II. REVIEW OF LITERATURE

A. Background

Coastal embayments are semi-enclosed areas of the coastline where the freshwater from groundwater and streams is mixed with saltwater from the adjacent oceans and bays. The areas ever-changing environment creates an ecological niche that is important for seagrass, scallops, oysters, soft/hard-shelled clams, and as breeding grounds for commercially important offshore fish (Cape Cod Commission, 2002).

In 1995 the commercial harvest from shellfish growing waters totaled 34.9 million kilograms (77 million pounds) of oysters, clams and mussels worth approximately 200 million dollars at dockside along the United States coastline (National Picture, 2002). One of the inhabitants of the coastal embayments is *Crassostrea virginica*, also known as the Eastern, American, or Atlantic oyster. The oyster was once plentiful enough in the Chesapeake Bay that seasonal harvests were in the millions of bushels. As recently as 100 years ago, oyster reefs were so massive that they posed a navigational hazard to ships. Today's oyster population is reduced to about 1% of its historic level (American Oyster, 2002).

The shellfish are often affected by loss of habitat due to environmental pollution including alga blooms, metal pollutants, and siltation. Shellfish, which include oysters, soft and hard-shelled clams, and mussels are filter-feeding organisms. Shellfish have a long history as vectors of infectious and sometimes dangerous diseases, ranging from typhoid fever and hepatitis to diarrhea and minor intestinal disorders. Shellfish pump large quantities of water through their bodies. Through this process contaminants can become concentrated in their tissues, harming the organism little but causing many problems for human consumers (MDE, 2003).

Raw consumption of shellfish may pose a health risk; therefore it is very important to protect the health of the consumer. The United States Environmental Protection Agency (USEPA) is directed by the 1972 Clean Water Act to maintain "water quality which provides for the protection and propagation of….shellfish…." (RL 992-500 as amended, Section 101(a)(2). 33 U.S.C. 1251)(Shellfish Protection, 2002). One section of the Clean Water Act is the Total Maximum Daily Load program (TMDL). A TMDL is essentially a prescription designated to restore the health of the polluted body of water by indicating the amount of pollutants that may be present in the water and still meet water quality standards set by federal and state governments (Press Release, 2002). The TMDL program for fecal bacteria stimulated a new and evolving methodology called BST. BST can be used to determine the sources of fecal contamination in coastal embayments that is causing such problems for the shellfish industry. The TMDL program accompanied by BST will ensure that the shellfish industry continues and that harvested shellfish are safe for human consumption.

B. History of Shellfish Industry

Centuries before TMDL or BST was developed, explorers journeyed across the Atlantic. Captain John Smith's maps, based on his 1607-1609 explorations, show 161 villages among the thirty-two kingdoms that made up Powhatan's Confederation. The Powhatan Confederacy was made up of tribes living in the coastal plain of Virginia. Chief Powhatan, whose real name was Wahunsonacock, united the tribes to form the Powhatan Confederacy. Captain John Smith later called him Powhatan. The Powhatans had about 12,000 people who lived in a 23,316 square kilometer (9,000 square mile) area. Of these, some 3,000 were probably warriors (Rountree, 1989). Among those kingdoms were the Indians of Pissaseck tribe near Leedstown; the Moraughtacunds near Marattico; the Cuttatawomen, on the Corotoman, the Wiccocomoco, on the Wicomico; the Cekacawon, on the Coan; and the Nominies near Nomini Bay. Native Americans had lived in the Northern Neck of Virginia for at least ten thousand years (Northern Neck VA, 2002). These areas in Virginia were important shellfish embayments to the Native Americans as well as to the present Virginia economy.

Many years before Captain John Smith first rounded Cape Henry in the early 17th century, which is today's Virginia Beach City, Virginia, the Algonquin Indians already called the Chesapeake Bay "Chesepiooc" for "great shellfish bay." By the late 19th century, during the "golden age" of oysters in the United States, an armada of fisherman were harvesting an estimated 54.4 million kilograms (120 million pounds) of oysters (meat weight) per year from the Chesapeake Bay. During the 20th century even this boundless resource came to an end. A total of 18.87 million kilograms (41.6 million pounds) harvested in 1954 was a recent high followed a shockingly low harvest of only 259 thousand kilograms (571 thousand pounds) in 1993. Since 1993 the once booming oyster industry along the East Coast has almost disappeared (Case Studies, 2002).

