Proceedings of the

INTERSTATE SEAFOOD SEMINAR

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PREFACE

The 1978 Interstate Seafood Seminar was held in Nags Head, North Carolina, from October 17 to 19. The seminar for Region III has been held almost annually since 1946. The first proceedings of the seminar was published in 1977. This proceedings is the second in the series.

Twenty-eight speeches were presented at the seminar; nineteen of these are included herein.

Many people were involved in preparing this publication. William Hess, Jr. gathered the manuscripts and speeches. Yvonne Holmes and Alice White transcribed speeches from tape and Jean B. Brewer and Barbara Barnett typed the camera-ready script. Dr. George J. Flick coordinated and conducted the seminar and provided the incentive for publishing the proceedings.

Chieko E. Hebard
Sea Grant Editor

CHOLERA*

Charles C. Conrad

Cholera has played an important role in the history of Louisiana. In 1832-33 over 6000 people in New Orleans alone died from this disease. This was the bleakest time in New Orleans history. The population was 55,000 and more than one-seventh of the population died from yellow fever, cholera, and other causes in a one-year period.(1) Over the next 40 years, several epidemics of cholera afflicted the population with each succeeding epidemic tending to be less virulent than the previous one. The dual horrors of yellow fever and cholera were the impetus behind the formation of the State Board of Health by the Louisiana Legislature in 1855.(2)

The U.S. has been spared of the 7th World Cholera Pandemic started in Asia in 1901. From 1911 until 1978 there was only one naturally acquired case of cholera in the continental U.S. This occurred in Port Lavaca, Texas, in 1973. No source was ever discovered.

On August 13, 1978, an Abbeville resident was admitted to the hospital with severe watery diarrhea. The hospital laboratory observed the strange occurrence of a pure culture of hemolytic colonies from a stool culture. An interested laboratory technologist continued to pursue the identification of the organism, and through biochemical reaction, determined that the bacterium was a Vibrio. The culture was sent to the state laboratory where it was further identified as a V. cholerae, then was forwarded to the National Center for Disease Control (CDC). The CDC confirmed the isolate as Vibrio cholerae O-1, Biotype Ei Tor, Serotype Inaba. Since mid-August, a total of 11 Vibrio cholerae infections have been identified, seven in Vermilion Parish and 4 in Lafayette Parish.

Characteristics of Patients

Three persons were totally asymptomatic and were only discovered through routine rectal swab cultures of case contacts. Five of the 11

*This paper was written by Donald Allegro, Charles Carraway, and Louise McFarland, Louisiana Health and Human Resources Administration.

patients were hospitalized. Six were male and five were female. Ages of patients ranged from 12 to 69. Duration of diarrhea was approximately two to seven days. All patients have recovered and none are in the hospital at the present time. The hospitalized patients were ill at home for 2-4 days before being admitted to the hospital. All of the hospitalized patients were severely dehydrated on admission and one was admitted in critical condition without obtainable blood pressure. This patient subsequently developed acute renal tubular necrosis secondary to prolonged hypotension. She required one week of dialysis before discharge.

The one common denominator in the histories of these patients was that they had all eaten crabs 2-5 days prior to becoming ill. The crabs were for the most part obtained privately from Louisiana waters stretching from Mud Lake west of Cameron to Vermilion Bay. The crabs were either boiled or steamed. Although no sanitary break in handling or cooking could be discovered in a few of the cases, it was felt that several patients had become ill by improper handling of cooked crabs. Cooked crabs were placed back in containers where the raw crabs had been stored and maintained unrefrigerated up to six hours before consumption. It is our presumption that proper cooking killed the cholera bacteria, but the crabs were re-contaminated when placed back in the original storage containers.

Surveillance

From the time of appearance of the first case of cholera, an extensive surveillance effort was set in motion by state and local health department personnel in cooperation with a team of investigators from the National Center for Disease Control in Atlanta, Georgia.

