ENVIRONMENTAL FACTORS INFLUENCING BACTERIAL LEVELS ASSOCIATED WITH *RANGIA CUNEATA* (GRAY) IN NORTH CAROLINA

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David P. Green
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Abstract

David P. Green. ENVIRONMENTAL FACTORS INFLUENCING BACTERIAL LEVELS ASSOCIATED WITH *RANGIA CUNEATA* (GRAY) IN NORTH CAROLINA. (Under the direction of Dr. Donald B. Jeffreys - Department of Biology and Dr. Bernard E. Kane, Jr. - Department of Environmental Health) Department of Biology, May 1980.

Rangia cuneata (Gray), 1831, is a mactrid clam that frequents oligo- to mesohaline systems of the mid-Atlantic and southern states. Recent interest in commercial harvesting and marketing the clam for human consumption as a fresh seafood product prompted an investigation into its sanitary quality. The purpose of this research was to: (1) extend the data base on *Rangia* fecal coliforms (FC) and standard plate counts (SPC) reported by Comar *et al*. (1979) from Albemarle Sound, N.C.; (2) investigate the effect of environmental factors on clam SPC; and, (3) determine SPC variance among clams.

A median MPN (most probable number) of 80 FC per 100 grams (g) was found in 92 *Rangia* samples collected over the 2 year study. A seasonal pattern in clam FC was shown. February through April had a median MPN of less than 20 FC/100g with no samples exceeding the federal and state standard of 230 FC/100g. Standard plate counts showed greater variability and ranged from mean monthly values of 30,000 SPC/g in January to 610,000 SPC/g in December.

A statistical analysis of clam SPC versus environmental factors gave a significant negative linear correlation between clam SPC and rainfall. Multivariate regressional analyses showed that 26.5 percent of the variation in clam SPC can be explained by variations in turbidity,

river index, water temperature, and salinity. The interaction of the observed environmental factors aids in understanding the large difference in mean *Rangia* SPC found at the 4 collection stations.

A field experiment conducted to assess the influence of sediment on clam SPC demonstrated the greater importance of factors associated with the water in determining SPC than with the sediment. High turbidity conditions caused by dredging did not significantly affect clam SPC. However, a large degree of variation among composite clam samples was found. Analyses of SPC variance among individual clams revealed a similarly large degree of variation.

High variance in SPC complicates the effort to market *Rangia* as a fresh seafood product. More frequent sampling of clams from open, high saline shellfishing waters supporting *Rangia* during the months of February through April is recommended. Failure to demonstrate a pattern in *Rangia* SPC may lead to alternative methods of marketing the clam such as depuration or pasteurization.

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INTRODUCTION

Rangia cuneata Gray, 1831, is a mactrid clam that frequents oligo- to mesohaline systems of the mid-Atlantic and southern states where its shell and meat are of ecological and economic importance. Rangia is a typical filter-feeding bivalve that obtains its food by concentrating suspended particulate matter and microorganisms. Hobbie (1977) states that in upper reaches of estuaries with large populations of this clam, it may be as effective in removing particulate matter from suspension as is coagulation. The clam is an important link in the estuarine food web by converting detritus to biomass (Darnell, 1958; Tenore et al., 1968) and supplying food to certain fish, crustaceans, and waterfowl (Suttkus et al., 1954; Cain, 1975; Comar et al., 1979). Its thick and heavy shell represents a sizeable "carbon sink" within the estuarine ecosystem.

Rangia shell deposits have been mined along the Gulf Coast for many years and used in construction, for roadbeds, and as a soil neutralizer in agriculture. Human consumption of Rangia meat has been documented in a number of locations (Shingley, 1893; Speck and Dexter, 1946; Hopkins et al., 1973).

The clam was commercially harvested and marketed for human consumption from North Carolina waters from 1964 to 1972 (Chestnut and Porter, 1976). The industry terminated when a large shipment of clam meat was rejected by New York public health authorities due to excessively high standard plate counts (SPC) and closure of extensive areas of shellfish growing waters by state shellfish sanitation authorities (Chestnut and Porter, 1976; Comar et al., 1979). Reopening of these waters in

October of 1976 renewed interest in marketing Rangia and prompted an investigation into the sanitary quality of clam meats.

Comar _et al_. (1979) found a median fecal coliform (FC) level of 80 FC/100g in 48 clam samples taken from Albemarle Sound, N.C. This value is well below the federal and state standard of 230 FC/100g. Standard plate counts approached, and at times exceeded, the 500,000 SPC/g market standard but were believed to reflect a naturally occurring high bacterial flora rather than an indication of product spoilage. Potential pathogens were occasionally detected at levels well below infectious doses. High SPC did not correlate with either indicator organisms or potential pathogens. They concluded that _Rangia_ did not pose any unique public health risk other than those normally associated with shellfish.

The purpose of this report is to: (1) extend the data base on _Rangia_ FC and SPC from Albemarle Sound, N.C.; (2) investigate the effect of environmental factors on clam SPC; and, (3) determine SPC variance among clams.

Review of Literature

Rangia cuneata has long been known to inhabit Gulf Coast estuaries and extended during the Pleistocene from Mexico to New Jersey (Richards, 1938). Since the Pleistocene the clam was absent along the East Coast until 1955 (Hopkins and Andrews, 1970) and its range extended from Texas to northwest Florida (Abbott, 1956). During the past 25 years, *Rangia* has been reported along the Atlantic Coast from Maryland (Pfitzenmeyer and Drobeck, 1964; Gallagher and Wells, 1969) to Florida (Woodburn, 1962). It was first found in North Carolina in 1955 (Wells, 1961). Other reports place the clam in Virginia by 1960 (Wass, 1972), in Maryland by 1964 (Pfitzenmeyer and Drobeck, 1964), and in the upper Chesapeake Bay by 1966 (Pfitzenmeyer, 1970). Hopkins and Andrews (1970) reviewed the most common explanations for the range extension of this species.

Early environmental research focused on easily discernable differences in the overlying water column, e.g., water temperature, salinity, and turbidity as the most important factors affecting benthic organisms (Gunter, 1956; Pearse and Gunter, 1957; Carriker, 1967). Sediment and related factors were shown by several investigators to affect the occurrence and distribution of bivalves (Bader, 1954; Thorson, 1957). More recent studies have been concerned with causes underlying observed relationships between benthic fauna and substrate (Swan, 1952; Tenore *et al*., 1968).

Fairbanks (1963) suggested that observed differences in population density, size, and shell weight of *Rangia* at two locations studied in Lake Pontchartrain, Louisiana, were attributable to differences in organic content of bottom deposits and organic matter in solution.

Tenore _et al_. (1968) showed in an _in situ_ experiment that high concentrations of organic matter and phosphate in sand sediments were favorable for growth in _Rangia_ while the same high concentrations in clay-silt sediments did not favor growth. In general, faster growth rates have been associated with bivalves in sand bottoms compared with rates in clay-silt bottoms where a heavier shell is often produced (Allen, 1954; Swan, 1952).

Peddicord (1976) found similar growth rates for _Rangia_ in the James River, Virginia. He determined the condition index (CI) of clams as defined by the ratio of dry tissue weight x 100 ÷ shell cavity volume in cm^3 (Peddicord, 1977). This ratio is influenced by the amount of stored glycogen and has been used as an indication of marketability. Highest CI was found at sand bottom stations where fastest growth rates were supported. Lowest CI was recorded at clay-silt stations where growth rate was slowest. A reciprocal transplant experiment showed that CI was associated with the water overlying mud stations rather than the substratum itself. He suggested the high suspended-solids concentrations in water overlying mud bottoms may be the cause of reduced CI. The CI also varied directly with salinity and with sediment particle size within study areas.

Peddicord attempted to explain the relationship of high suspended-solids concentrations to growth rates and CI of clams at mud stations. He suggested that clams may continue to pump and filter water regularly with an increase in the cleansing of the filtering apparatus. Clams, on the other hand, may reduce or stop filtering completely during periods of high suspended solids. In either case the result would be a net decrease in available energy to the clam by increased expenditure due to added cleansing or reduced input through loss of food during non-filtering periods.

Loosanoff and Tommers (1948) and Loosanoff and Engle (1947) showed that the pumping rate of the eastern oyster (Crassostrea virginica) varied inversely with suspended silt concentrations and had a reduced pumping rate when exposed to high concentrations of microorganisms. Pratt and Campbell (1956) found an increase in pseudofeces production by the hardshell clam (Mercenaria mercenaria) when placed in a substrate of high clay-silt content. Johnson (1971) and Rhoads and Young (1970) demonstrated increases in growth rates for the slipper limpet (Crepidula fornicata) and the hardshell clam, respectively, when elevated in trays above the substrate. Growth was greater for molluscs held in clear water compared with those in the more turbid water near the bottom.

All of these findings tend to support Peddicord's contention of reduced pumping rates for bivalves exposed to high turbidity or suspended solids concentrations. This would indicate slower growth rates and lower CI because of increased energy demands and loss of food through greater pseudofeces production.

Actually very little is known concerning the relative importance of food types in the diet of adult bivalves. Loosanoff and his associates (in Fairbanks, 1963) classified pelecypod larvae into 2 general types based on food requirements. Oyster larvae were apparently able to utilize only a limited number of marine bacteria while clam and mussel larvae thrived on nearly any organism small enough to be ingested. Darnell (1961) suggested that, under natural conditions, detritus and its associated organisms represent an important food source. Tenore *et al.* (1968) found that *Rangia*, though morphologically typical filter-feeders, are capable of obtaining organic matter and phosphate from the sediment. They suggest that this may be possible either by direct ingestion of the sediment or by feeding on bacteria associated with these materials. Cate and Hemingway (1979) have shown that *Rangia* are capable of assimilating ^{14}C from labeled bacteria into their body tissues. Wilbur and Yonge (1964), Jorgensen (1966), and Jorgensen (1975) reviewed potential food sources available to suspension-feeding organisms in general.

Another important consideration in marketability potential is the sanitary quality of the product. Because bivalves and other filter-feeding shellfish are capable of concentrating organisms in the overlying water column, they may accumulate bacteria of fecal origin (Howser, 1965). Shellfish, when consumed in a raw or slightly heated state, thus transmit a number of enteric diseases found in domestic waste and polluted waters. The history of the standard methods for determination of the sanitary quality of shellfish was reviewed by Comar (1979).

The Food and Drug Administration's (FDA) National Shellfish Sanitation Program (NSSP) has established a fecal coliform MPN (most probable number) limit of 230 FC/100g for shucked oysters. This standard does not specifically apply to all shellfish. However, the National Shellfish Sanitation Program does suggest the MPN limit of 230 FC/100g and the market standard of 500,000 SPC/g be applied to other shellfish as well (Read, 1977). The basic concept behind NSSP is to control the safety of shellfish by preventing contamination of their environment (Hunt, 1979). The agency adheres to the long standing public health concept that if fecal wastes are found in shellfish waters then disease organisms may also be present. The NSSP coliform standard for shellfish growing waters states that: "The coliform median MPN of the water does not exceed 70/100ml and not more than 10 percent of samples ordinarily exceed an MPN of 230 per 100 ml for a 5-tube decimal dilution test in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions" (Howser, 1965). This standard and other NSSP guidelines are not designed to prevent utilization of shellfish resources but outline the conditions under which shellfish can be safely harvested and marketed (Hunt, 1979).

A national survey conducted by FDA's Division of Microbiology in 1974-76 determined mean SPC and FC levels for the eastern oyster at the retail market and the softshell clam (*Mya arenaria*) and hardshell clam at the wholesale market (Read, 1977). A mean SPC level of 6,800/g was determined for 350 samples of softshell clams incubated at 35 °C. Hardshell clams had a mean of 950 SPC/g in 1130 samples. Oysters exhibited a much larger mean of 380,000 SPC/g in 1337 samples, but the difference in retail to wholesale market levels must be taken into consideration which precludes any comparative value of these sets of data. Of 2817 samples analyzed in the survey, 53, 96, and 99 percent of the oysters, softshell, and hardshell clams, respectively, met the wholesale market standard of 500,000 SPC/g. Mean FC levels per 100 g were 34, 7.5, and 3.5 for oyster, softshell, and hardshell clam with 89, 95, and 99 percent, respectively meeting the 230 FC/100g federal standard.

Comar *et al.* (1979) cite Gilbert who reported an average of less than 10,000 SPC/g for shellfish from North Carolina waters other than Rangia destined for market. In investigating the sanitary significance of the microbial flora associated with Rangia in Albemarle Sound, N. C., Comar et al. found SPC much higher than the value reported by Gilbert. Means ranged from 31,000 SPC/g in October to 4,900,000 SPC/g in December. No statistical correlation was shown between clam SPC and month or water temperature. There was, however, a relationship between total counts and collection site during the 12 month study. Highest SPC were found at freshest water stations with a mean of 640,000 SPC/g at the most inland site. The most saline station had a mean of 56,000 SPC/g. The study confirmed preliminary reports by state shellfish sanitation authorities of unusually high SPC in the clam. However, high SPC reported by Comar et al. were not an indication of product spoilage since all samples were iced immediately and analyzed within 24 hours of collection. The data suggest that Rangia possess a naturally high bacterial flora.

One final consideration concerning marketability depends on whether existing populations can support an industry. Tenore (1972) recorded a density of 275/m^2 in portions of the Pamlico River and cited densities of up to 300/m^2 in the Neuse River (Porter, in Tenore, 1972). Gray and Winkler (1977) attributed lower levels of the clam in the Pamlico than previously reported to the severe winter of 1976. The greatest density was in South Creek at 200/m^2. Davis (1979) recorded densities of up to 800/m^2 in areas of the upper Pamlico River during the summer of 1979. The average clam size in higher density areas was 25 mm. Haven (1977) reported an average density of 250/m^2 for Rangia in the waters of Virginia and estimates that up to 10,000 clams of small size may be found per m^2. He described the clam as one of the more under utilized resources in the state of Virginia today.

Description of Study Areas

Three major oligo- to mesohaline systems, the Albemarle Sound, the Tar-Pamlico River, and the Neuse River are located in eastern North Carolina (Figure 1). They are characterized by low salinity, high turbidity, and shallow water. Lunar tides are small (10-15 cm) because of the dampening effect of the Outer Banks and Pamlico Sound and are often overshadowed by strong wind tides of up to 1 m (Hobbie, 1971). The total drainage basin for the Albemarle Sound is 47,733 km^2 (Giese et al., 1979) while those of the Tar-Pamlico and Neuse River basins are 11,137 and 16,058 km^2, respectively (Bowden and Hobbie, 1977).

