some lost of virus and site 5 should show the lowest level of virus isolation; this occurred during the June - December 1987 sampling period, but not during the 1988 samplings when isolations at site 5 was approximately equal to the total number of viruses found at sites 1,4 and 6 (see Table 3C). Tidal movement in the estuary as well as rainfall and boat traffic probably combine to mix the waters sufficiently making it difficult to ascertain a defined pattern of virus movement. Thus, isolations appear random, and it is difficult to evaluate the actual source of an isolate. A table similar to Table 3, when prepared for FC counts, shows an identical trend. Fecal coliform measurements are also highly variable at each site and during the different months of the year. Counts do not drop off toward site 5, indicating that mixing promotes movement and transport of this bacterial population as it did for virus levels. The dye studies performed in June 1987 also support these results. Dye releases at varying points throughout the bay demonstrated that wastewater effluents could travel up to 6 miles during one ebb tide. Dye levels indicated that our site 5 (on the border of Back Bay and Biloxi Bay) was in a location that was continuously swept by treated wastewater. Basically, the dye plume followed the boat channel, which would include all of our

Table 8. Correlational Statistics: FC and Virus Levels vs. all Sites by Date

	Fecal Coliforms	Viruses	MPD
06-17-87	.407	.552	.832
07-20-87	.147	.355	.586
08-17-87	.018	.517	.623
09-28-87	.050	.623	.091
10-26-87	.024	.771	.293
11-09-87	.239	.446	-
12-14-87	.217	.148	.297
01-28-88	.752	.619	-
02-25-88	.174	.414	-
03-17-88	.427	.021	.575
04-26-88	.339	.109	.925
05-23-88	.200	.231	.160
06-15-88	.740	.169	.332
07-12-88	.706	.362	.306
08-04-88	.352	.321	.382
09-23-88	.651	.273	.377
10-06-88	.459	.976	.404
11-11-88	.619	.923	.912
12-09-88	.444	.321	.092

Critical Values for r (n-2) = .456 (.05); .575 (.01)

Table 9. Correlational Statistics: Viruses, Fecal Coliforms, and Mean Particle Diameter vs. Temperature by Site

Site	Viruses	Fecal Coliforms	Mean Particle Diameter
1	.200	.230	.123
2	.048	.022	.086
3	.177	.082	.568
4	.191	.009	.146
5	.274	.027	-
6	.230	.007	.154

Critical Values for r (n-2) = .456 (.05); .575 (.01)

sampling sites with the exception of site 6.

One of the possible reasons for the variation in virus associated suspended solids is adsorption or reabsorption following changes in the conditions which exist in the bay at any given point in time. Unattached virus or viruses attached to particulates smaller than 0.2 microns would not have been isolated during this study. If adsorption/reabsorption occurs it would influence the virus count and result in data variability. Such problems should be addressed in a future study.

Retrospectively, Back bay or similar estuaries were not suitable for the strict fulfillment of the objectives of this study. From a more practical standpoint, a location with one wastewater source, a single, well defined channel, and several miles of sampling area would better serve to define the question of virus transport. Most small estuaries receiving wastewater pollution, however, are similar to Back Bay and it is expected that similar problems of virus variability due to mixing would be encountered.

Discussion:

This investigation was designed to evaluate the level of solids-associated virus in a polluted Mississippi estuary. The research hypothesis was consistent with previous studies which related virus persistence in the estuarine environment to virus attachment to particulates. This study was unique, however, in that it was an attempt to relate particulate size and composition to virus isolations, persistence and transport. Of three major estuaries evaluated for this study, only Back Bay contained low, but consistent levels of solids-associated virus.

In the laboratory, viruses adsorb to a variety of substrates and bonding is strongly influenced by pH, ionic composition, quantity of dissolved organic matter, organic particulates such as bacteria and fecal residues, and the presence of non-reactive end products of the breakdown of plant materials such as fulvic and humic acids. Both laboratory and field studies have shown that, depending on environmental conditions, virus numbers are partitioned at any one point in time and place between the water column, the suspended solids fraction and the flocculating solids particulates. Bound virus has been shown to survive longer than solids-associated virus, thus rapid water movement would be required for free, infectious virus to reach non-polluted shellfish growing or recreational waters. If the physical/chemical conditions which enhanced virus adsorption to particulates do not rapidly change in an estuary, virus should remain attached to the suspended fraction and eventually be deposited into the Gouy sediment layer and then into the anaerobic sediment fraction.

Suspended solids thus appear to be the most important particulate fraction which relates to virus persistence and transport. Collection of this fraction from the water column by filtration is a most efficient method, although sedimentation at 4 C would probably retrieve a greater percentage of the particles smaller than .1 microns. The bag-type unit utilized during this investigation was easy to manipulate and retained the majority of suspended matter. Following the filtration of 100 gallons of estuarine water, bags were coated with approximately 1/8 in of suspended material, but the filtration rate was not slowed. Thus, larger volumes, perhaps 300 gallons, could be filtered to examine estuarine particulates for virus content.