The boom began as railroads opened new markets to a burgeoning population and food preservation improved with the use of artificial ice, which appeared around 1880. Once eaten only by the wealthy, except in local harvest areas, oysters became a very popular fresh food, cheaper than beef, poultry, or fish. In every town there seemed to be oyster parlors, oyster cellars, oyster saloons, oyster bars, and oyster lunchrooms (Case Studies, 2002).

The demand for oysters declined rapidly in the early 1900's when increased concerns over sanitation and diseases like typhoid drove the consumers away. The result was a sharp reduction in harvest that caused widespread hardships and failures among the oyster businesses.

In the Chesapeake Bay and other regions, the systematic loss of habitat through siltation and over fishing also contributed to decreasing harvestable shellfish (Case Studies, 2002).

C. Shellfish Growing Waters

Thirty-six percent of the United States waters classified for shellfish growing are in the Gulf of Mexico region, followed by the twenty-five percent in the Mid-Atlantic. Nineteen percent of the classified waters are found in the North Atlantic region, eighteen percent in the South Atlantic region, and less than two percent in the West Coast region. In the three Atlantic regions are located, eighty percent or more of all shellfish growing waters approved for harvest. In the Gulf of Mexico and West Coast regions, however, less than half of all shellfish beds are approved for harvest. The nation's commercial shellfish harvest from these classified waters consists of primarily two oyster species, a dozen species of clams, and blue mussels (Regional Contrasts, 2002).

The following tables show the United States classified shellfish growing waters as of 1995. In Table 1, the number of classified shellfish acres is shown along with the percentage of limited shellfish harvested. In Table 2, the top 10 estuaries along with the state where they are located, is listed with the classified shellfish growing estuary accompanied by the classified number of acres in the estuary. The states listed in Table 2 in bold indicate these states found on the East Coast of the United States. The classification status is based on sanitary surveys of pollution sources conducted by the National Shellfish Sanitation Program (NSSP).

Table 1. 1995 Classified United States Shellfish Growing Waters (National Picture, 2002)

State	**Classified Hectares (x 1,000)**	**% Harvest Limited**
Maine	750	16
New Hampshire	25	10
Massachusetts	626	27
Rhode Island	115	14
Connecticut	149	66
New York	459	14
New Jersey	298	29
Delaware	132	23
Maryland	583	9
Virginia	668	6
North Carolina	1134	15
South Carolina	317	10
Georgia	77	63
Florida	585	79

Table 2. 1995 Classified Shellfish Growing Waters of the Contiguous United States (National Picture, 2002)

Top 10 Estuaries	**State(s)**	**Estuary**	**Classified Hectares (X 1000)**
1	**Maryland and Virginia**	Chesapeake Bay Albemarle/Pamlico	1026
2	**North Carolina**	Sounds Breton/Chandeleur	783
3	Louisiana	Sounds	482
4	Louisiana	Mississippi Sound	441
5	**New York and Connecticut**	Long Island Sound	328
6	**Delaware and Maryland**	Delaware Bay Atchafalaya/Vermillion	206
7	Louisiana	Bays Terrebone/Timbalier	202
8	Louisiana	Bays Lower Laguna	166
9	Texas	Madre	161
10	**Maine**	Penobscot Bay	155