Albemarle Sound, with an area of 1,160 km^2, is situated along northeastern North Carolina (N.C. Wildlife Commission, 1969). A number of tributaries empty into the sound of which the largest are the Roanoke and Chowan Rivers having watersheds of about 25,100 and 12,700 km^2, respectively (Bowden and Hobbie, 1977). The sound itself extends 89.5 km from Roanoke Island to the US 17 bridge at Edenton. Its nearest connection to the Atlantic Ocean is a 37 km straight line distance from Roanoke Island to Oregon Inlet. The average depth of the sound is 4.5 m and is 13 km in average width. Annual rainfall for the area is 115-140 cm.

Salinity is low when compared with other coastal estuaries and an inverse relationship exists between salinity and river flow (Bowden and Hobbie, 1977). Water temperatures range from 0-34 ^{o}C. Unlike the Tar-Pamlico and Neuse River estuaries, little stratification of salinity, temperature, and dissolved oxygen takes place (Bowden and Hobbie, 1977). Albemarle Sound is described as a partially to well mixed estuarine system.

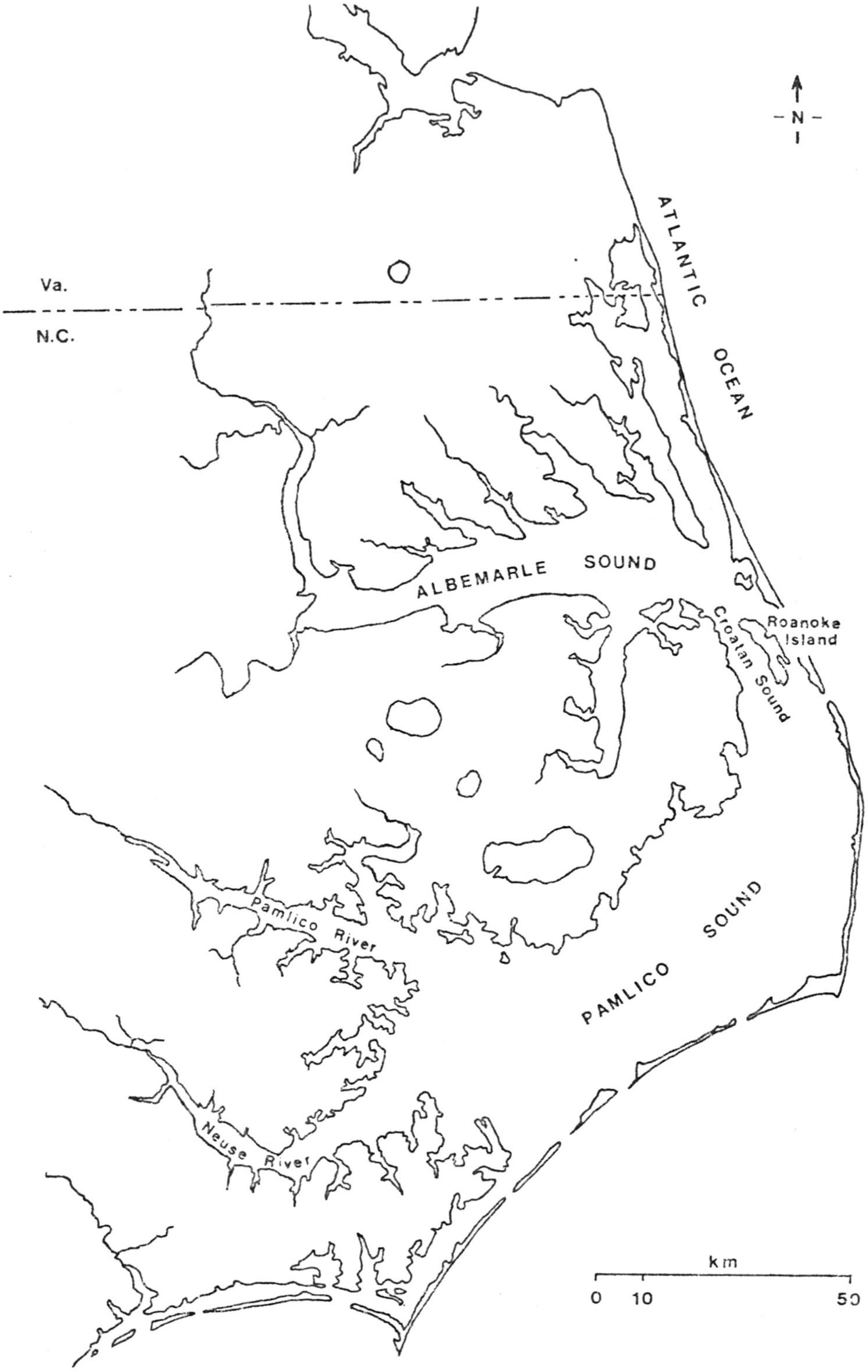


Figure 1. Eastern North Carolina showing location of Albemarle Sound, the Pamlico, and Neuse Rivers.

The watershed is composed of wetlands, farms, and forests. Agricultural crops include tobacco, peanuts, soybeans, and corn. There is very little urbanization with the largest population center at Elizabeth City (approximately 10,000). Major industries are wood and paper production and a fertilizer plant on the Chowan River.

The Tar-Pamlico River estuary extends some 65 km from Washington, N.C., to its mouth in the Pamlico Sound. The average depth is 3.5 m and is 12.5 km at its greatest width. The major tributary of the estuary is the Tar River which has a watershed of 8,008 km^2 (Hobbie *et al.*, 1975). Average rainfall for the area is 122 cm per year.

Salinity can vary from 0-20 $^{o}/oo$. As a result of the Coriolis effect, much of the freshwater flow occurs along the south side of the estuary (Hobbie, 1970). Temperatures range from 3 to 34^{o} C. Turbidity in the system causes the 1 percent light penetration level to be at 1 m in the upper and middle reaches of the estuary and at 4 m in the lower parts (Hobbie *et al.*, 1975). The estuary stratifies irregularly and deoxygenation can occur until broken up by strong winds (Hobbie, 1970).

Only 40 percent of a total population of 300,000 in the basin is urban (Hobbie *et al.*, 1975). The majority of the area is farmland (71 percent) with tobacco as the chief product. The major industry is a large phosphate mining operation (Texasgulf Industries) with another company now under construction (N.C. Phosphate, Inc.). The phosphorus level in the estuary is naturally high so that the amount released by industry has little added effect on the biology of the river (Hobbie, 1971).

MATERIALS AND METHODS

Sampling Stations and Duration

Albemarle Sound/Roanoke Sound (January - December 1979).--The objective of this survey was to extend the data base on *Rangia* SPC, FC, and TC. Clam and water samples were collected monthly during 1979. The four collection stations on the Albemarle and Roanoke Sounds from the previous study (Comar *et al.*, 1979) were retained in this investigation.

Criteria for selection of the stations were based on variation in bacterial counts of overlying water, variation in salinities, and distance from areas of human habitation (Comar, 1979). The first three stations were in water open to shellfishing, while the fourth was located in a closed area (Figure 2). Salinities during the investigation ranged from an average of 2.5 $^{o}/oo$ at Station 1 (range of 0.5 to 5.5 $^{o}/oo$) to an average of 0.2 $^{o}/oo$ at Station 4 (range of 0.0 to 0.5 $^{o}/oo$). Stations 1 and 4 were located near areas of residential and recreational homes utilizing septic tanks for sewage disposal, while stations 2 and 3 were at least 1 km from human habitation.

Pamlico River/Bath Creek (February - April 1979).--A field experiment was conducted during March and April at Bath, N.C., to investigate the importance of sediment and its microbial flora on bacterial levels associated with clams. The area for the investigation (Figure 3) was chosen for proximity to the laboratory and its well established clam populations both in sand and in clay-silt bottom types. The sand bottom station (Station 1) was located 30 m from shore in water averaging 1 m in depth. This station was open to wind and tidal influences and re-

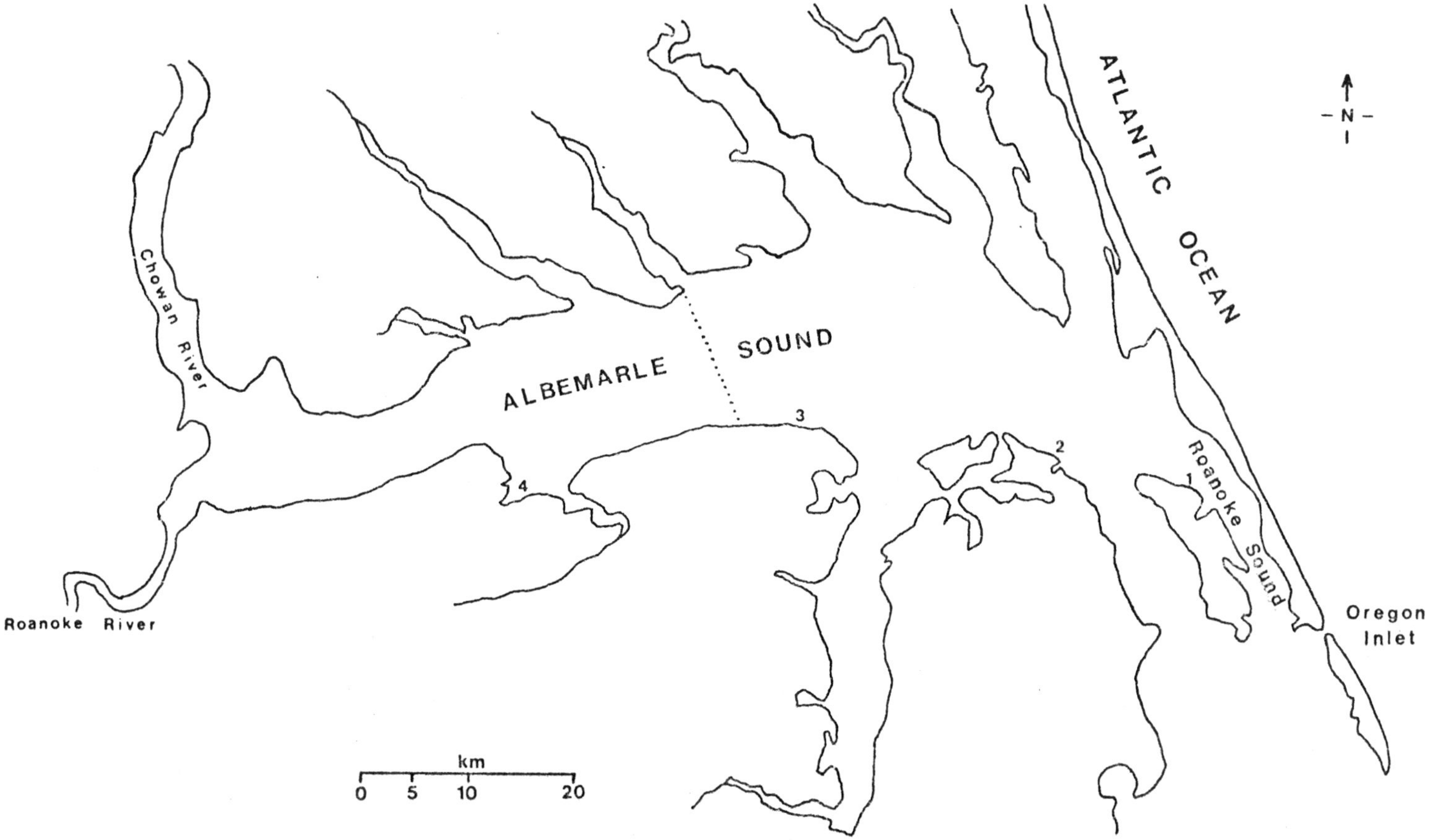


Figure 2. Location of sampling stations on the Albemarle Sound. The area east of dotted line was open to shellfishing.