A) Patient Investigation: Each case was questioned in great detail and all close contacts had rectal swabs done and serum drawn for vibriocidal antibody testing. Generally, there was good agreement between serology testing and rectal swabs. Immigration from Asian countries into Vermilion Parish was reviewed. Since there had been no immigration since April, 1978, the possibility of recent immigrants introducing cholera was extremely remote. Also there had been no recent cases of diarrhea in foreigners admitted to local hospitals in Vermilion Parish.

B) Sewage Surveillance: Swabs were placed in town sewage systems throughout Southcentral and Southwestern Louisiana. This surveillance is continuing at regular intervals and the only positive samples have been in towns where known patients reside. Every positive sewage swab could be explained by a patient and these positive sewage systems quickly became negative on repeat testing. From this information, it appeared that our sewage surveillance was quite specific and that cholera was occurring only as sporadic cases and was not a widespread phenomenon.

C) Water Samples: Municipal water systems were tested throughout Southern Louisiana and have been found negative for fecal contamination and cholera.

D) Environmental Sampling: Water and live seafood samples were taken from all implicated Louisiana coastal waters. From hundreds of samples of live crabs, oysters, and shrimp, only one positive sample of shrimp was found. Some patients had leftover seafood. These seafood samples were cultured and only one refrigerated crab was positive for cholera. Swabs were placed in coastal and inland waterways suspected of carrying cholera. Only one swab was positive in the old Intracoastal Canal between Vermilion Bay and White Lake in Vermilion Parish.

E) Serologic Survey: One hundred and five samples were obtained from Abbeville residents at random. Eight had titers of 1:160 or greater and three had titers of 1:320 or greater. Supposedly, an elevated vibriocidal antibody titer would be evidence for a recent cholera infection. But this test is not specific, though highly sensitive. It can be positive with prior infections with Brucella citrobacter and other organisms, which limits its usefulness as an indication of exposure to cholera.

F) Lab Experiments with Cholera: Crabs were placed overnight in cholera-infected water in the lab. The next day they were cooked. Boiling for up to eight minutes did not kill the cholera organisms, but at eight minutes of boiling the crabs were red and the meat was firm. This points out the inadequacy of using these criteria to determine how long crabs should be cooked. Ten minutes or more of full boiling did kill the cholera bacteria in the samples. Non-pressure steaming of crabs for up to 30 minutes did not kill the organisms. Freezing infected crabs will lower the number of organisms but will not eliminate cholera from infected samples.

Characteristics of Cholera

Classically, it is taught that cholera presents as a severe dehydrating diarrhea. Cholera, however, can present as a spectrum from asymptomatic to mild to life-threatening disease. It has been estimated that, with cholera caused by the El Tor biotype, 75% of cases are asymptomatic and only 2% have severe diarrhea. The other 23% have mild to moderate diarrhea. Text books state that the incubation period can be as long as 5-7 days, but in almost all cases, the incubation period is from several hours to 3 days.[3] Mucus in the stool imparts the characteristic "rice-water" appearance. Although there is no absolute diagnostic features of cholera, some criteria are helpful. Vomiting is often present, but usually occurs after the onset of diarrhea, which is the reverse of staphylococcal food poisoning where vomiting precedes diarrhea. High fever and severe abdominal pain are uncommon in cholera but often are prominent features of salmonellosis, shigella, and amebic dysentery. Painful muscle cramps in cholera are probably secondary to electrolyte imbalance. Again, it must be stressed that the severe "rice-water" diarrhea of "cholera gravis" leading to hypotension, electrolyte imbalance, and death is only the tip of the iceberg. In endemic areas, cholera should be considered in the differential diagnosis of any mild non-specific diarrheal illness.

Cholera is not easily spread person-to-person, probably because of the large inoculum necessary to cause disease. Vibrios will not survive when dried and therefore, inanimate objects are not important in transmission. Flies may transport vibrios physically from excreta to food, but since human excrement is generally treated or deposited underground and food kept in screened areas, flies are not felt to be an important means of transmission except in very poor sanitation areas.