D. Shellfish Diseases and Pollution

In the late 1950's, harvest dropped significantly again when a new disease named multinucleated sphere unknown, (MSX) caused by a protozoan called *Haplosporidium nelsoni,* began to kill large numbers of oysters in the Chesapeake Bay as well as other regions along the east coast. The protozoan *Perkinsus marinus*, recorded by the National Oceanic and Atmospheric Administration, caused another fatal oyster disease (dermo-*cystidium)* that gained strength in the early 1980's, further crippling the oyster industry. Although these diseases do not make consumption of oysters unsafe, they continue to cause devastating population decline in the Chesapeake Bay and other regions, typically killing oysters before they reach a harvestable size. MSX and dermo-*cystidium* are caused by natural occurring single-celled parasitic organisms that are not directly associated with human activities, but may be more prevalent under polluted conditions (Case Studies, 2002). More recently MSX and dermo-*cystidium* have been creating problems for the northeastern states. In the year 2000 MSX and dermo-*cystidium* impacted the oyster industries of New Jersey. The oyster industry may be revitalized at some future date when the MSX and dermo-*cystidium* situations are eliminated or perhaps the oysters can be improved through genetic engineering (Jersey Shore, 2002). There are hopes that new technology will enable scientists to develop a MSX and dermo-*cystidium* resistant oyster, which would help reestablish the deterioration of the East Coast oyster industry.

The Chesapeake Bay is not the only Region with shellfish harvesting restrictions. Northumberland County in the Northern Neck of Virginia has two condemned rivers where shellfish cannot be harvested. These two rivers were the old homes for the Virginia Native Americans, the Little Wicomico and the Coan. Not only are these two rivers affected but also the Potomac shoreline between these two points. On the 23rd of February 2002, Virginia and Maryland environmental officials planned to extend an oyster harvest ban in the Potomac River and its tributaries, which included the Little Wicomico and the Coan, until at least the 4th of March 2002. Oyster harvests in the Potomac and its tributaries were banned due to a widespread bloom of potentially toxic algae. The algae, known as *Dinophysis acuminata*, which produces the toxin okadaic acid, has been blamed for gastrointestinal illnesses (or diarrhetic shellfish poisoning [DSP]) in other parts of the world. Virginia and Maryland are not the only states that have been affected by the same alga bloom (Roanoke Times, 2002). Maine has also recently experienced increased DSP activity (Marine Biotoxins, 2002). Algal blooms have occurred from the East Coast of Nova Scotia, Canada, to the tip of Florida where DSP has been associated mostly with mussels, oysters and scallops (FDA, 2002).

Along with algae, human and natural sources can cause shellfish closures. In Jacksonville, Florida, high amounts of fecal bacteria found in the St. John's River closed shellfish beds in November 1997, and today less than fifteen percent of the shellfish beds remain open in Florida (Patterson, 1997). In the mid 1970's, New Jersey eliminated fourteen small and outdated wastewater treatment plants discharging to the lower Navesink river to improve the shellfish beds after more than twenty years of harvest restrictions (State of Coast, 2002). The increase in the human population has also affected the shellfish industry and one example is in Cape Cod, Massachusetts, where the shellfish bed closures increased from 288 hectares (712 acres) in 1980 to 2,928 hectares (7,235 acres) in 1992 (Cape Cod Commission, 2002). With an increase in human population, the use of pesticides, oil, grease, and fertilizer also increased. These non-point source pollutants accumulate in run-off that blend together as they make their journey over lawns, parking lots, crop lands, streets, feedlots and other surfaces into waterways where shellfish live. Rainstorms can intensify the polluted run-off problem by overwhelming old sewer systems, which sometimes results in the discharge of raw or inadequately treated sewage into rivers and bays (Oceana, 2002). Humans, together with the natural environment, can cause damage to the shellfish beds furthering the deterioration of the shellfish industry.

The NSSP, a cooperative and non-voluntary alliance among states, arose from a series of public health initiatives formulated in the mid- 1920s following a widespread outbreak of typhoid fever in which 1,500 cases and 150 deaths were reported. The United States Public Health Service subsequently recommended sweeping changes in the harvest and distribution of shellfish, including inspection of shellfish growing areas and scientific investigations of disease outbreaks. Of a particular importance with respect to typhoid fever and fecal contamination was the initiation of research to find a bacterial indicator to measure the quality and safety of shellfish growing waters (National Picture, 2002).