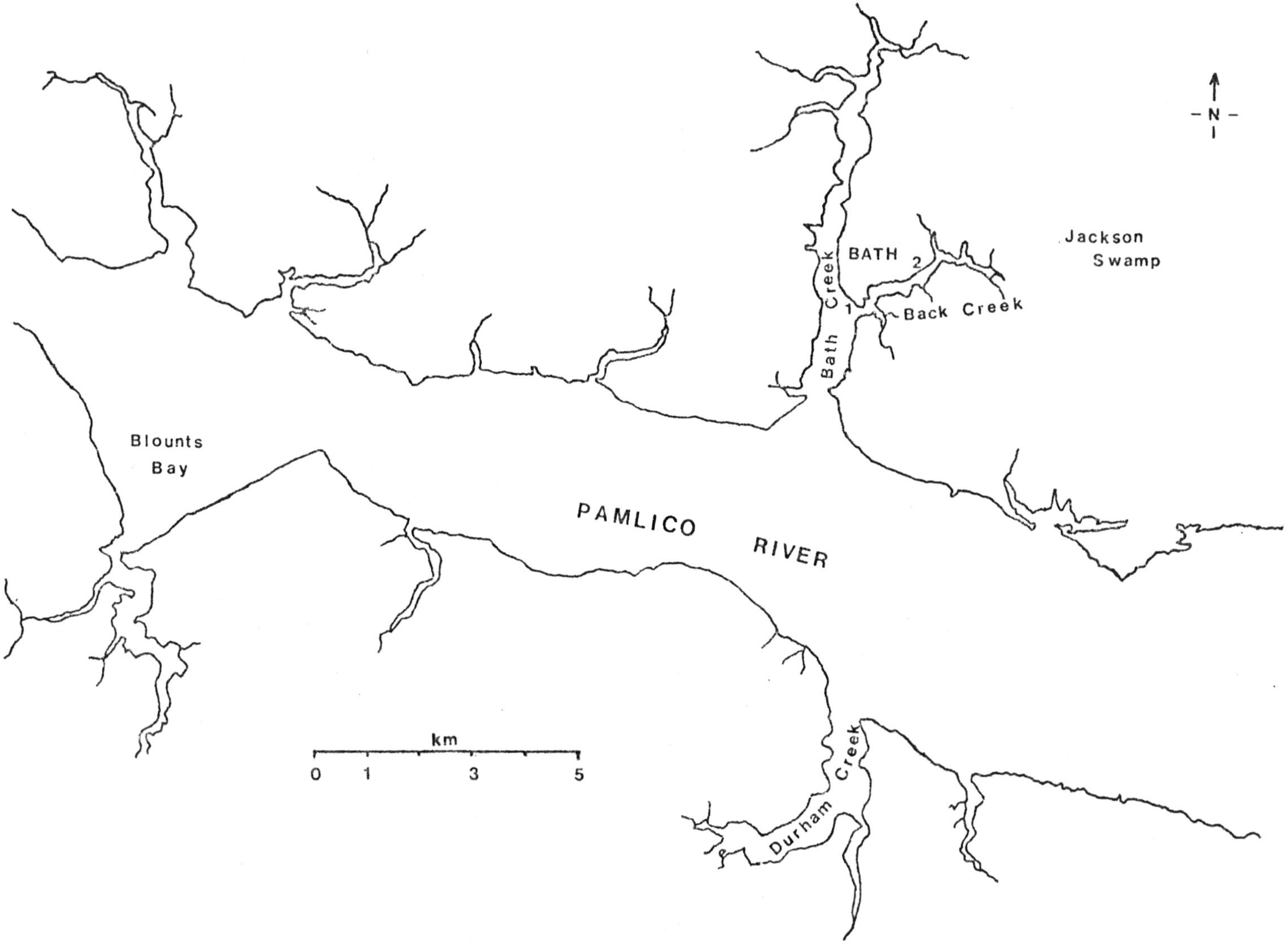
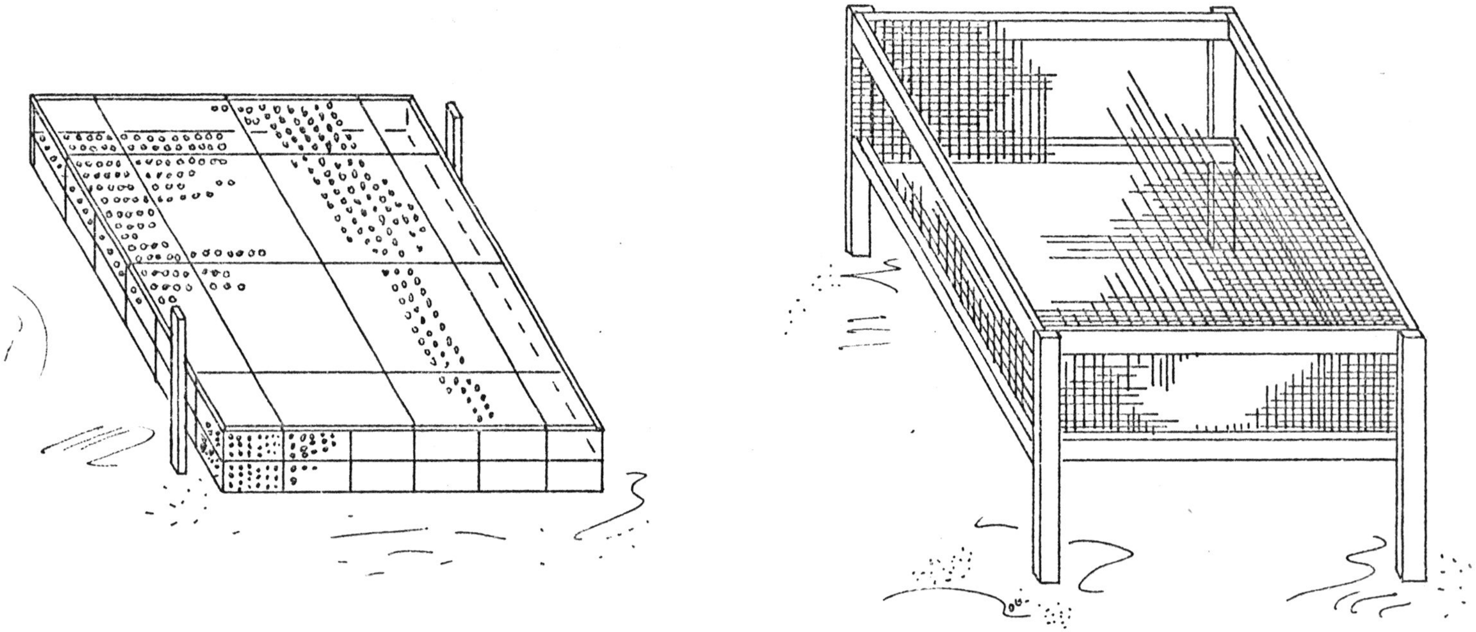


Figure 3. Bath Creek area showing collection stations.

ceived a large amount of water flow from Bath Creek. Station 2 was on Back Creek, an eastern tributary of Bath Creek draining from Jackson swamp. It was located in a small cove, well protected from winds by dense vegetation including trees. The bottom was a clay-silt mixture rich in organic matter probably derived from the nearby shoreline vegetation. Land drainage at this station was substantially less than at the sand bottom station. Water temperature and salinities were similar for both stations and ranged from 10 to 22 ^{o}C and 0.5 to 5.0 ^{o}/oo during the investigation. Turbidity varied slightly between stations and ranged from 14.5 to 60 NTU during the study period.

Preliminary sampling was conducted during February. Routine clam, water and sediment samples were collected from both stations on 11 March, 1979 before initiating the field experiment. Perforated, polyethylene trays measuring 0.75 m^2 were used to hold clams above the bottom surface thus preventing reburrowing. Clams were collected from both stations independently and divided into 3 groups of 60. One group from each station was placed in a separate polyethylene tray and enclosed by strapping a second tray on top of the first. The double-tray arrangement was inverted, placed on the bottom surface where clams were originally collected, and anchored to the sediment with two 30 cm wooden pegs (Figure 4). Inverting the trays held the clams 6 cm above the bottom surface. The groups are referred to as resident tray (RT) clams and are distinguished by station.

The remaining groups were transported to stations differing in sediment type from where they were collected. One transported group at each station was placed in a similar double-tray arrangement and anchored



Inverted Double Tray

Wood-frame, Wire Box

Figure 4. Experimental tray and overhead box design in Bath Creek experiment.

to the sediment 5 m from the RT groups. They are referred to as transported tray (TT) clams and are differentiated by the station where they were placed. The last group of 30 clams at each station was air dried and marked with fingernail polish to aid in later identification. They were placed on the bottom surface 5 m from the tray groups and allowed to reburrow in the substrate. The groups were retained in similar 0.75 m^2 areas by wooden-framed, wire boxes positioned above (Figure 4). They are referred to as transported sediment (TS) clams and again differentiated by station.

Sampling during the 4 week experiment involved the collection of water (W), sediment (S), undisturbed resident sediment (RS) clams, and the experimental clam groups (RT, TT, TS) from both stations (Figure 5).

Pamlico River/South Creek (July - October 1979).--Increased turbidity conditions created during a hydraulic dredging operation were monitored to assess the effect on clam bacterial levels. North Carolina Phosphate, Inc., dredged 650 m^3 of material during the summer of 1979 from South Creek on the Pamlico River. An existing channel on the creek was lowered by an average of 43 cm and a basin or barge slip was cut into the nearby marsh. Clam, water, and sediment samples were collected from the barge slip area before, during, and after dredging on South Creek. Sand tailings and dredge spoil were pumped into a settling pond before being directed to a discharge point on the Pamlico River (Figure 6). Clam, water, and sediment samples were collected from this area during and after discharge of settling pond overflow into the river. Water temperatures ranged from 20 to 32.5 ^{o}C and salinities from 2.5 to 7.0 ^{o}/oo during the study. Turbidities on South Creek increased slightly over ambient levels

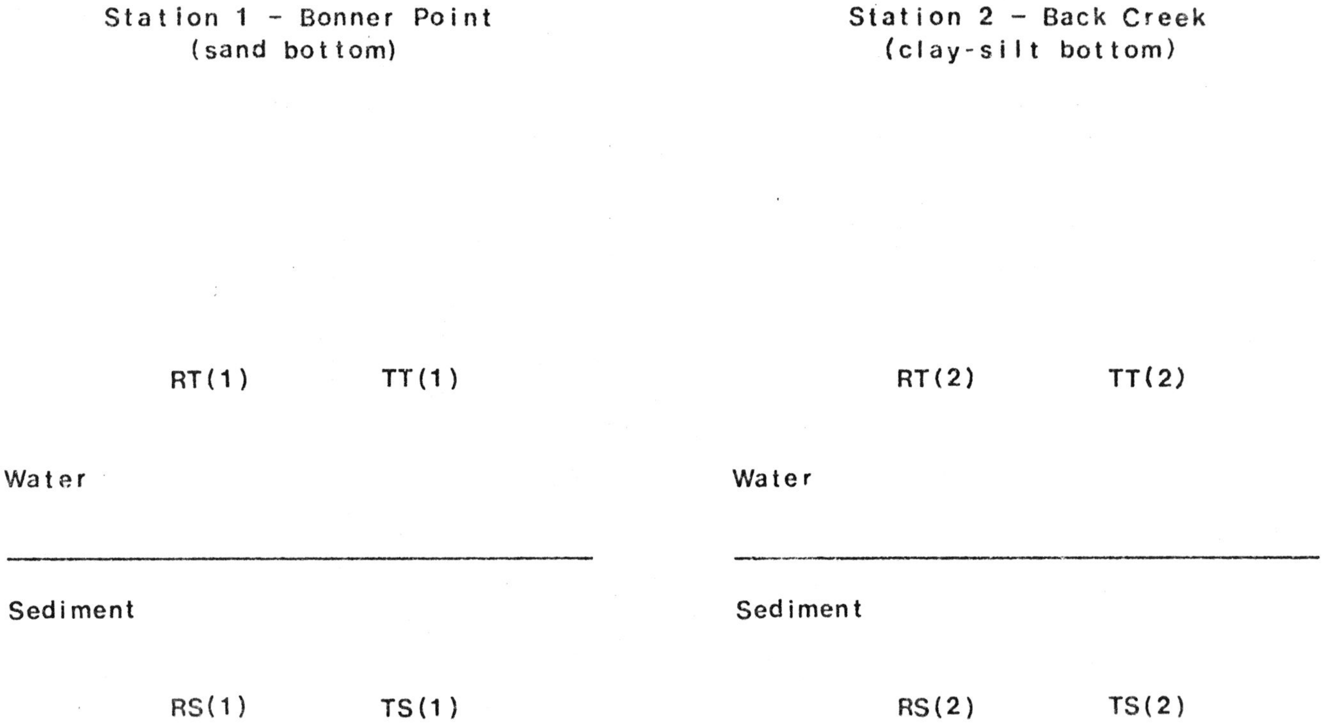


Figure 5. Bath Creek experimental design.

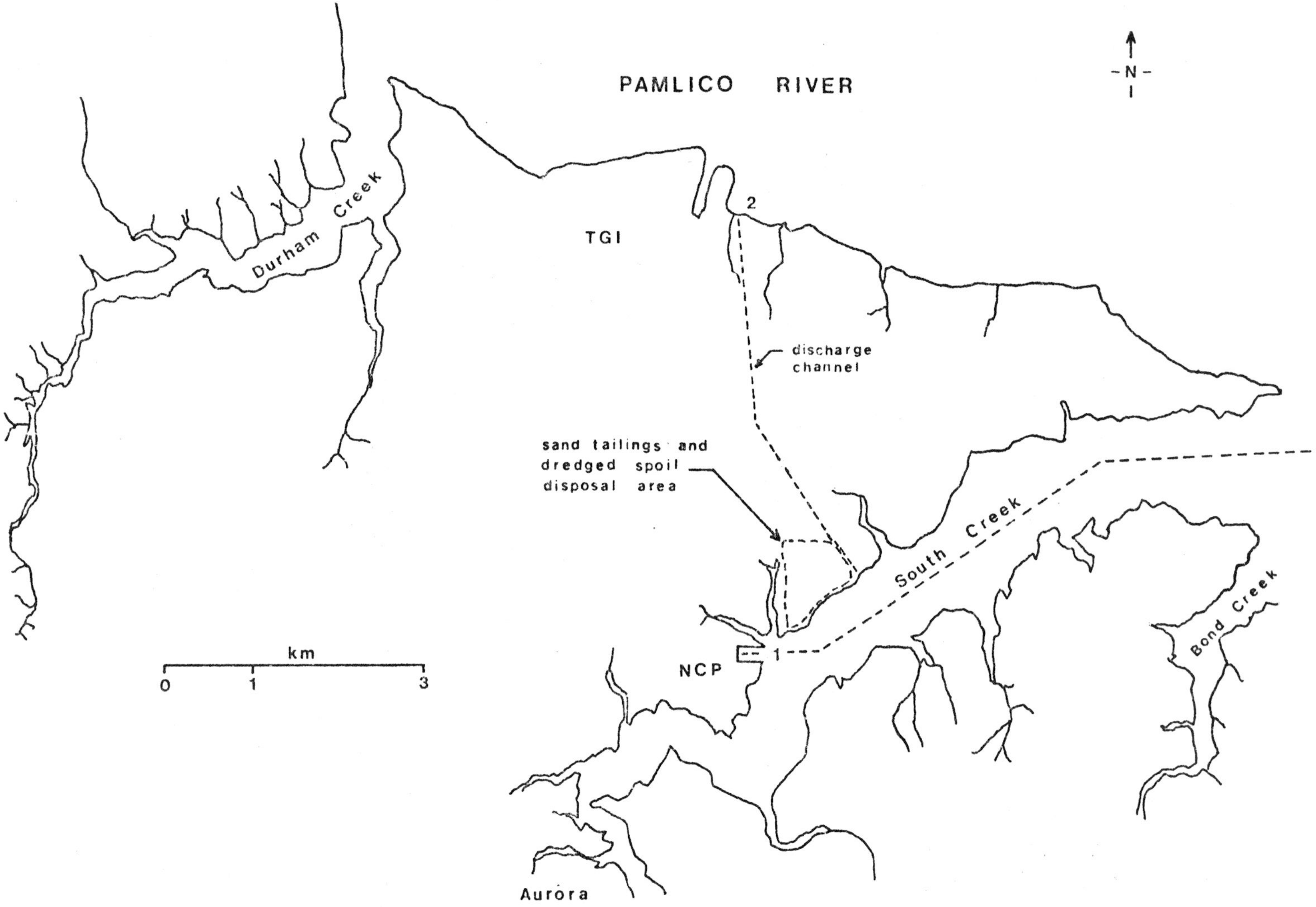


Figure 6. South Creek with reference to the navigation channel and collection stations.

during dredging while a significant increase in turbidity was found during discharge into the Pamlico River.