Most patients eliminate the vibrio organisms within a few days if treated with antibiotics and within 1-2 weeks if untreated. There have been isolated reports of protracted vibrio excretion and even of one long-term carrier, Cholera Dolores.[4] However, these reports identify what is felt to be an extremely rare phenomenon.

Vibrios are inactivated at acid pH, and those who have had their gastric acidity barrier modified or eliminated by gastric surgery or

antacid therapy seem to be particularly susceptible to cholera infection. Also lab animals who have had their normal intestinal flora altered by broad-spectrum antibiotics have been shown to be more susceptible to cholera.

Treatment

"Cholera gravis," the severe dehydrating form of cholera, is a medical emergency. Appropriate fluid management is the cornerstone of therapy and should be instituted immediately. Cholera diarrhea is generally isotonic containing about 135 meq/l of Na+, 13 meq/l of K+, 105 meq/l of Cl^-, and 45 meq/l of HCO_3^-. The best single IV replacement fluid is Ringer's lactate with 10-15 meq KCl supplementation. Normal Saline (0.9%) with 1 amp of bicarbonate per liter and 10-15 meq KCl/l can also be used.[5] IV fluids should be given in quantities sufficient to replace the fluid that the patient has lost through vomiting and diarrhea. Fluid requirements may be enormous with some patients requiring over 20 liters in 24 hours. Electrolytes should be followed carefully, and if the patient appears acidotic, arterial pH should be monitored if available. Antibiotics shorten the duration of diarrhea and the period of excretion of vibrios. Tetracycline is the drug of choice given in doses of 250 mg orally every 6 hours for 5 days. This should be started when vomiting subsides. Furazolidone is preferable for children and pregnant women. Parenteral antibiotic treatment is unnecessary and may actually be dangerous. Parenteral cephalosporins and aminoglycosides can accentuate renal insufficiency in patients with severe dehydration. Kaopectate, antispasmodics, and other anti-diarrheal agents are of no known value in treating cholera. In children with cholera, physicians should be alert to the possibility of severe hypoglycemia which can present as convulsions or coma.

It is unnecessary and undesirable to use stringent isolation protocols for cholera patients. Close personal contacts of cholera patients are not in jeopardy if simple handwashing and careful disposition of the patients' excreta are enforced. No masks, gowns, or physical isolation is warranted.

Recommendations and Plans

(1) Surveillance of sewage, water, and live seafood (oysters, crabs, shrimp) will be continued indefinitely. All 48 commercial establishments that process crabs will have raw and cooked crabs sampled as well as the effluent from the washings of the crabs.

(2) Crabs are safe to eat if handled and cooked properly. Recommendations for the handling and cooking of crabs are as follows: Crabs should be boiled for a full 15 minutes. This 15-minute period should begin not when the crabs are put in the boiling water, but after the water they have been put into comes to a rapid boil again. Cooked crabs should not be placed back into the containers in which they were originally stored before cooking. Utensils used in handling cooked crabs should be kept separate and not used for raw crabs. Steaming of crabs is not recommended at the present time. These recommendations have been forwarded to all seafood restaurants in southern Louisiana.

(3) The State Central Lab in New Orleans and its two regional labs in Lake Charles and Lafayette and five other hospitals in southern Louisiana have begin routine plating of stool and rectal swabs from all diarrheal illnesses on thiosulfate-citrate-bile salt agar (TCBS). This medium is specific for vibrios (cholera and non-cholera vibrios). Since we have had cases of Vibrio cholerae and Vibrio parahaemolyticus this year in Louisiana, we recommend that every hospital in southern Louisiana obtain TCBS and routinely plate on this medium all stools submitted for routine culture. If colonies grow out on the TCBS, the specimens can be submitted to the Regional or State Health Department labs. Specific tests can be done on those to determine if the vibrios isolated are truly V, cholerae or one of several non-pathogenic strains of vibrio.