The DSS surveys and classifies the shellfish bed waters in Virginia according to the guidelines set by the NSSP, the United States Food and Drug Administration (FDA), and the Shellfish Industry. The Virginia Department of Health (VDH), accompanied by the DSS, has supported a research project to determine the sources of fecal contamination that have condemned the Little Wicomico River and the Coan River shellfish beds. The project will provide regulatory officials with data on sources of fecal contamination and seasonally of those sources in estuarine rivers that contain shellfish beds. These results will allow better planning in the future regarding designing sampling plans that incorporate seasonal trends for other shellfish TMDL projects. Millions of dollars are spent on the Chesapeake Bay investigating restoration options alone. Some scientists believe that at least partial recovery is possible. Other states have their own restoration to keep their shellfish industry clean, open, and harvestable for human consumption.

The shellfish industry is important to many of the US states along the East Coast to maintain a stable economy. Virginia for instance has an economy that is highly diversified and ranges from mining coal to raising cattle to hosting the largest concentration of computers communication firms in the United States. The major sources of agricultural income are now poultry, dairy goods, and cattle, raised especially in the Valley of Virginia. The coastal fisheries of Virginia are large. Virginia has a $500 million seafood industry, supporting over 14,000 jobs (VADGIF, 2003), and bringing in primarily oysters and crabs (Infoplease, 2002). Shellfish are also an economic asset for Florida. In addition to playing a key ecological role, the annual value of shellfish to the seafood industry in Florida exceeds $20 million, with as many as 2,500 people employed in the harvesting, processing, and distribution of the shellfish. The most common shellfish found in Florida waters are clams and oysters (American Oceans, 2002). The shellfish harvesting waters impact the economy, which is why the importance of saving these marine animals is very valuable.

E. Monitoring and Source Tracking in Shellfish Embayments

In order to save the shellfish industry, the USEPA implemented the TMDL process in coastal areas, which is the driving force behind bacterial source tracking development. BST is a new methodology that is being used to identify sources of fecal pollution in water. Fecal bacteria are isolated from water samples at particular sites, and BST methodology is then employed to determine the sources of those bacteria. The bacteria may be either of human, livestock, or wildlife origin. BST is also called fecal sourcing or fecal typing in scientific literature (Hagedorn, 2002).

The objective of microbiological monitoring of the water quality in shellfish beds is to provide evidence of protection against transmission of water-borne infectious diseases. The presence of any microbial pathogens in coastal waters presents potential health risks. There is no worldwide agreement as to what criteria should be used to measure the safety of shellfish. Human health problems associated with shellfish consumption are recognized internationally and have been recorded since medieval times (Formiga-Cruz, M. *et al.*, 2002). It has been suggested in many countries, like France and the United Kingdom, that the shellfish standard should be based on the direct examination of shellfish and not on the growing waters. There have been several outbreaks of illness due to assumption of bivalve shellfish within the legal limits of bacterial standards allowed by the current legislation (Formiga-Cruz, M. *et al.*, 2002). Bacteriological measurements of shellfish beds' water quality must be based on the detection of fecal contamination from all sources of warm-blooded animals (Bacterial Indicators, 2002).

Total and fecal coliforms have been used extensively for many years as indicators for determining the sanitary quality of surface, recreational, and shellfish growing waters. In recent years, scientists have learned more about the ways in which the coliforms' ecology, prevalence, and resistance to stress differ from those of many pathogenic microorganisms they are proxy for (Scott *et al.*, 2002). These differences are so great that they limit the utility of the coliforms as indicators of fecal pollution. Therefore, additional microbes have been suggested for use as alternative indicators, including *E. coli*, enterococci, and *Clostridium perfringens* (Scott *et al.*, 2002).

Potential sources of fecal bacteria are generally grouped into three major categories: human, livestock, or wildlife. In most urban watersheds, a fourth category of pets, or dogs may be added. An example is the watershed along the Coan and Little Wicomico rivers that the potential sources of fecal bacteria have been split into four categories, human, livestock, wildlife, and pets. Each source produces unique, identifiable strains of fecal bacteria because the intestinal environments and selective pressures to which the bacteria are subjected to differ from source to source (USEPA, 2002).