Field Collections

Clam, Water, Sediment.--Sampling throughout the investigation was near shore in water averaging 1 m in depth. Clam samples were collected with a clam rake having a tine spacing of 20 mm or by hand during the warmer months. Size of the clams ranged from 30 - 65 mm in the Albemarle/ Roanoke Sounds; 30 - 70 mm in the Bath Creek study; and, 25 - 65 mm in the South Creek area. Twelve to fifteen clams were collected per sample and each sample, was sealed in a separate plastic bag. Water samples were obtained at 0.5 m depths using autoclaved polypropylene bottles. Polycarbonate core tubes having an inner diameter of 45 mm were used to collect sediment samples. All clam, water, and sediment samples were placed on ice immediately after collection for transport back to the laboratory.

Total Organic Carbon.--Water for total organic carbon (TOC) analysis was collected during the South Creek study. Water samples were obtained at 0.5 m depths in acid-washed, 50 ml polyethylene bottles. All samples were iced immediately for transport back to the laboratory.

Water Temperature, Salinity, Turbidity.--Water temperature and salinity were obtained during the investigation with a YSI Model 33 S-C-T meter. Turbidity was recorded at 0.5 m depths using a Model DRT - 150 turbidimeter (H.F. Instruments, Bolton, Ont.).

Discharge Index, Wind, Rainfall.--In order to gage the relative amount of water flowing over collection stations, a discharge index (DI) was determined according to a method modified from Comar _et al_. (1979). Data from select monitoring stations were obtained from the U.S. Geological Survey in Raleigh, N.C. A total DI for the sample date and three previous days was calculated from river discharge data at two creeks along the Chowan River, the Ahoskie and Potecasi Creeks. The DI was used as the single index reading for collection dates. A sample calculation for DI is in Appendix C - Part III. The values are used as a model to indicate the relative amount of freshwater drainage into the sound from areas adjacent to the collection stations. Wind data were obtained from the National Oceanic and Atmospheric Administration's (NOAA) weather station at Norfolk, Virginia. Velocity and direction on collection dates were used to indicate possible increases in turbidity within collection areas as a result of wave action. The amount of rainfall was included on collection dates as an indication of non-point source runoff from surrounding areas.

Flow Rate, Turbidity Grids.--In order to characterize the amount of turbidity caused by sand tailings discharge into the Pamlico River, flow rate determinations and turbidity grids were determined on 2 occasions during August. Transects along the drainage channel nearest the discharge point (DP) were made to determine cross-sectional areas of the water mass. Velocity was determined by timing floating objects (oranges) on one occasion and following a plug of rhodamine dye on the other over a known distance. The flow rate for sand tailings discharge was determined in m^3/sec. Dilution and distribution of discharge over collecting areas was characterized by constructing turbidity grids. Eleven transects were made at 100 ft. intervals at the discharge point and extended 500 ft. on either side. Transects extended 500 ft. out from shore and turbidity readings were recorded at 55 points over the grid. Water samples for turbidity determination were collected at 0.5 m depths from a boat. A summary of the flow rate determinations and turbidity grids is in Appendix E - Part III and IV.

Individual Clam Analysis.--Additional collections were made to analyze the variation among individual clams compared with composite samples of 12 clams. Clam and water samples were collected from Station 4 on the Albemarle Sound, from Bath Creek, and from the discharge point on the Pamlico River. At least 25 clams were collected for individual and composite clam sample analyses on each occasion. All samples were treated as described earlier. Water temperature, salinity, and turbidity were recorded during each collection.

Laboratory Analysis

Clam, Water, Sediment.--All clam, water, and sediment samples were kept on ice and processed within 24 h of collection. Bacteriological analyses included standard plate counts (SPC), total coliforms (TC), and fecal coliforms (FC). A flow diagram depicting the analyses performed is in Figure 7. Media used during laboratory analyses are listed in Appendix B. All samples were processed in accordance with Recommended Procedures for the Examination of Sea Water and Shellfish (APHA, 1970). At least 12 clams per station were thoroughly scrubbed and shucked to obtain the

LABORATORY ANALYSES FLOW CHART

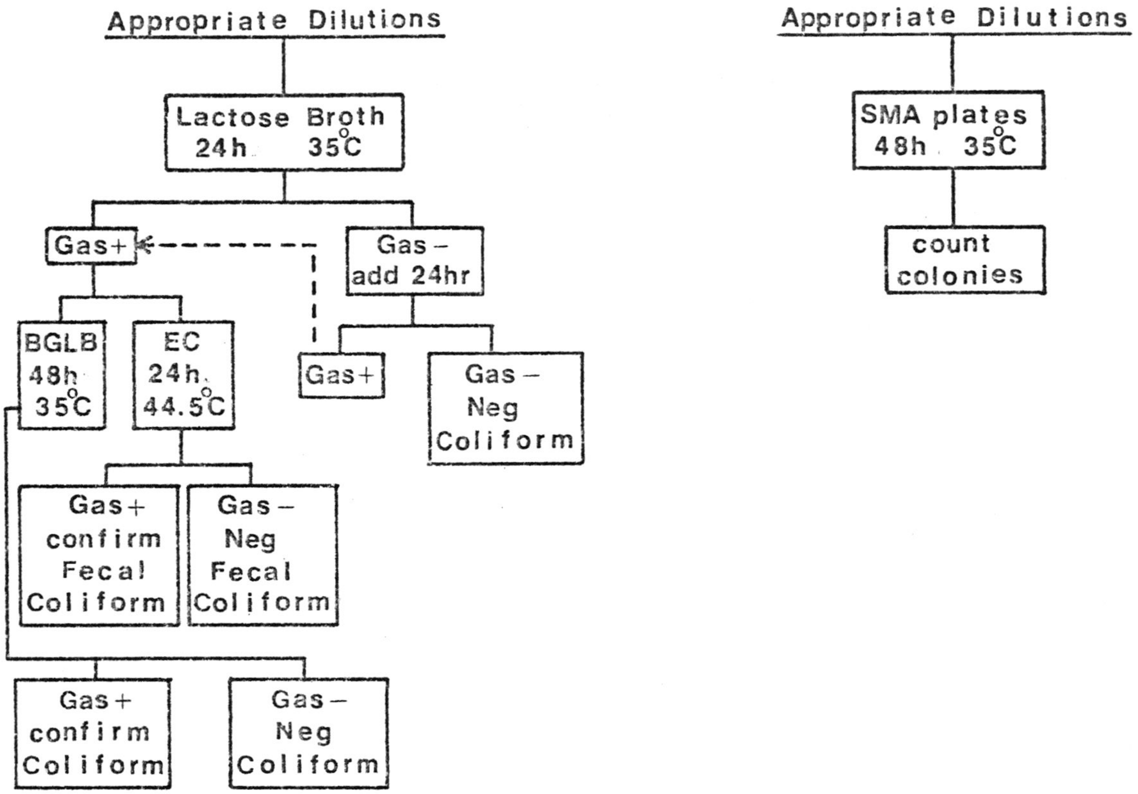


Figure 7. Bacteriological procedures used for determinations of SPC, TC, and FC in clam, water, and sediment samples.

required 50 g minimum sample. Clam meat and liquor were blended with an equal amount, by weight, of sterile phosphate buffered water. Decimal dilutions of clam samples to 10^{-4} g/ml were made in phosphate buffered water. Water samples were carried to decimal dilutions of 10^{-3} ml/ml. Sediment was analyzed by aseptically measuring 50 g from the top 5 cm of core samples and diluting with an equal amount of buffered dilution water in a sterile beaker. The mixture was swirled for 30 seconds and dilutions made to 10^{-4} g/ml in phosphate buffered water.

Individual Clam Analysis.--To determine variance in SPC among clams, individual clams were analyzed similarly to composite clam samples with the exception of the initial dilution factor prior to blending. In order to facilitate blending with such small amounts of sample, a 9:1 phosphate buffered water to clam meat ratio was employed instead of the 1:1 ratio described above. Decimal dilutions were carried again to 10^{-4} g/ml in phosphate buffered water.

Standard Plate Count.--Standard plate counts for clam, water, and sediment were obtained by inoculating 1 ml portions of the various dilutions with standard methods agar (SMA). Samples were pour-plated with SMA and incubated at 35 °C for 48 ± 3 h. Bacterial colonies were counted with a Quebec Colony Counter and multiplied by the appropriate dilution factor to obtain the SPC per g or ml of sample.

Indicator Organisms.--The standard 5-tube multiple dilution method was employed to determine TC and FC MPN's for all samples. Regular clam, individual clam, and sediment samples were run at 10^{0} to 10^{-3} dilutions while water samples were analyzed from 10^{+1} to 10^{-2} dilutions. The presumptive coliform media used was lactose broth. Gas positive presumptive tubes were inoculated at 24 ± 2 and 48 ± 3 h into brilliant green lactose bile (BGLB) broth and EC broth tubes. A gas positive BGLB tube within 48 ± 3 h at 35 °C confirms the presence of TC's. While gas formation in EC broth within 24 ± 2 h at 44.5 °C in a water bath was considered confirmation of FC.

Total Organic Carbon.--Water for TOC analysis was collected in acid-washed, polyethylene bottles. Samples were placed on ice for transport back to the laboratory where they were stored in a cold room (4 °C) until time of analysis. A Beckman Model 915 Total Organic Carbon Analyzer in the Centralized Environmental Laboratory of the Department of Biology was used to determine TOC with the assistance of Martha N. Jones. Water samples were acidified with 0.2 ml of 12 N HCl to convert the inorganic carbon to carbon dioxide and purged with nitrogen gas to drive off the dissolved CO_2 in the water. A 100 μ ml amount of sample was used for analysis of TOC. Analyses were repeated at least twice for each sample and averaged to obtain the ppm of TOC per sample.

RESULTS AND DISCUSSION

Base Line Study

For the 2 year investigation of the sanitary quality of *Rangia* meats from Albemarle Sound, N.C., 26 of 92 samples or 28.3 percent exceeded the 230 FC/100g federal and state standard. The median MPN value for clam FC was 80/100g. Figure 8 shows mean clam FC MPN's by month for each collection station. Clams from Station 1 exhibited the highest FC with 11 of 23 samples exceeding the FC standard. A median MPN of 230 FC/100g was found for clams from this site.

Station 1 was located on the northeast side of Roanoke Island at the mouth of a 100 m long sand spit running parallel to the island. It was the closest collection site to Oregon Inlet and exhibited a range in salinity from 0.5 to 18 $^{o}/oo$. The highest clam FC were observed at the most saline station. This was not expected as explained by Comar *et al.* (1979). Lower FC generally occur at higher salinities (Ketchum *et al.*, 1952; Carlucci and Pramer, 1960) and greater distance and time of exposure from sources of river discharge (Kittrell and Furfari, 1963; Lin *et al.*, 1974; Faust *et al.*, 1975). Containment of water within the sand spit and poor mixing with water in the nearby sound are possible explanations of these results. A shoreline survey for septic tank malfunctioning proved negative with the location of disposal fields making this possibility of contamination remote (Comar *et al.*, 1979).

Median water TC and FC MPN's over the 2 year study at Station 1 again exhibited the highest indicator levels (Table 1). The median MPN was 170 TC/100 ml and 10 of 23 samples or 43.5 percent exceeded the MPN of 230 TC/100 ml. Station 1 also exceeded proposed NSSP fecal coliform

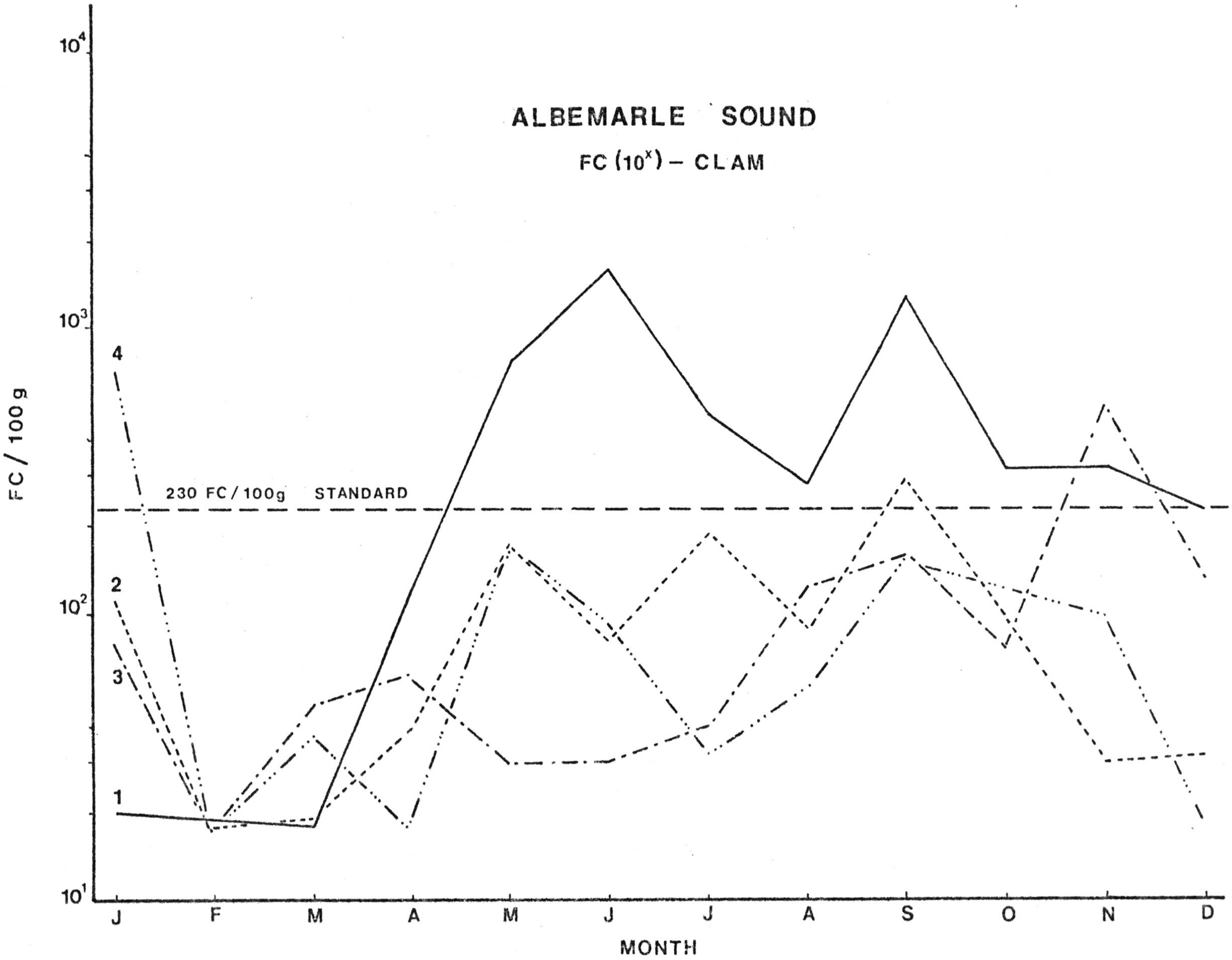


Figure 8. Mean fecal coliform MPN's per 100 grams in Rangia meats from the 4 stations.