(4) Cholera Vaccination: Vermilion and Lafayette Parishes are temporarily defined as cholera-infected areas by the World Health Organization. Travelers from these parishes will be able to visit Europe, Mexico, and most other major tourist centers without cholera shots. However, the following countries will require cholera shots from travelers from these parishes: Albania, Angola, Brunei, Cape Verde, China (Taiwan and People's Republic), Egypt, Fiji, Iran, Iraq, Laos, Libya, Arab Jamahiriya, Madagascar, Mali, Nauru, Pakistan, Panama, Pitcairn Island, Qatan, Ryukyu Islands, Saint Helena, Seychelles, Swaziland, Yemen, and Zambia. Five

countries that always require cholera vaccination from all travelers are Malawi, Maldines, Mozambique, Papua New Guinea, and Saudi Arabia.[6] It is not recommended that cholera shots be given indiscriminately to all travelers. Under the most favorable circumstances, the vaccine is only 50-60% effective and then only for a few months. Also there are mild to moderate side effects of the shots. Almost all recipients develop a sore arm and some develop constitutional symptoms, e.g., malaise, generalized aches, and slight fever lasting 24 to 48 hours, sometimes severe enough to require absence from work.

Discussion

It is still unclear how the implicated crabs became infected with cholera bacteria. It is known that different types of vibrios (e.g. V. parahaemolyticus) are common inhabitants of our coastal waters, and it is possible that cholera has been with us a long time and only recognized now because it was fortuitously tested for. Crabs were obtained from several different widely spaced fishing areas and it is hard to imagine a single common source contaminating all the areas. We may have a few more isolated cases, but we do not expect a major outbreak. Cholera is not propagated in areas where there are safe drinking water and adequate sewage disposal. As long as these are maintained in proper condition, it is extremely unlikely that we will be faced with a major public health threat from cholera.

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MONITORING OF IMPORTED SEAFOOD PRODUCTS

Richard Dawson

The subject of my talk is imported seafood products and the involvement of the Food and Drug Administration in this area. Under Section 801 of the Food, Drug, and Cosmetic Act, imported products are treated in the same manner as domestic products. Our district investigators review lists of products offered for entry into the U.S. to determine what products are being offered and that they meet the same regulations as domestic products. They conduct wharf examinations to actually see if products meet our requirements. If collected samples violate the FD & C Act, they are detained. The cause of detention varies from violating the Food Product Labelling Act to products being decomposed or adulterated with pesticide, chemicals, or other reasons.

FDA's strategy for the enforcement of the regulations on imported products revolves around surveillance of ships, planes, ports and products offered for entry. In 1972, FDA implemented a new import coverage strategy designed to increase surveillance. One tool of that strategy is the mobile laboratory which we set up in particularly large ports to examine merchandise right at the port, thus reducing the time for analysis drastically. A decision can then be made more rapidly as to whether the product should be released or detained.

In 1972, we instituted the "circuit rider" program. On the Canadian border from Maine to New York, there are a lot of very small ports, actually very small towns, where truck shipments enter the United States from Canada. In addition, a larger amount of merchandise is entered through Canadian ports, then subsequently entered into the United States through some of these small ports. Previously, we gave very little coverage in this area. Starting in 1972, however, we stationed our people at these small ports from time to time, and sampled and reviewed all the merchandise that was entered.

We also utilize saturation sampling. In this instance, we pay attention to one particular product from a particular country and sample that product at all of our ports for a certain period of time.

This gives us a good overview of how that product meets the requirements of the FD & C Act's regulations.

Currently, a vast amount of firms are shipping via containerized cargo. This method of shipping has posed many problems for us. How could we sample a closed container being shipped inland? We now have agreements with various shipping agents to do our sampling at various breakdown points where the containers are stored prior to shipping.