Back Bay was an excellent estuary to demonstrate solids-associated virus due to the number of sources of viral input. However, the amount of mixing in the estuary and the short residual time of tidal water made an analysis of virus transport difficult to evaluate. Table 3A clearly shows that virus isolations are common throughout the estuary, indicating either that input into the estuary is continuous or that mixing of estuarine water produces a virus dispersion effect which precludes a determination of virus source. The small number of plaques per analysis (76% of the analyzes containing between 0 and 10 plaques) indicates that input during the year is low, or

that the mixing phenomenon dilutes higher virus levels at certain places in the estuary. Transport of virus to site 5 did occur as indicated in Table 3C, where the average per month was 2.3 during the period of June to December 1987, and which increased to 6.4 per month during the period of January to December 1988. Mixing, tidal movement, and the movement of water primarily along the channel of the Bay, demonstrate that virus transport to oyster growing waters of Biloxi Bay and beyond is a definite consideration. The dye plume was found to reach the middle of the bay side of Deer Island, and it is assumed that under the correct conditions, virus could be transported at least this distance.

As noted by the results shown in Table 4, virus numbers infrequently correlated with physical, chemical and geologic parameters. No pattern developed which would suggest a trend consistent with an indirect or direct statistical relationship. This implies that if virus data concerning an estuary is need as a public health measurement, no substitute parameter will serve as a suitable replacement. Basically, the same can be said for the fecal coliform count (Table 5). Although this standard procedure is much simpler and more economical than a virus test, there does not appear to be any statistical validity to a relationship between this test and the physical, chemical or geological measurements examined. In addition mean particle diameter could not be correlated with the physical and chemical measurements, but showed a 50% correlation with specific clay mineral concentrations at each of the sites tested. Since mean particle diameter did not correlate with salinity, pH, turbidity, CEC, % organic matter, and % organic matter, it is assumed that these parameters do not readily affect particle size in a natural environmental situation or that they have a complicated, interactive relationship to particle diameter. The meaning of this lack of correlation will require further experimentation.

Of the parameters tested, rainfall demonstrated the strongest correlations. Correlation of virus versus rainfall on the day of sampling and the three preceding days were very strong indicators, but this occurred only at site 6. Site 5 virus isolations showed a weak, positive correlation with rainfall, but only the cumulative rainfall of the day of sampling and the 3 preceding days. Fecal coliforms, however, displayed multiple positive correlations at sites 3 and 5, but only on the days prior to the day of sampling, and were consistent with the virus correlations. Mean particle diameter did not appear to relate to rainfall. These data again suggest that virus isolations are individual entities which must be measured separately, and should not depend on indicator parameters such as the amount of rainfall.

The overall lack of correlations during this investigation demonstrate the complexity of relationships between microbiological and other measurable parameters in estuarine waters. Although it has been stated by numerous investigators, this study again underscores the need to isolate viruses from

estuarine areas which receive human wastewater and for which a possible public health problem exists. The technology is available and economically feasible for routine estuarine sampling.

Summary and Conclusions

Viruses associated with the suspended solids fraction of estuarine waters can be readily estimated by filtration of one hundred gallon volumes followed by standard extraction and plaque assay procedures.

Back Bay of Biloxi was shown to contain low levels of virus during most months of the year.

Suspended-solids associated virus levels ranged from a low of 0 to a high of 35. Seventy six percent of the analyses contained between 0 and 10 isolates. Only five percent of the samples contained greater that 21 isolates.

Viruses were isolated from the mouth of the Bay. To reach this point required transport of the viruses on suspended solids.

No correlation could be demonstrated between virus levels, fecal coliform counts, mean particle diameter, and a variety of physical, chemical and geological measurements.

Rainfall correlated strongly with virus and fecal coliform concentrations at specific sampling sites, but did not correlate with rainfall at the same sites. Mean particle diameter did not correlate with rainfall.

A strong correlation did not exist between the day of sampling at a particular site and the levels of fecal coliforms, virus, or the mean diameter of the suspended particles. Similarly, no relationship was observed between viruses, fecal coliforms, and mean particle diameter versus the temperature of the water.

The lack of correlation between the microbial parameters, mean particle diameter, and a variety of physical, chemical, and geologic measurements strongly suggests that estuarine mixing causes constant redistribution of microorganisms, and random isolation patterns.

Enteric virus isolation from suspended solids is the best method to evaluate the potential health hazard of polluted estuarine water.

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Checklist Summary:

In Main Document:

1. Title Page, Principal Investigators, Sea Grant Project Number, USM project numbers, duration of the project, Period covered by this report

2. Body of Report, including Research Hypothesis, Introduction Review of Literature, Methods and Procedures, Results, Discussion, Tables, Literature Cited.

Additional Information concerning the total project:

Tabulations:
No. field exercises: 23
Person Hrs. Expended: 3937
Dollars Encumbered: $71,289
Percent Completion of Objectives: 95

Problems:
The proposed timetable for the project was completed. A minor number of samples or data were accidentally lost during the study, but did not significantly affect the analysis.

Changes during the Investigation:
The project was completed with essentially no changes in the sampling protocol.

Publications:
No publications to date; one in preparation.