Table 1. Water total coliform MPN's per 100 ml and number exceeding NSSP regulations. Fecal coliform MPN's per 100 ml and number exceeding proposed NSSP regulations.

Factor	Station 1	 2	 3	 4
Median MPN TC/100ml	170	23	46	33
No. exceeding MPN 230 TC/100ml	10	2	1	4
Percent of total	43.5	8.7	4.4	17.4
Median MPN FC/100ml	8	4	7	8
No. exceeding MPN 43 FC/100ml	7	3	2	1
Percent of total	30.4	13.0	8.7	4.3

guidelines for shellfishing waters with 7 of 23 samples or 30.4 percent beyond the upper limit of 43 FC/100 ml. The argument for a fecal coliform standard for shellfishing waters is based on differentiating between fecal contamination of water and ambient total coliform levels from natural sources of minor sanitary significance (Hunt, 1975). The proposed guidelines of a median MPN of 14 FC/100 ml with less than 10 percent exceeding 43 FC/100 ml has yet to be adopted by NSSP. However, our results indicate that water at Station 1 does not meet either the "70 TC standard" or the proposed "14 FC standard" of the National Shellfish Sanitation Program.

The seasonal pattern in Rangia FC observed by Comar et al. (1979) was strengthened by the additional sampling. During the months of February through April the 24 clam samples collected from all four stations had a median MPN of less than 20 FC/100g. No samples exceeded the 230 FC/100g standard during this period (Table 2). The water MPN for total coliforms was 23 TC/100 ml with only 2 of 24 or 8.3 percent exceeding 230 TC/100 ml. Based on the federal and state standard of 230 FC/100g for shucked oysters, the months of February through April appear to be the safest time for harvesting Rangia as a fresh seafood product from open shellfishing waters in Albemarle and Roanoke Sounds.

Standard plate counts during the investigation exhibited greater monthly variability than FC. Mean SPC for the 2 year study ranged from 30,000 SPC/g in January to 610,000 SPC/g in December (Table 3). The study again confirmed higher SPC in Rangia when compared with the typical values of less than 10,000 SPC/g for oysters reported by Gilbert and cited by Comar et al. (1979). The data support the suggestion by

Table 2. *Rangia* FC per 100 gram (10^x) for the 2 year study, month, and site.

Month	Site 1	2	3	4	(10^x)
Jan	20	110	80	700	110
Feb	19	18	18	18	18
Mar	18	19	48	38	28
Apr	120	40	60	18	48
May	770	170	30	170	160
Jun	1,600	80	30	90	140
Jul	490	190	40	32	110
Aug	280	88	120	55	110
Sept	1,300	290	160	150	310
Oct	320	94	77	120	130
Nov	320	30	510	99	64
Dec	230	33	130	19	66
(10^x)	200	68	70	67	-

Table 3. *Rangia* SPC per gram (10^x) for 1979, 1977-78, and the combined 10^x by month.

Month	Site	$\frac{SPC}{g}$	log	mean log (x)	1979 10^x	1977-78 10^x	2 year 10^x
Jan	*	*	*	*	*	30,000	30,000
Feb	1	1,100	3.041	4.148	14,000	210,000	54,000
	2	4,100	3.613				
	3	87,000	4.940				
	4	100,000	5.000				
Mar				4.554	36,000	190,000	83,000
Apr				4.685	48,000	300,000	120,000
May				4.995	99,000	470,000	220,000
Jun				4.521	33,000	150,000	70,000
Jul				4.988	97,000	56,000	74,000
Aug				5.071	120,000	63,000	87,000
Sept				4.572	37,000	140,000	73,000
Oct				5.236	170,000	31,000	73,000
Nov				5.204	160,000	72,000	110,000
Dec				4.878	76,000	4,900,000	610,000

Comar et al. (1979) that *Rangia* possess a naturally high bacterial flora.

Standard plate counts are used by several commercial markets to indicate product spoilage and reduced shelf life. Economic loss (Willis Brother's Seafood as cited by Comar et al. (1979) has occurred as a result of these naturally high *Rangia* SPC.

The second objective of this study was to determine what environmental factor or combination of factors affect *Rangia* SPC. The statistical evaluation of the relationship between clam SPC and environmental factors was performed through multi-variate regression analysis. Prior to this analysis, product-moment correlation coefficients were determined for all bacteriological and environmental factors measured because of the possible interdependence of variables. Scattergrams were made of all significant linear correlations found and regression lines were determined (Table 4). All bacteriological data was log transformed before analysis to provide a better approximation of the biological processes.

Linear correlation coefficients and their significance levels are shown in Table 5 for clam SPC versus environmental factors and water quality indicators. A significant negative linear correlation at $P < 0.05$ was found in clam SPC vs. rainfall within 24 h of collection. No significant correlation was shown for any other environmental factor or water quality indicator and clam SPC.

The level of bacteria (SPC) associated with *Rangia* was assumed to be a function of the various environmental characteristics of the water column. A step-wise multiple regression (SPSS computer package, version 7) was used to test the hypothesized functional dependence, $y = F(x_1;$

Table 4. List of all significant linear correlations found in the Albemarle/Roanoke Sound study.

Factor y	Factor x	Number of Observations (n)	Correlation Coefficient (r)	Regression Line Equation
WTEMP	TURB	48	- 0.4051 **	y = - 0.207x + 22.756
"	DI	88	- 0.1987 *	y = -114.776x + 19.125
"	RNFL	88	0.2615 **	y = 6.196x + 16.181
"	WS	88	- 0.2906 **	y = - 0.796x + 23.004
SAL	STA	88	- 0.4759 **	y = - 1.179x + 0.227
TURB	STA	48	0.2900 *	y = 4.604x + 11.438
"	WS	48	0.2904 *	y = 1.358x + 13.406
DI	WS	96	- 0.1844 *	y = - 0.001x + 0.019
RNFL	WS	96	- 0.2295 *	y = - 0.027x + 0.395
Log CSPC	STA	91	0.4760 **	y = 0.345x + 4.151
"	RNFL	91	- 0.2054 *	y = - 0.423x + 5.096
"	Log SSPC	48	0.4726 **	y = 0.511x + 3.076
"	Log STC	48	0.2453 *	y = 0.414x + 4.342
Log CTC	WTEMP	84	0.2188 *	y = 0.029x + 2.823
"	DI	92	0.3288 **	y = 20.543x + 3.012
"	WS	92	- 0.3642 **	y = - 0.107x + 3.991
"	Log WSPC	90	0.3701 **	y = 0.644x + 1.416
"	Log CFC	92	0.2738 **	y = 0.409x + 2.467
"	Log WTC	92	0.3102 **	y = 0.431x + 2.554

* $P < 0.05$

** $P < 0.01$

Table 4. (continued)

Factor		Number of Observations (n)	Correlation Coefficient (r)	Regression Line Equation
y	x			
Log CTC	Log WFC	92	0.2948 **	y = 0.417x + 2.894
Log CFC	STA	92	- 0.2867 **	y = - 0.167x + 2.364
"	WTEMP	84	0.2861 **	y = 0.020x + 1.571
"	SALIN	84	0.2445 *	y = 0.054x + 1.826
"	TURB	44	- 0.4157 **	y = - 0.014x + 2.191
"	Log WSPC	90	0.3019 **	y = 0.340x + 0.956
"	Log WTC	92	0.4449 **	y = 4.134x + 1.267
"	Log WFC	92	0.5763 **	y = 0.545x + 1.465
"	Log SFC	48	0.3080 *	y = 0.819x + 0.862
Log WSPC	DI	90	0.2966 **	y = 10.676x + 2.723
"	Log WTC	90	0.5885 **	y = 0.471x + 2.088
"	Log WFC	90	0.4772 **	y = 0.389x + 2.513
Log WTC	TURB	44	- 0.2641 *	y = - 0.009x + 1.752
"	DI	92	0.2015 *	y = 9.053x + 1.535
"	Log WFC	92	0.6357 **	y = 0.646x + 1.074
Log WFC	WTEMP	84	0.2871 **	y = 0.021x + 0.484
"	SALIN	84	0.2124 *	y = 0.049x + 0.768
"	RNFL	92	0.2006 *	y = 0.352x + 0.813
"	WS	92	- 0.1789 *	y = - 0.037x + 1.138

* $P < 0.05$
** $P < 0.01$

Table 4. (continued)

Factor		Number of Observations (n)	Correlation Coefficient (r)	Regression Line Equation
y	x			
Log SSPC	STA	48	- 0.3149 *	$y = - 0.227x + 4.689$
"	WTEMP	40	- 0.3149 **	$y = - 0.047x + 4.952$
"	Log STC	48	0.3918 **	$y = 0.611x + 2.879$
Log STC	Log SFC	48	0.4462 **	$y = 0.939x + 0.701$

* $P < 0.05$

** $P < 0.01$

Table 5. Linear correlation coefficients and significance levels for clam SPC per gram versus environmental factors and water quality indicators.

Factor	Number of Observations (n)	Correlation Coefficient (r)	Significance (P)	Regression Line Equation
WTEMP	83	0.0066	0.476	
SALIN	83	- 0.1780	0.054	
TURB	43	- 0.0564	0.364	
DI	91	0.1206	0.127	
RNFL	91	- 0.2054	0.025	$y = -0.423x + 5.096$
WTC	91	- 0.0329	0.378	
WFC	91	0.0092	0.466	

x_2; x_3; x_4; x_5) (Snedecor, 1978 as cited in Gerba et al., 1979). The results are shown in Table 6.

The environmental factors for which regression coefficients were found statistically significant at $P < 0.01$ were (1) turbidity, (2) discharge index, (3) water temperature, and (4) salinity. The first factor, turbidity, accounted for approximately 8.0 percent of the variation in clam SPC. Discharge index accounted for 6.5 percent while water temperature and salinity accounted for 6.7 and 5.2 percent respectively. This brings the total R^2 value to 26.5 percent for the four factors considered. The addition of rainfall to the five variable model increases R^2 by less than 0.1 percent. Thus, a relationship between clam SPC and some environmental factors exist with less than a one percent chance that this association was found by accident alone.

There are significant negative correlations for turbidity and discharge index compared with water temperature (Table 7). Turbidity was negatively correlated with water TC. Water FC were significantly correlated with water temperature and salinity. All of this points to interdependence between variables, i.e., during cold weather (winter) high turbidity and high discharge index are expected while warm water (summer) supports a lower discharge index and high FC. Rainfall accounts for some non-point runoff resulting in high water FC and low clam SPC. The positive correlation between water FC and salinity was discounted due to the unnaturally high FC found at Station 1.

Of the environmental factors studied by multivariate analysis, salinity and turbidity demonstrated negative influence on clam SPC. Turbidity was affected by discharge index and wind initiated wave action

Table 6. Multiple regression of clam SPC per gram and environmental factors.

Variable*	Significance of each variable	Multiple R	R Square	R Square Change	Simple R	Overall F	Overall Significance P
TURB	0.001	0.28214	0.07960	0.07960	- 0.28214	14.619	0.010
DI	0.001	0.38055	0.14482	0.06521	- 0.23157	7.046	0.001
WTEMP	0.001	0.46128	0.21278	0.06796	0.19770	12.917	0.001
SALIN	0.001	0.51447	0.26468	0.05190	- 0.07984	5.549	0.001
RNFL		0.51513	0.26536	0.00068	- 0.05509	0.083	0.001

* Variable abbreviations are listed in Appendix A.

Table 7. Product-moment correlation matrix of select measured variables.

Variable	WTEMP	SALIN	TURB	DI	RNFL	WTC	WFC	CSPC
WTEMP	1.0000							
SALIN	0.1039	1.0000						
TURB	- 0.4051**	- 0.1648	1.0000					
DI	- 0.1987*	- 0.1562	0.1371	1.0000				
RNFL	0.2615	- 0.1600	0.0176	0.1491	1.0000			
WTC	0.0762	0.0113	- 0.2641*	0.1667	0.1002	1.0000		
WFC	0.2871**	0.2124*	- 0.1944	0.0807	0.2006*	0.6357**	1.0000	
CSPC	0.0066	- 0.1780	- 0.5640	0.1500	- 0.2054*	- 0.0329	0.0092	1.0000

* $P < 0.05$
** $P < 0.01$

as well ($r = 0.2904$, $P < 0.05$). Water temperature showed positive influence on clam SPC. Examining the interaction of these observed factors may improve understanding the relationship between clam SPC and collection stations.

The mean SPC value for clams taken from the most saline station over the 2 year study was 30,000 SPC/g (Table 8). The most inland site (Station 4) had the largest mean SPC value at 420,000 SPC/g. These data reflect lower clam SPC under higher salinity conditions. Combined with the positive correlation of water temperature with clam SPC, it is possible the best conditions for harvesting *Rangia* as a fresh seafood product are high salinity and low water temperatures. The mean SPC for the months of February through April for all stations were 54,000, 83,000, and 120,000, respectively. This period coincides with the seasonal pattern in *Rangia* FC levels previously shown. Thus, based on this 2 year study, the months of February through April would be the best time for harvesting and marketing *Rangia* as a fresh seafood product. Further in-depth sampling during these months should be conducted to determine the variability in clam samples from high salinity, open shellfishing waters.

Field Experiment

A field experiment was conducted to assess the influence of sediment and its associated factors on *Rangia* SPC. This investigation was initiated in response to the suggestion by Tenore *et al*. (1968) that *Rangia* are capable of obtaining organic matter and phosphorous from the sediment either by direct ingestion of the sediment or by feeding on microorganisms associated with these materials. Comar *et al*. (1979)

Table 8. *Rangia* SPC per gram (10^x) for 1979, 1977-78, and the combined 10^x by station.

Station	Month	SPC/g	log	mean log (x)	1979 10^x	1977-78 10^x	2 year 10^x
1	Jan	*	*	4.175	15,000	59,000	30,000
	Feb	1,100	3.041				
	Mar	32,000	4.505				
	Apr	8,600	3.935				
	May	8,100	3.908				
	Jun	1,800	3.255				
	Jul	28,000	4.447				
	Aug	52,000	4.716				
	Sept	29,000	4.463				
	Oct	110,000	5.041				
	Nov	61,000	4.785				
	Dec	6,700	3.826				
2				4.605	40,000	66,000	51,000
3				5.092	120,000	120,000	120,000
4				5.426	270,000	640,000	420,000

found a highly significant correlation between clam and sediment SPC ($P < 0.01$).