We have also intensified our coverage of importers who are found to be consistently handling suspect products. In these instances, we utilize the saturation sampling technique. This increased sampling program has resulted successfully in numerous detentions including such seafood products as shrimp. In some cases, we have "block listed" various products from various countries. This procedure results in detention of the offered product upon entry into the country, without sampling or analyzing the product!

Another way we are trying to increase our sampling program and our assurance of acceptable products entering this country is the development of cooperating agreements and certification programs, such as shellfish sanitation programs, with foreign countries. For example, we had large amounts of decomposed tuna from Japan being offered for entry in 1972. We sent to Japan a team of experts who worked with the Japanese setting up an inspection program. Now the Japanese inspect any shipment going to the U. S. on a lot-by-lot basis. This has resulted in a better product being shipped. We encourage expanded foreign inspection of products shipped to the U. S.

Our foreign inspection program, that is, the on-site inspection of manufacturers, currently is limited to the drug and device area. We do, however, expand that program to include food when we are requested. At this time, we do not have any legal authority to inspect foreign food plants. This is the major difference between domestic food products and imported food products and in the way they are treated or inspected by FDA. However, as mentioned earlier, if we do encounter continuing problems, we assist when requested, and naturally also stop or detain violating products. For instance, in 1973, pistachio nuts coming from Iran and Turkey were being detained. We were requested and

went over and set up a self inspection program, working with the governments and the industry. Since then, we have not encountered serious problems and pistachio nuts are being allowed entry. Another example, frog legs from India, has not worked as well. We were having problems with salmonella. We went to India and set up a sanitation program. For a while it worked. However, it has since deteriorated and we are now back to lot-by-lot examination.

We are currently in the process of developing procedures for supplying the U. S. embassies with information on imported and exported products. We have given some thought in the past to setting up attaches within the embassies around the world. We are still in the process of evaluating this proposal and are not prepared to implement it at this time.

In England, two persons recently died of botulism after eating canned salmon from Alaska. As a result, the English banned all shipment of canned salmon from the United States. We immediately worked with our embassy in England and the English government on this problem. The ban currently applies only to canned salmon shipped from the suspect plant in Falls Castle, Alaska, and does not cover all the salmon shipped from the U. S.

We have tried as much as possible to open our line of communication between industry groups and foreign governments. An example of this liaison can be seen in the case of certain French cheese implicated in a case of food poisoning on a plane from Paris to New York. We learned that the cheese was contaminated with E. coli and that, in fact, most of the incoming cheese of the same type was also contaminated with the organism. We then worked with the French embassy, the French government, and the industry to correct this situation. I am happy to report that the situation has improved considerably.

The Food and Drug Administration also encourages exchange of personnel between governments. Recently, I was on an assignment to Kuwait. I spent about seven months there working with the Kuwaiti government on legislation and reviewing their inspection procedures. As you may know, Kuwait is a large exporter of shrimp. At the time I was there, FDA was detaining an abnormally large amount of shrimp

from Kuwait. In fact, all shrimp from Kuwait was suspect. However, I worked with the Kuwaiti government and the industry with the result that the shipment of decomposed shrimp from Kuwait was terminated.

I appreciate the time you have given me to express some of the procedures FDA is currently utilizing to monitor entry into the country of products under our jurisdiction. It is an extremely difficult program to manage and our people are doing their best. We recognize that improvement is needed, and are making changes when necessary. If any of you have suggestions, I would be happy to discuss them.

NEED FOR STANDARDS FOR FREE LIQUOR IN OYSTERS

J. W. Ferguson

I am Chairman of the Board and General Manager of J. W. Ferguson Seafood Co., Inc. and the Zero Seafood, Inc. of Remlik, Virginia. Ninety percent of the seafood we produce are oysters in some form; fresh oysters, fresh frozen oysters, frozen oyster fritters, and frozen breaded oysters. We produce seed oysters and shucking oysters on the 7,000 acres of oyster planting ground we manage. Of the 80,000 bushels of adult oysters we process each year, 50,000 bushels are grown on our own grounds. Our company has been in the oyster business since 1948 and distributes oysters over the entire United States and part of Canada. We have had many problems within the oyster industry.