The BST methods can be divided into three basic groups molecular, biochemical, and chemical. The method that was be used to detect the fecal contamination, *Eschericia coli*, in the Little Wicomico and Coan rivers is ARA. Antibiotic resistance analysis is a biochemical (phenotypic) BST method pioneered around 1996 for BST work (Hager *et al.*, 2002). Molecular BST techniques appear to be more precise in source identification, but proponents of ARA have demonstrated that it provides comparable results.

Currently, there is increasing interest in the potential for molecular fingerprinting methods to be used not only for detection but also for identification of fecal contamination sources. Molecular methods have been applied to the study of microbial ecology of environmental systems for years. These methods are now being applied to help improve our waters by identifying problem sources and determining the effect of implemented remedial solutions, management, and remediation of water pollution would be more cost effective if the correct sources could be identified (Simpson, *et al.*, 2002).

A differentiable characteristic, or fingerprint (such as antibiotic resistance or DNA), must be selected to identify various strains of bacteria. A representative library of bacterial strains and their fingerprints must then be generated from the human and animal sources that may impact the water body. Indicator bacteria fingerprints from the polluted water body are compared to those in the library and assigned to the appropriate source category based on fingerprint similarity. (USEPA, 2002).

The size of the database or library of bacterial isolates has not been determined for a specific water-body. Known fecal sources are required for accurate source prediction in a given watershed but the amount of those known fecal sources is not yet known. Considerations in the development of the BST library include the size of the watershed, diversity of animal species, and human sources that may significantly impact water quality, and the heterogeneity of the population within a given source species. In many studies, the number of isolates required to develop the known source database may make up the majority of the total isolates analyzed, constituting a large fraction of the total cost for the study (USEPA, 2002).

The ARA method is based on the patterns of antibiotic resistance found in bacteria from human and animal sources. The principle behind the method holds that human fecal bacteria will demonstrate greater resistance to certain antibiotics, and animal fecal bacteria will be resistant to others. Humans are exposed to a different set of antibiotics than are farm animals. Although agricultural species routinely receive antibiotics, those for cattle differ from those for pigs, which differ from those for poultry, and so on. Wild animals receive relatively little exposure to antibiotics; thus their fecal bacteria should not exhibit substantive antibiotic resistance. Dr. Valerie J. Harwood describes the ability of the bacteria to grow on different antibiotics as a type of "fingerprint"(Hager *et al.*, 2002).

Fecal bacteria are grown in wells in microtiter trays and then replica-plated onto a series of agar plates, each containing on specific antibiotic concentration. Forty-eight cultures can be transferred to each agar plate simultaneously with a stainless steel replicator. After incubation, each isolate is scored for growth or no growth on each plate and resistance pattern emerges that can be used in source differentiation (Hagedorn, 2002). The results form an organism-specific resistance pattern that can be used to identify individual sources of bacterial contamination.

ARA has also been used to perform source tracking on both enterococcus and *E coli*. States are beginning to monitor for contamination from distinct sources as BST is an emerging field and economical analyses have only recently become available. With increased availability, the use of BST throughout the TMDL process will improve public perception and lead toward solutions that are more effective (Hagedorn, 2002). BST development is so new that no research comparing BST methods either chemical, biochemical, molecular, or identifying their relative strengths and weaknesses have yet been completed.

The shellfish industry over the next decade has a chance to survive due to the large amount of funds being set aside for the health of estuaries, especially shellfish embayments, along the East Coast of the US. The importance of keeping shellfish beds open is very crucial for fishing, tourism, recreation, and economic health. Oysters, clams, and mussels are important key organisms to help determine the health of an ecosystem. In order to keep shellfish safe to consume, after being properly cooked, will be based on the TMDL and BST requirements set by federal and states governments to preserve the health of the coastal and shellfish environment. In the future, more and more states will be adopting the BST methodology to ensure the safety of their estuaries and other waterways. Just as the Native Americans used the shellfish embayments off the East Coast to sustain their lives ten thousand years ago, the United States should invest to keep these shellfish embayments open for years to come.