Clams held above the bottom sediment but exposed to similar water conditions as undisturbed resident sediment clams had higher SPC (Figure 9). Statistical analysis using a paired sample mean test (Wilcoxon) showed a significance of $P = 0.0625$. Failure to demonstrate significance at the $P < 0.05$ level was limited by sample size (4, weekly samples). Clams transported from the sandy bottom station and placed on the bottom at the clay-silt station showed a similar significance level at $P = 0.0625$ for SPC compared with undisturbed resident clams. No difference was shown in SPC for clams transported from the sand bottom and held in trays above the clay-silt bottom when compared with resident sediment clams.

Comar et al. (1979) hypothesized that <u>Rangia</u>, belonging to the infauna group of organisms, may filter water laden with larger quantities of suspended sediments than the epifaunal filter-feeding oysters. This could account for the higher SPC characteristic of <u>Rangia</u> since sediment SPC are known to be as much as 1,000 times higher than water SPC. If clams were feeding directly on the sediment, then clams held above the bottom surface would take in lower numbers of bacteria per unit of material ingested. The fact that suspended clams had higher SPC compared with undisturbed sediment clams does not disprove the possibility of sediment ingestion but does suggest the greater importance of related factors in the immediate water column. The difference may possibly be associated with higher pumping rates, increased retention of organisms from the water, or stress related increases in SPC when clams are

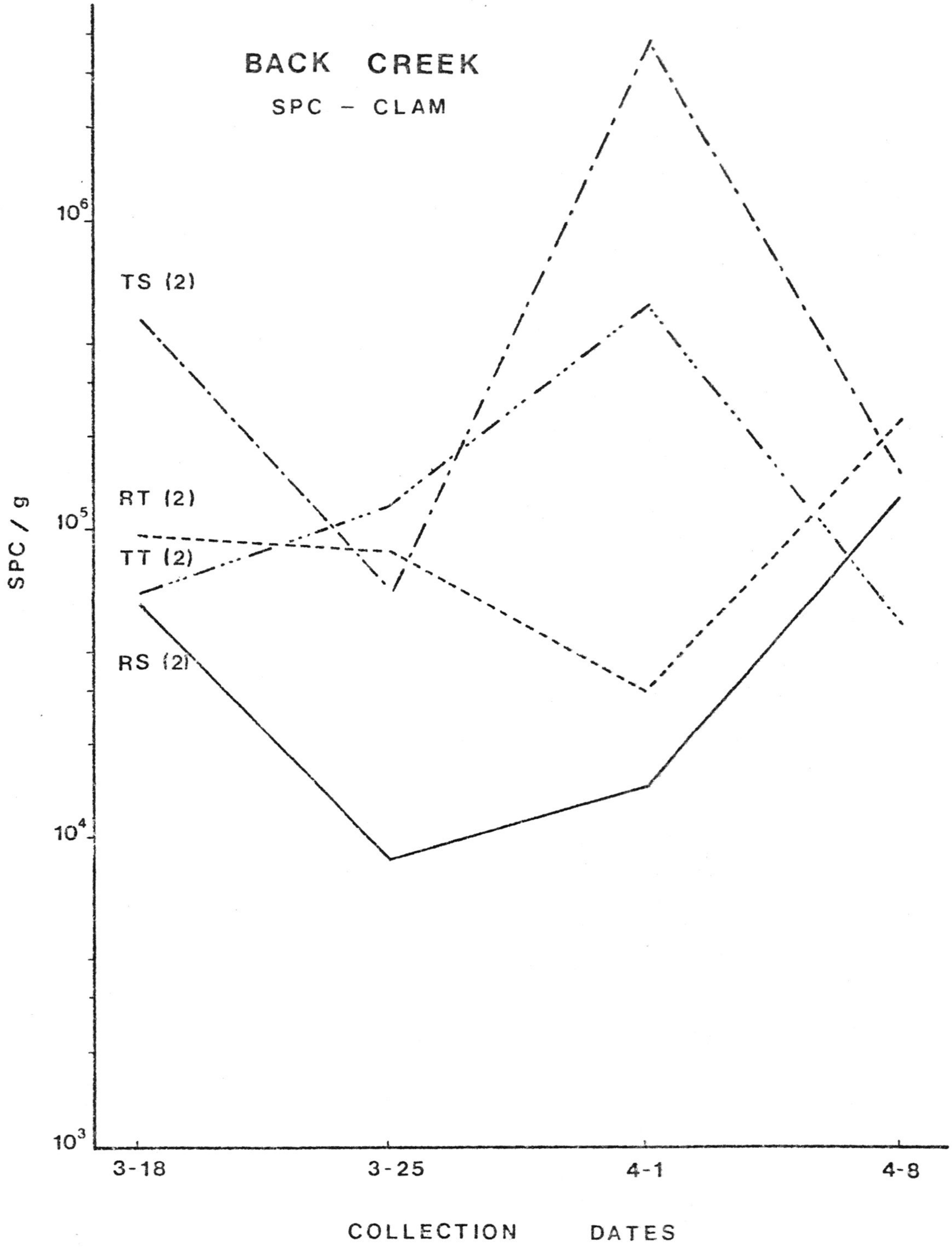


Figure 9. Clam SPC per gram at the clay-silt station during the field experiment.

removed from their usual substrate.

Faster pumping rates and increased growth are associated with molluscs held in clear water compared with those in the more turbid water characteristic of the bottom surface (Loosanoff, 1961; Rhoads and Young, 1970; Johnson, 1971). Though the relative importance of bacteria as a food item is not fully understood, increased pumping rates are viewed as a greater intake of material contained in the water column. Galtsoff (1964) determined the pumping rate for the eastern oyster to be up to 30 liters per h, averaging between 11-20 liters/h. Raising clams above the sediment-water interface would increase pumping rates and facilitate concentration of suspended materials including bacteria in the water column. Higher SPC in *Rangia* held above the bottom surface may reflect the ability of the clam to filter the medium more effectively than *Rangia* would in the sediment.

Transport of clams from one sediment type to another may also affect their ability to filter the medium. Peddicord (1977) demonstrated that *Rangia* moved from clay-silt bottoms and placed in sand bottoms showed dramatic increases in condition index. He believed that this was caused by a positive factor present in water over sand bottoms or some inhibitory factor associated with clay-silt bottoms. The difference in suspended-solids concentrations at the two sediment types was suggested as the explanation.

Clams transported from the sand bottom to the clay-silt bottom (Back Creek) displayed a dramatic increase in SPC during the third week of the study. Resident clams in tray and sediment failed to show this increase (Figure 9). Sediment clams at the sand bottom station did not display any dramatic difference in SPC during any time of the study

(Figure 10). Comparison of suspended clams at the sand bottom station is not possible because of the loss of these trays during adverse wind conditions.

The dramatic increase of SPC in transported sediment clams from the sand bottom compared to resident sediment clams at the clay-silt bottom may be in response to higher suspended-solids characteristic of clay-silt bottoms. Clams normally associated with water conditions above sand bottoms may not be able to cope with the higher suspended-solids levels at the clay-silt bottom site. Clams taken from clay-silt sediments and placed in the sand bottom area failed to show any dramatic increase in SPC. This may reflect their ability to filter the medium over sand bottom stations better than their counterparts are capable of filtering the medium over clay-silt bottom types. It appears that clam SPC is influenced more by the nature of the overlying water column in areas where they are found than the sediment itself.

Dredging Study

The opportunity to study the effects of high turbidity conditions on clam SPC arose when North Carolina Phosphate, Inc., undertook a dredging operation in South Creek, N.C. Environmental and bacteriological data before, during, and after dredging in the creek are summarized in Table 9. Water SPC were observed to increase dramatically during dredging which coincided with increases in turbidity. Total organic carbon levels were relatively higher after dredging. Clam TC showed little difference during dredging but had a mean MPN of 1,600 TC/100g after the operation was complete. Clam SPC were not changed by the

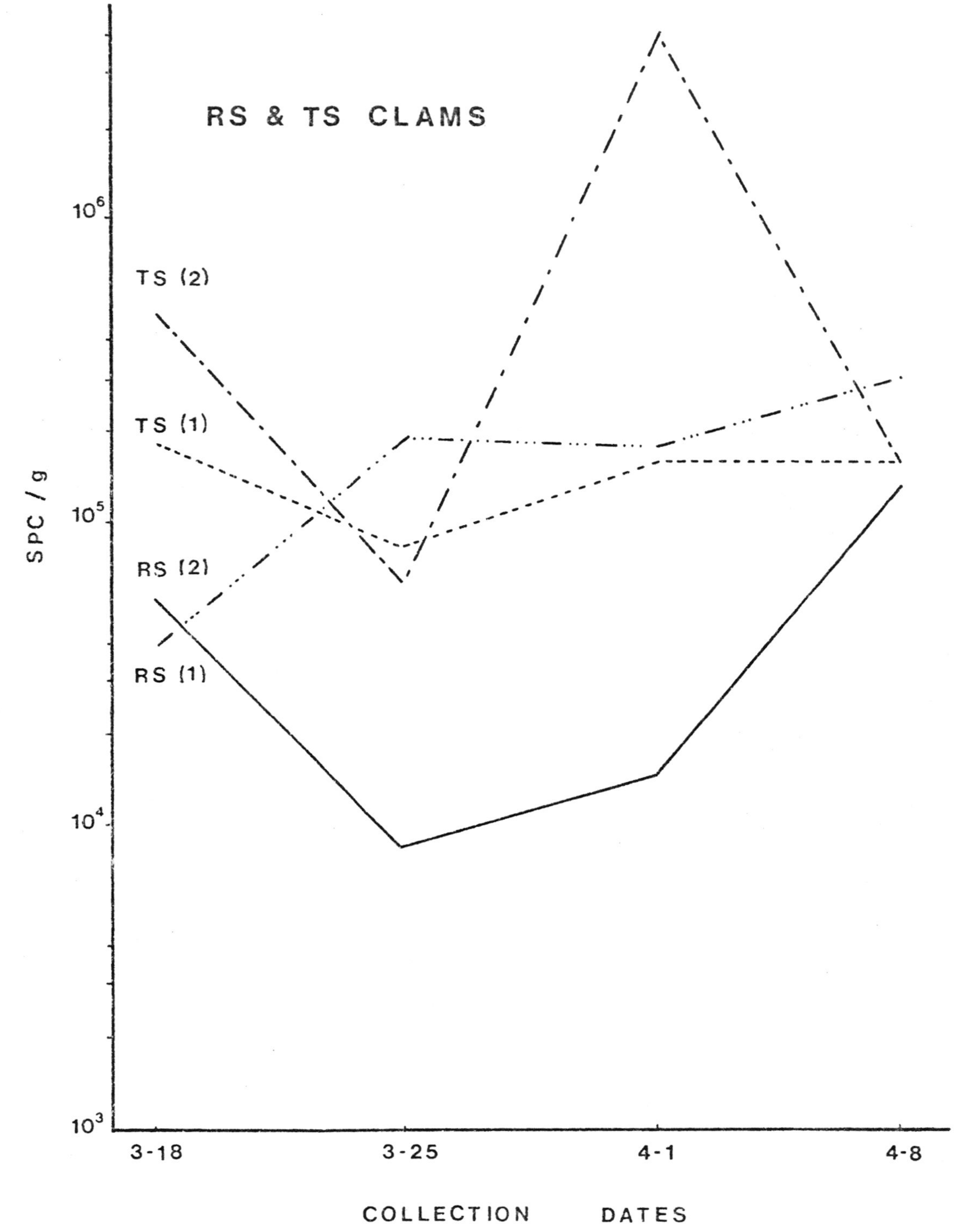


Figure 10. Resident and transported sediment clam SPC per gram at both collection stations during the field experiment.

Table 9. Summary of South Creek/Dredge Site area.

Parameter		Dredging	
	Before	During	After
No. of Samples	6	4	6
WTEMP	28.0 - 28.5	29.0	20.0 - 22.0
SALIN	2.5 - 4.0	6.0 - 6.1	5.3 - 6.1
TURB	12.0 - 16.5	15.0 - 34.0	5.5 - 9.5
TOC	9.38 - 11.8	9.49 - 11.9	10.3 - 15.4
CLAM SPC	5,100 - 110,000	25,000 - 36,000	27,000 - 77,000
10^x	33,000	31,000	42,000
CLAM TC	130 - 1,100	130 - 1,100	490 - 3,300
10^x	500	360	1,600
WATER SPC	260 - 990	1,700 - 22,000	200 - 550
10^x	590	89,000	390
WATER TC	2 - 23	130 - 330	33 - 140
10^x	15	210	60

conditions created during dredging at this station. There was a range of 25,000-36,000 SPC/g during dredging compared with ranges of 5,100-110,000 SPC/g before and 27,000-77,000 SPC/g after dredging. Mean clam SPC levels before, during, and after dredging were about the same.

The SPC of clams collected during and after discharge of sand tailings overflow into the Pamlico River did not show any significant correlation with turbidity. The amount of discharge and its pattern of dispersion in the river was characterized by flow determinations and turbidity grids (Appendix E. Part III and IV). Though the dispersion was influenced by wind and tide action, samples were identified by location of collection relative to the discharge point. Total organic carbon, water TC, water FC, and clam FC levels were correlated with turbidity; r = 0.8073, 0.7233, 0.6002, and 0.6254, respectively.

Clam SPC variability that can be observed in samples collected under similar environmental conditions is shown in Table 10. These data reflect a composite sample of 12 clams homogenized together in a blender. Clam SPC varied from 16,000 SPC/g to 1,200,000 SPC/g in composite samples collected within 600 feet of each other. Turbidity, salinity, and water temperature were similar for the two samples.

The same high degree of variability was found after dredging was completed and discharge over the collection area ceased by approximately 4 weeks. Composite clam SPC were found to vary from 20,000 SPC/g to 1,900,000 SPC/g in samples collected within 300 ft. of each other. These data demonstrate the high degree of composite clam SPC variability that can be expected under similar environmental conditions.

Table 10. Examples of composite clam variation in samples collected during and after spoil discharge into Pamlico River.