The growing of oysters to an adult size is a major accomplishment in itself. We have to cope with storms over the water, floods from the land, changes in the salinity of the water and so on. When the salinity of the water remains below five parts per thousand for ten days, all the oysters on the bottom die. We have had a fungus disease in oysters that destroyed all the oysters in some areas. We are hopeful that this problem will be controlled so that the oyster industry can survive.

The problem I want to speak with you about is something that must be controlled if the oyster industry is to continue to have a market for its product. Because of the particular characteristic of the animal, the oyster meat is very delicate in its makeup. It is about 85% liquid and 15% solids if it comes from the water with high salinity, and 90% liquid if it comes from the low salinity water of nine to twelve parts. Because of variation in the water content of the oyster meat, the Food and Drug Administration has been reluctant to regulate the amount of free drain liquor in fresh packed oysters.

The difference in the water content is about 10% at most. But the oysters which contain as much as 30 to 40% free drain liquor are being put on the market. Now what does this do to the retail market, the largest food market we have in the country?

Any manager of a retail market can tell you that the sale of fresh oysters has dropped as much as 50% in the last decade while the sale of most other foods has increased. For instance, a housewife buys a 12 oz. can of select oysters for $2.40 and finds only twelve small oysters in the can, when there should have been 20 to 23 nice size oysters. She is not going to continue to buy oysters and I can't blame her. Because of the loss of this market, we of the oyster industry are selling mostly to institutional users of oysters.

Before the oyster industry finds itself withering on the vine, so to speak, I believe the state and federal agencies should step in and solve this problem for us. Regulations for the percentage of free drain liquor could be something like this: Fresh oysters should not contain more than 15% of free drain liquor at the time of shipment or at the time of delivery, provided that the draining is done when the temperature of oysters is 40°F or lower. My company has had printed on all of our fresh oyster cans this wording: "This package contains 85% or more solid oyster meats."

The oyster industry and state and federal agencies should be more concerned about the quality of oysters on the market. There used to be competitive marketing of oysters on quality and price. But now the industry is competing only on price, and you can surely sell faucet water at a lower price than oysters. Let's say a can of oysters with net weight of 12 oz. retails at $2.40 and has the free drain liquor content of 12%. Then, drained oysters, 88% or 10.56 oz. of the total weight, cost $.2286 per oz. But the same 12 oz. can selling at retail for $2.10 and draining 35% free drain liquor contains only 7.80 oz. of drained oysters, costing over $.26 per oz. of oyster meat.

Now you can see that while a good quality 12 oz. can of oysters is sold for $2.40, the price cutter can sell the same size can for $2.10 by adding water and make $.03 per ounce or $4.08 per gallon more. At present, our wholesale price of a 12 oz. can of select oysters is $1.90, or $18.83 per gallon, with eleven cans per gallon. The poor quality 12 oz. can priced at $1.60 at wholesale would gross $22.40 per gallon, with 14 cans per gallon. After deducting the cost of can and freight, it would net $20.33 per gallon.

My company will continue to pack wholesome and high quality oyster products. But we are concerned with the very unhealthy condition of the oyster industry as a whole. The oyster industry should be encouraging more people, including young people, to eat oysters, because oysters are healthful and a good buy. But this is not the case at all, and something must be done about it.

A REVIEW OF BIOLOGICAL AND ECOLOGICAL FACTORS INFLUENCING THE SURF CLAM (Spisula solidissima) AND THE OCEAN QUAHOG (Arctica islandica)

Dexter S. Haven

Introduction

This discussion highlights some of the important factors relating to the biology and distribution of the arctic quahog (Arctica islandica). The distribution of this species is fairly well known. Few studies, however, have been published relating to its growth, spawning habits, and other factors. The reason for this is that, only during the last three years, have significant quantities of the ocean quahog been landed. This species is caught by the same gear as is used for surf clams (Spisula solidissima). The range of the two species are similar; both are subject to nearly the same hydrographic conditions. Therefore, this discussion will include information on both species.

Gear Used to Harvest Surf Clams and Ocean Quahogs

Both species are caught in large box-like dredges which are dragged over the bottom and emptied at intervals on the deck of the boat. The dredges loosen the clams from the bottom with powerful jets of water pumped to the dredge through a hose at the rate of 1500 to 3500 gallons per minute. The blade of the dredge which slices through the substrate ranges from 30 to 84 inches in width (Ropes, Chamberlin and Merrill, 1969). Today, even larger dredges are used.

A typical surf clam dredge boat operating off the Virginia coast catches from 3 to 682 bushels per hour (Ropes and Ward, 1977). The National Marine Fisheries Service uses a conversion factor of 17 pounds of meats per bushel. However, the edible meat content for clams caught off the Virginia coast averages 12.55 pounds per bushel since the visceral mass is removed prior to processing (Loesch, 1977).

The typical catch of a dredge boat fishing for A. islandica ranges from 600 to 1800 bushels per day. The latter figure represents the usual

capacity of the boat. Most boats fishing for surf clams and ocean quahogs return to port with their catch within 24 to 48 hours.

A History of the Surf Clam Fishery

The history of the surf clam fishery can be divided into five periods. The early period was one of limited use of this enormous resource. It began about 1870 and by 1942, annual landings had reached only 753,000 pounds. Most of these clams came from the New Jersey, Long Island area.

The second period started during the years of World War II when there was a rapid expansion of the clam fishery. By 1947, landings reached 5,329,300 pounds.

The third phase was characterized by a rapid rise in landings. The fleet used better equipment and increased in size. This put greater fishing pressure on the mid-Atlantic populations. By 1966, total East Coast landings had increased to 45.1 million pounds.

The fourth stage was marked by a further increase in numbers of vessels and an increase in landings. Of great significance was the expansion of the fishing area to include waters off Delaware, Maryland and Virginia (Table 1). In this period, landings in the Chesapeake region increased from 1.1 to 63.6 million pounds. Total landings from all areas reached an all time high in 1974 of 96.1 million pounds (Bruce G. Nichols, Personal Communications).

The beginning of a decline was marked in 1974 in landings for both Chesapeake and Mid-Atlantic regions. By 1976, landings had dropped to only 51.0 million pounds. Studies by NOAA, the states and regional regulatory groups showed that overfishing was occurring. Catch per unit of effort was declining. Because overfishing was evident, quotas on landings--1,800,000 bushels per year--were imposed by the Secretary of Commerce under the Fisheries and Conservation Management Act. The plan was designed to allow surf clam populations to rebuild to the levels which would eventually allow annual harvesting of 50 million pounds, the present estimate of the maximum sustainable yield (MSY). Surf clam boats now work only one day or one-half a day a week.

Table 1. Landings of Surf Clam Meats in Millions of Bushels - 1966 to 1977*

Year	Total	Mid-Atlantic	Chesapeake
1966	45.1	43.8	1.1
1967	45.0	43.0	0.06
1968	40.5	35.2	5.3
1969	49.5	42.2	7.3
1970	67.3	52.6	14.6
1971	52.5	40.1	12.3
1972	63.4	32.6	30.7
1973	82.3	31.5	50.7
1974	96.1	32.4	63.6
1975	86.9	47.8	39.1
1976	49.1	27.9	21.2
1977	51.0	26.8	24.2

*NOAA data (personal communications, Bruce G. Nichols, State Fish Pier, Gloucester, Mass.).

The Ocean Quahog Fishery

The ocean quahog fishery is still in developmental stages. It began slowly in Rhode Island where 1.5 million pounds were landed by 1946. By 1976, landings were still only about 5 million pounds (Federal Register, 1977). As surf clams began to decline in abundance, and as quotas were imposed, however, the industry turned to the ocean quahog. The latest information available suggests that about 18 million pounds of ocean quahogs were landed in 1977 (Bruce G. Nichols, Personal Communications).