STA			SPC/g	TC/100g	FC/100g
Date: 08-30-79					
I.	DP x 1200 ft.	Water	1,000	94	17
	TURB 7.0	Clam	13,000	2,600	170
II.	100 ft. E x 100 ft.	Water	12,000	170	49
	TURB 40.0	Clam	16,000	1,700	220
III.	300 ft. E x 100 ft.	Water	16,000	790	490
	TURB 60.0	Clam	90,000	3,300	170
IV.	300 ft. E x 600 ft.	Water	19,000	490	70
	TURB 40.0	Clam	1,200,000	2,200	120
Date: 10-29-79					
I.	300 ft. E x 100 ft.	Water	440	79	/ 2
	TURB 16.0	Clam	55,000	1,100	230
II.	350 ft. E x 500 ft.	Water	230	33	/ 2
	TURB 12.0	Clam	1,900,000	33,000	20
III.	500 ft. E x 300 ft.	Water	190	17	/ 2
	TURB 12.0	Clam	20,000	130	/ 20

Individual Clam Analysis

The great variability of SPC data during the 2 year study plus the above data raise the question of whether the erratic high counts are a reflection of the general environmental conditions or some individual members of the population having extremely high counts, thus biasing the data.

Whether or not these unusually high composite clam SPC were the result of a single high bacterial count clam in the group of 12 or reflected similar SPC in all 12 clams was tested by analysis of individual clams. Table 11 contains the results of three separate collections and the determination for variance among clams.

Arithmetic means and medians of 9 clams analyzed individually for SPC and FC can be compared with composite samples of 12 clams collected under identical environmental conditions. Variance among clams was determined for the 3 collections and standard deviations are given for clam SPC and FC. The first collection was made during May from Station 4 on the Albemarle Sound while the second was obtained during the same month from Bath Creek. The last collection was taken from the discharge point of the dredging operation on the Pamlico River in November. Water temperature and salinity conditions were similar for the 3 collections. Turbidity was slightly higher at the discharge point when compared with the other 2 collections. Variances among clams were so large that one standard deviation from the means in collections 2 and 3 were greater than the mean values themselves.

This large degree of variance makes predicting clam SPC impossible. A very large degree of variance can be expected from individual

Table 11. Summary of individual clam analyses.

Factor	Collection 1	 2	 3
Date	5-8-79	5-30-79	11-12-79
WTEMP	20	22	21
SALIN	0	0	2.3
TURB	32	33	59
Water			
SPC/ml	150	1,000	59,000
FC/100	∠ 2	13	240
Clam			
Composite			
SPC/g	*	85,000	180,000
FC/100g	*	∠ 20	340
Clam			
Individual			
SPC/g			
Mean	32,000	22,000	240,000
Median	19,000	3,600	150,000
Variance	0.92×10^9	2.70×10^9	61.19×10^9
Standard Deviation	30,000	52,000	250,000
FC/100g			
Mean	∠ 200	55	110
Median	∠ 200	20	110
Variance	NA	2,500	1,800
Standard Deviation	NA	50	43

clams within a composite clam sample. Large individual clam variance and a high degree of composite clam variance further complicate the possibility of marketing *Rangia* as a fresh seafood product. Composite clam variance under high salinity, cold water conditions in open shellfishing areas should be determined because of the importance placed on SPC by market standards. If a reduced degree of variability occurs in composite clam SPC during the months of February through April, it would support harvesting *Rangia* as a fresh seafood product during this time.

The failure to predict clam SPC under certain environmental conditions may force adoption of alternative marketing methods of *Rangia* such as pasteurization or depuration. Further research into composite clam variance is warranted.

SUMMARY

The extended data base from this research on bacterial levels associated with *Rangia cuneata* provides additional evidence of a seasonal pattern in clam FC. A mean MPN of less than 20 FC/100g was found over the 2 year period for the months of February through April. No clam samples exceeded the federal and state MPN standard of 230 FC/100g during this time. The study shows that *Rangia*, when harvested during the months of February through April from open shellfishing waters in Albemarle Sound, N.C., meet suggested indicator standards set by the National Shellfish Sanitation Program for shucked oysters. Evidence based on this standard and the absence of infectious levels of potential pathogens detected by Comar *et al*. (1979) support the suggestion that *Rangia* pose no unique public health risk other than those normally associated with shellfish.

Clam SPC approached and at times exceeded the 500,000 SPC/g market standard throughout the 2 year period. The study reaffirms high SPC in *Rangia* meats reported by Comar *et al*. (1979). Multi-variate regressional analyses of clam SPC versus the environmental factors measured show that only 26.5 percent of the variance in clam SPC can be explained by the combined effects of turbidity, discharge index, water temperature, and salinity. This association shows a significance of less than one percent by chance alone. While clam monthly mean SPC ranged from 30,000 SPC/g in January to 610,000 SPC/g in December, mean SPC by collection station ranged from 30,000 SPC/g at Station 1 to 620,000 SPC/g at Station 4. The large difference in clam SPC between the two stations is believed to be the result of a combination of the measured environmental factors (turbidity, discharge index, water temperature, salinity) and other unknown factors.

The investigation on the effect of sediment on clam SPC showed the greater importance of water-associated factors than the substratum itself. High turbidity conditions caused by dredging did not affect clam SPC. However, a large degree of composite clam variation was found in samples collected from the discharge point during and after discharge into the Pamlico River.

Composite clam and individual clam SPC data show the high degree of variance that can be expected with Rangia. Any potential market wishing to exploit the large numbers of this clam found in our coastal waters will have to deal with the initially high SPC and large degree of SPC variance.

The low FC found in Rangia meats during the months of February, March, and April coincide with SPC of 54,000, 83,000, and 120,000 SPC/g, respectively. More frequent sampling and variance determinations for composite and individual clams during these months may indicate whether the clam can successfully be marketed as a fresh seafood product. Failure to harvest Rangia with sufficiently low SPC to allow time for transport to markets may make alternative marketing methods necessary. The feasibility of depuration of Rangia to reduce SPC is now under investigation. Pasteurization of shucked clam meats is another alternative that may be employed. The potential of this seafood resource within our coastal area is great.

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APPENDICES

Appendix A: Glossary of Abbreviations

BGLB	brilliant green lactose bile broth
C	clam
^{o}C	degree(s) Celsius
^{14}C	radio-carbon fourteen
CI	condition index
cm	centimeter(s)
cm^3	cubic centimeter(s)
CO_2	carbon dioxide
cumec	cubic meter/second
DI	discharge index
E	east
EC	EC broth
FC	fecal coliforms
FDA	Food and Drug Administration
ft	feet
g	gram(s)
HCL	hydrochloric acid
h	hour(s)
km	kilometer(s)
km^2	square kilometer(s)
LB	lactose broth
m	meter(s)
m^2	square meter(s)
m^3	cubic meter(s)
μ	micro-
ml	milliliter(s)
mm	millimeter(s)
MON	month
MPN	most probable number
N	nitrogen
NA	not applicable
NCP	North Carolina Phosphate, Incorporated
NOAA	National Oceanic and Atmospheric Administration
NSSP	National Shellfish Sanitation Program
NTU	nephelometric turbidity units
P	probability
$^{o}/oo$	parts per thousand
ppm	parts per million
R^2	coefficient of determination
RNFL	rainfall (inches)
RS	resident sediment clams
RT	resident tray clams
S	sediment
SALIN	salinity ($^{o}/oo$)
SMA	standard methods agar
SPC	standard plate counts
STA	station
TC	total coliforms

Appendix A: (continued)

TGI	Texasgulf Industries
TOC	total organic carbon
TS	transported sediment clams
TT	transported tray clams
TURB	turbidity (NTU)
W	water
WD	wind direction (360° radius)
WS	wind speed (miles per hour)
WTEMP	water temperature (°C)
*	no data available

Appendix B: Laboratory Media and Reagents

Brilliant green lactose bile broth (BGLB)

EC broth (EC)

Lactose broth (LB)

Phosphate buffered dilution water

Standard methods agar (SMA)

Appendix C: Albemarle/Roanoke Sounds
Part I. Summary of Physical Data

STA	WTEMP	SALIN	TURB	WD	WS	RNFL	DI
Date: 01-20-79							
1	2.8	4.0	16.5	18	9.9	0.27	.0364
2	3.9	3.0	14				
3	4.4	3.2	32				
4	5.0	0.5	33				
Date: 02-11-79							
1	1.1	2.5	18	04	10.4	0.01	.0111
2	0	1.5	72				
3	0.5	1.0	91				
4	1.1	0.5	50				
Date: 03-23-79							
1	14.4	1.9	16	18	9.9	0.01	.0115
2	13.3	1.0	41				
3	13.9	1.3	25				
4	13.3	0	25				
Date: 04-27-79							
1	17.5	2.7	20	18	3.5	0.56	.0128
2	21.0	0.2	48				
3	21.0	0	46				
4	22.0	0	36				
Date: 05-14-79							
1	22.8	1.2	13	04	3.9	1.18	.0252
2	23.3	0.2	34				
3	23.3	0	26				
4	22.8	0	25				
Date: 06-11-79							
1	26.7	1.9	7.8	33	2.0	1.44	.0256
2	26.7	0.5	18				
3	26.7	0	20				
4	26.1	0	11				

Appendix C: Albemarle/Roanoke Sounds
Part I. (continued)

STA	WTEMP	SALIN	TURB	WD	WS	RNFL	DI
Date: 07-23-79							
1	28.0	5.5	7.5				
2	27.0	2.0	7	23	8.8	0.09	.0018
3	29.0	0.2	6				
4	29.0	0	11				
Date: 08-05-79							
1	32.0	4.2	7				
2	31.0	1.0	16	35	3.3	0.01	.0029
3	31.0	0.5	18				
4	32.0	0	24				
Date: 09-25-79							
1	22.8	3.0	15				
2	22.8	1.5	32	05	14.9	0.65	.0016
3	23.3	1.5	24				
4	21.1	0.5	60				
Date: 10-25-79							
1	15.6	2.0	7				
2	15.6	1.3	12	01	2.8	0.0	.0034
3	16.6	0.5	13				
4	16.7	0.3	11				
Date: 11-25-79							
1	21.1	1.0	3.5				
2	17.8	0.3	6	18	7.8	0.0	.0009
3	17.2	0	4.5				
4	17.2	0	14				
Date: 12-10-79							
1	11.1	0.5	8				
2	11.1	0	13.5	22	7.1	0.17	.0079
3	11.1	0	20				
4	11.1	0	18				

Appendix C: Albemarle/Roanoke Sounds
Part II. Summary of Bacteriological Data

STA	SPC/g	TC/100g	FC/100g
Date: 01-20-79	*	*	*
Date: 02-11-79			
1-W	140	8	< 2
1-C	1,100	70	< 20
2-W	230	5	< 2
2-C	4,100	20	< 20
3-W	750	9	5
3-C	87,000	40	< 20
4-W	730	14	< 2
4-C	100,000	20	20
Date: 03-23-79			
1-W	69	23	< 2
1-C	32,000	70	< 20
2-W	100	5	< 2
2-C	13,000	83	< 20
3-W	100	5	< 2
3-C	110,000	160,000	< 20
4-W	140	13	< 2
4-C	*	470	< 20
Date: 04-27-79			
1-W	150	23	5
1-C	8,600	220	110
2-W	420	70	70
2-C	8,700	470	20
3-W	240	17	8
3-C	800,000	11,000	45

Appendix C: Albemarle/Roanoke Sounds
Part II. (continued)

STA	SPC/g	TC/100g	FC/100g
4-W	840	23	23
4-C	92,000	92,000	< 20
Date: 05-14-79			
1-W	2,000	1,100	110
1-C	8,100	1,700	460
2-W	570	33	23
2-C	80,000	160,000	230
3-W	170	23	8
3-C	460,000	≥ 240,000	45
4-W	520	8	8
4-C	320,000	8,100	130
Date: 06-11-79			
1-W	2,700	1,600	33
1-C	1,800	2,400	790
2-W	1,600	23	13
2-C	41,000	≥ 24,000	< 20
3-W	1,700	49	33
3-C	66,000	490	< 20
4-W	2,400	31	23
4-C	250,000	700	< 20
Date: 07-23-79			
1-W	590	170	130
1-C	28,000	3,500	1,100
2-W	620	23	5
2-C	160,000	2,400	490

Appendix C: Albemarle/Roanoke Sounds
Part II. (continued)

STA	SPC/g	TC/100g	FC/100g
3-W	450	13	2
3-C	80,000	230	20
4-W	630	23	5
4-C	250,000	110	20
Date: 08-05-79			
1-W	640	8	8
1-C	52,000	1,100	330
2-W	650	23	8
2-C	95,000	790	70
3-W	1,300	46	11
3-C	61,000	1,300	110
4-W	4,000	23	< 2
4-C	640,000	7,900	< 20
Date: 09-25-79			
1-W	12,000	460	130
1-C	29,000	7,000	490
2-W	720	17	< 2
2-C	20,000	330	50
3-W	460	79	2
3-C	16,000	330	50
4-W	3,000	460	8
4-C	210,000	3,300	110
Date: 10-25-79			
1-W	2,400	240	240
1-C	110,000	1,400	1,300

Appendix C: Albemarle/Roanoke Sounds
Part II. (continued)

STA	SPC/g	TC/100g	FC/100g
2-W	860	130	49
2-C	180,000	2,400	490
3-W	580	240	49
3-C	120,000	9,200	330
4-W	810	130	14
4-C	370,000	2,200	790
Date: 11-25-79			
1-W	490	49	< 2
1-C	61,000	840	460
2-W	290	5	2
2-C	77,000	90	< 20
3-W	220	33	< 2
3-C	340,000	940	330
4-W	260	33	2
4-C	410,000	210	20
Date: 12-10-79			
1-W	380	240	7
1-C	6,700	3,500	40
2-W	140	5	5
2-C	71,000	90	60
3-W	190	17	11
3-C	140,000	1,400	330
4-W	510	33	33
4-C	490,000	490	< 20

Appendix C: Albemarle/Roanoke Sounds
Part III. Discharge Index-Sample Calculation

Gage Stations - U.S. Geological Survey

1. Potecasi Creek near Union, N.C.
   Drainage basin of 495 km^2.

2. Ahoskie Creek at Ahoskie, N.C.
   Drainage basin of 148 km^2.

DI determination for January, 1979:

Date	Mean Daily Flow (ft^3/sec) Potecasi	Ahoskie
Jan 17	124	989
18	95	833
19	73	678
20*	68	541
Totals	360	3041

- total discharge/total drainage area/days

= 3401 ft^3/sec / 643 km^2 / 4

= 1.3223 ft^3/sec/km^2

- converted to cumecs (X 0.028)

= 1.3223 X 0.028

= 0.0364 cumecs/km^2

* collection date

Appendix D: Pamlico River/Bath Creek
Part I. Summary of Physical Data

STA	WTEMP	SALIN	TURB
Date: 02-24-79			
1	10	5.0	14.5
2	12	4.3	22
Date: 03-11-79			
1	12	1.2	60
2	13	0.5	47
Date: 03-18-79			
1	13	2.2	48
2	16	2.0	35
Date: 03-25-79			
1	14	2.2	35
2	16	2.0	21
Date: 04-01-79			
1	21	1.0	28
2	22	0.5	21
Date: 04-08-79			
1	18	1.0	24
2	20	0.5	26

Appendix D: Pamlico River/Bath Creek
Part II. Summary of Bacteriological Data

STA	SPC/g	TC/100g	FC/100g
Date: 02-24-79			
1-W	425	23	< 2
1-S	36,000	230	80
1-RS	49,000	170	< 20
2-W	33,000	140	23
2-S	190,000	790	230
2-RS	400,000	≥ 24,000	80
Date: 03-11-79			
1-W	5,700	540	79
1-S	130,000	4,600	490
1-RS	77,000	22,000	460
2-W	6,300	540	33
2-S	240,000	2,100	80
2-RS	200,000	35,000	20
Date: 03-18-79			
1-W	1,300	23	23
1-S	140,000	9,200	700
1-RS	40,000	2,800	90
1-RT	50,000	790	70
1-TS	180,000	7,000	40
1-TT	*	*	*
2-W	2,500	49	23
2-S	300,000	11,000	330
2-RS	57,000	18,000	20
2-RT	97,000	700	< 20
2-TS	490,000	240,000	50
2-TT	62,000	6,200	< 20

Appendix D: Pamlico River/Bath Creek
Part II. (continued)

STA	SPC/g	TC/100g	FC/100g
Date: 03-25-79			
1-W	760	22	8
1-S	67,000	3,500	140
1-RS	194,000	6,400	50
1-RT	*	*	*
1-TS	86,000	3,500	< 20
1-TT	*	*	*
2-W	1,100	23	23
2-S	150,000	11,000	490
2-RS	8,700	5,400	50
2-RT	88,000	700	230
2-TS	63,000	2,600	230
2-TT	120,000	5,400	170
Date: 04-01-79			
1-W	460	11	2
1-S	34,000	230	50
1-RS	180,000	≥ 24,000	20
1-RT	*	*	*
1-TS	160,000	24,000	80
1-TT	*	*	*
2-W	920	33	8
2-S	180,000	700	80
2-RS	15,000	790	70
2-RT	30,000	950	230
2-TS	≥ 4,000,000	1,300	18
2-TT	530,000	54,000	20
Date: 04-08-79			
1-W	490	13	2
1-S	110,000	2,400	230
1-RS	300,000	790	70
1-RT	*	*	*
1-TS	160,000	8,100	< 20
1-TT	*	*	*

Appendix D: Pamlico River/Bath Creek
Part II. (continued)

STA	SPC/g	TC/100g	FC/100g
Date: 04-08-79			
2-W	1,500	33	23
2-S	400,000	1,700	230
2-RS	130,000	490	130
2-RT	230,000	25,000	70
2-TS	160,000	3,500	70
2-TT	49,000	700	45

Appendix E: Pamlico River/South Creek (Dredge Site)
Part I. Summary of Physical Data

STA	WTEMP	SALIN	TURB	TOC
Date: 07-13-79				
1 - Mouth of Creek	28.0	4.0	16.5	9.38
2 - Southeast/Barge Slip	28.5	2.7	13.0	11.8
3 - Northeast/Barge Slip	28.0	3.0	16.5	10.8
Date: 07-26-79				
1 - West/Barge Slip	28.0	2.5	16.5	11.4
2 - North/Barge Slip	28.0	3.2	12.0	11.1
3 - Channel/Barge Slip	28.5	3.8	16.0	10.2
Date: 08-30-79				
1 - West/Barge Slip	29.0	6.0	15.0	9.49
2 - West/Barge Slip	29.0	6.1	29.0	9.57
3 - South Point/Barge Slip	29.0	6.1	34.0	11.9
4 - East/Barge Slip	29.0	6.1	17.0	9.72
Date: 10-15-79				
1 - West/Barge Slip	22.0	5.3	9.5	15.4
2 - South/Barge Slip	22.0	5.8	8.5	11.6
3 - East/Barge Slip	22.0	6.1	9.0	10.5
Date: 10-29-79				
1 - West/Barge Slip	20.0	6.0	5.5	11.2
2 - South Point/Barge Slip	20.0	6.0	7.5	10.3
3 - East/Barge Slip	20.0	6.0	8.5	11.5

Appendix E: Pamlico River/South Creek (Dredge Site)
Part II. Summary of Bacteriological Data

STA	SPC/g	TC/100g	FC/100g
Date: 07-13-79			
1-W	260	2	< 2
1-C	5,100	490	220
2-W	480	17	11
2-C	24,000	490	50
2-S	170,000	490	40
3-W	510	23	2
3-C	64,000	130	20
Date: 07-26-79			
1-W	980	23	8
1-C	110,000	490	< 20
2-W	990	23	23
2-C	80,000	1,100	80
3-W	670	23	23
3-C	17,000	900	80
Date: 08-30-79			
1-W	8,100	240	79
1-C	25,000	130	20
1-S	130,000	7,900	2,400
2-W	21,000	330	46
2-C	36,000	1,100	80
3-W	22,000	130	49
3-C	32,000	490	230
4-W	1,700	180	31
4-C	*	230	130
4-S	54,000	4,600	790

Appendix E: Pamlico River/South Creek (Dredge Site)
Part II. (continued)

STA	SPC/g	TC/100g	FC/100g
Date: 10-15-79			
1-W	540	140	17
1-C	32,000	3,300	< 20
2-W	260	49	2
2-C	44,000	2,300	< 20
3-W	200	49	11
3-C	27,000	630	< 20
Date: 10-29-79			
1-W	550	79	33
1-C	31,000	2,800	210
1-S	150,000	340	60
2-W	470	33	5
2-C	58,000	2,300	1,300
3-W	460	49	8
3-C	77,000	490	80
3-S	100,000	490	230

Appendix E: Pamlico River/South Creek (Discharge Point)
Part I. Summary of Physical Data

STA	WTEMP	SALIN	TURB	TOC
Date: 07-26-79				
1 - Channel/Roadside	30.0	3.0	100.0	17.5
2 - Discharge Point	30.0	3.0	110.0	24.5
3 - 300 ft. E x 200 ft.	28.5	3.9	35.0	*
Date: 07-31-79				
1 - Discharge Point	31.0	3.8	100.0	19.1
2 - 250 ft. E x 400 ft.	*	*	21.0	*
3 - 250 ft. E x 600 ft.	*	*	38.0	*
Date: 08-14-79				
1 - Discharge Point	27.0	5.5	108.0	13.4
2 - 250 ft. E x 600 ft.	28.0	7.0	12.0	7.54
Date: 08-24-79				
1 - Discharge Point	32.5	*	190.0	18.0
2 - DP x 300 ft.	*	*	130.0	*
3 - DP x 500 ft.	*	*	80.0	*
4 - 100 ft. E x 100 ft.	*	*	180.0	*
5 - 300 ft. E x 100 ft.	*	*	130.0	*
6 - 300 ft. E x 500 ft.	*	*	60.0	*
Date: 08-30-79				
1 - DP x 1200 ft.	29.5	6.2	7.0	*
2 - 100 ft. E x 100 ft.	29.5	6.2	40.0	*

Appendix E: Pamlico River/South Creek (Discharge Point)
Part I. (continued)

STA	WTEMP	SALIN	TURB	TOC
3 - 300 ft. E x 100 ft.	29.5	6.2	60.0	*
4 - 300 ft. E x 500 ft.	29.5	6.2	40.0	*
Date: 10-15-79				
1 - 350 ft. E x 500 ft.	21.0	3.2	12.5	10.6
2 - 350 ft. E x 100 ft.	21.0	3.2	14.0	8.6
3 - 100 ft. E x 100 ft.	21.0	3.2	14.0	8.94
Date: 10-29-79				
1 - 300 ft. E x 100 ft.	20.0	6.5	16.0	9.5
2 - 350 ft. E x 500 ft.	20.0	6.5	12.0	10.5
3 - 500 ft. E x 300 ft.	20.0	6.5	12.0	9.23

Appendix E: Pamlico River/South Creek (Discharge Point)
Part II. Summary of Bacteriological Data

STA	SPC/g	TC/100g	FC/100g
Date: 07-26-79			
2-W	64,000	490	49
3-W	16,000	79	23
3-C	13,000	490	50
Date: 07-31-79			
1-W	33,000	23	13
2-W	9,800	33	33
2-C	52,000	4,900	110
3-W	18,000	33	23
3-S	660,000	3,300	790
Date: 08-14-79	*	*	*
Date: 08-24-79			
1-W	4,600	4,900	220
2-W	2,900	2,300	79
3-W	2,600	170	14
4-C	130,000	17,000	1,100
5-C	39,000	13,000	170
6-C	13,000	940	50
Date: 08-30-79			
1-W	1,000	94	17
1-C	13,000	2,600	170
2-W	12,000	170	49
2-C	16,000	1,700	220

Appendix E: Pamlico River/South Creek (Discharge Point) Part II. (continued)

STA	SPC/g	TC/100g	FC/100g
3-W	16,000	790	490
3-C	90,000	3,300	170
4-W	19,000	490	70
4-C	1,200,000	2,200	120
Date: 10-15-79			
1-W	520	130	5
1-C	30,000	7,900	< 20
2-W	1,200	110	13
2-C	56,000	13,000	140
3-W	3,000	110	8
3-C	86,000	13,000	80
Date: 10-29-79			
1-W	440	79	< 2
1-C	55,000	1,100	230
2-W	230	33	< 2
2-C	1,900,000	33,000	20
3-W	190	17	< 2
3-C	20,000	130	< 20

Appendix E: Pamlico River/South Creek (Discharge Point)

Part III. Flow Determinations

Date: 08-14-79

Area*

Distance (ft)	Depth (ins) 1	3	Mid	3	1	Width (ins)
0	6	17	19	13	3.5	260
300	8	21	21.5	20.5	8	248
600	9	16	16	17	10	250
900	9.5	15	15	15	6	240
1200	12	13	13	12.5	8	253
1500	8	15	15	14	7.5	244

Velocity**

Object	Position	Time
1	Mid	19:14
2	Mid	19:30
3	Shore	21:30
4	Shore	*

Flow Determination

$$Q = \text{Area} \times \text{Velocity}$$

$$= 26.2\ \text{ft}^2 \times 1.05\ \text{ft/sec}$$

$$= 27.51\ \text{ft}^3/\text{sec}$$

- converted to m^3/sec

$$= 27.51\ \text{ft}^3/\text{sec} \times 0.0283$$
$$= 0.78\ \text{m}^3/\text{sec}$$

* Area was determined by graphing.

** Velocity = 1500 ft / 1205 sec
= 1.24 ft/sec

- corrected for surface flow
= 1.24 ft/sec x 0.85
= 1.05 ft/sec

Appendix E: Pamlico River/South Creek (Discharge Point)

Part III. (continued)

Date: 08-24-79

Area*

Transect	Depth (ins)						Width (ins)
	1	3	5	5	3	1	
1	8	18	16	16	12	6	209
2	8	12	11.5	12	11	7	188

Flow Determination

Q = Area x Velocity

$= 15.4 \text{ ft}^2 \times 0.68 \text{ ft/sec}$

$= 10.47 \text{ ft}^3\text{/sec}$

- converted to m^3/sec

$= 10.47 \text{ ft}^3\text{/sec}$

$= 0.30 \text{ m}^3\text{/sec}$

Velocity**

- determined with rhodamine plug, distance of 900 ft.

* Area was determined by graphing.

** Velocity

= 900 ft / 1330 sec

= 0.68 ft/sec

No corrected flow required.

Appendix E. Pamlico River/South Creek (Discharge Point)

Part IV. Turbidity Grids

						Turbidity					
Distance*(ft.)	500	400	300	200	100	DP	100	200	300	400	500
Date: 08-14-79											
100	26	34	52	76	76	100	110	94	66	66	58
200	33	42	51	86	103	120	110	78	40	41	38
300	31	32	31	30	47	70	55	40	27	24	31
400	11	12	12	12	26	24	18	17	21	23	19
500	10	10	11	13	12	13	14	14	15	15	19
Date: 08-24-79											
100	46	66	130	360	180	190	480	205	*	*	*
200	22	40	40	80	150	140	170	195	*	*	74
300	17	20	35	86	90	130	150	180	95	105	70
400	12	20	50	68	100	98	140	150	100	130	130
500	14	28	60	70	95	80	130	130	100	110	115

* This table reads east (E) to west.

Appendix F: Individual Clam Analysis
Part I. Summary of Physical Data

RUN	WTEMP	SALIN	TURB
Date: 05-08-79			
1	20	0	32
Date: 05-30-79			
2	22	0	33
Date: 11-12-79			
3	21	2.3	59

Appendix F: Individual Clam Analysis
Part II. Summary of Bacteriological Data

SAMPLE	SPC g	TC 100g	FC 100g
Date: 05-08-79			
W	150	23	< 2
S	190,000	1,300	130
C(12)	*	*	*
C-1	15,000	2,300	< 200
C-2	7,900	800	< 200
C-3	99,000	2,700	< 200
C-4	14,000	6,800	< 200
C-5	65,000	200	< 200
C-6	29,000	1,100	< 200
C-7	12,000	7,800	< 200
C-8	30,000	< 200	< 200
C-9	19,000	7,800	200
Date: 05-30-79			
W	1,000	33	13
C(12)	85,000	160,000	< 20
C-1	5,700	20	< 20
C-2	3,600	230	50
C-3	2,700	110	< 20
C-4	3,800	230	130
C-5	2,700	50	< 20
C-6	160,000	270	20
C-7	11,000	50	< 20
C-8	2,800	170	80
C-9	2,100	790	140
Date: 11-12-79			
W	59,000	350	240
C(12)	180,000	7,900	340
C-1	150,000	3,300	110
C-2	260,000	2,300	130
C-3	220,000	7,000	130
C-4	110,000	2,300	170

Appendix F: Individual Clam Analysis
Part II. (continued)

SAMPLE	SPC/g	TC/100g	FC/100g
C-5	100,000	1,700	140
C-6	49,000	280	83
C-7	100,000	1,100	$<$ 20
C-8	300,000	790	80
C-9	860,000	$\geq$ 24,000	80