Range and Habitat of the Two Species

Surf clams occur from close to shore out to depths of about 60 m from the Gulf of St. Lawerence to South Carolina. In the northern part of this range, they occur close to shore and also far out over George's Bank. To the south, the densest populations occur in the deeper waters. Off Long Island, the average depth is about 21.2 m; off Virginia, it is 32.1 m.

The ocean quahog is a boreal species and lives on both sides of the Atlantic Ocean. On the East Coast, the southern limit is North Carolina. It lives further offshore than the surf clam from 15 to 234 m, with the largest concentration between 25 to 60 m.

Surf clams and the ocean quahog both inhabit a wide range of bottom type from gravel to sand and mud. Both species typically live with their posterior end about 1/2 inch below the sediment surface, and with their fused siphons extending into the water column.

The ocean quahog does not move significant distances as an adult. In contrast, surf clams have the ability to move about on the bottom and even glide short distances through the water. They do this by expelling water from the mantle cavity by opening and closing their shells (Merrill and Ropes, 1969). Small clams 1 to 2 inches can reach an elevation of .25 m off the bottom by the action of their foot and shell (John N. Kraeuter, Personal Communications).

Surf clams may aggregate in shallow waters along the offshore edge of sand bars. Clams from these locations are sometimes driven ashore by storm waves and deposited on the beach. In 1964, for example, at Wallops Island, Virginia, clams averaged 20 per square foot along a two mile stretch of beach (Ropes, Chamberlin and Merrill, 1969).

Temperature and Salinity

Surf clams and ocean quahogs live in the cooler high salinity waters. Recent studies by NOAA show that average bottom temperatures in September off the Long Island, New England coast where most surf clams occur, range from about 14C along shore, to about 8-12C in deeper waters. During the same month, they occur along shore in Virginia where temperatures range from 25C where small populations exist along the inlets to about 8-14C in deeper waters.

Reproduction - Larval Life

Surf clams begin to spawn at one year of age. There are two spawning periods. The first (major) period occurs from mid-July to early August, and the second (minor) period from mid-October to early November (Ropes, Chamberlin and Merrill, 1969). In the laboratory, surf clam larvae have been reared to setting size in 19 days at 22C. Available evidence shows that recruitment to the fishery is not regular, and that the occurrence of dense populations may be the result of a single successful year class.

The ocean quahog spawns from June to October with a peak in August. Little is known about recruitment rates (Federal Register, 1977).

Age and Growth

Techniques have been developed to determine the age of surf clams and ocean quahogs by examining a cross-section of the shell surface. Studies show that shallow water populations of surf clams from the New Jersey region reach a length of 94 mm in five years and about 110 mm in 11 years. Few clams over 10 years are found inshore. Offshore populations from the same region grow faster. At 5 years, they average about 114 mm and at 11 years, 146 mm. Growth slows after 10 years, reaching about 150 mm by 15 years. Offshore clams are long-lived and commonly attain ages of 25 years and occasionally 30 years. At this latter age, they may average about 165 mm (Jones, Thompson and Ambrose, 1978). Surf clams seldom exceed 207 mm.

Little is known with certainty about the age composition of ocean quahog populations. A preliminary study, however, indicates that this species may first enter the fishery at 60 years and that the average

age of those now being landed may be up to 110 years. Their maximum size is about 127 mm.

Adverse Aspects of the Environment

Surf clams and ocean quahogs have no known fungus diseases such as MSX or Dermocystidium. Both species, however, are consumed by predators which include fish, moon snails, conchs and crabs.

Surf clams from along the Virginia and South Carolina coast may be infected with immature nematode worms which do not cause economic concern until infected with the hyperparasite Urosporidium spisuli. When this protozoan sporulates, the spores give the nematode a brownish-black color making it visible in the clam tissue. All spores are killed at 5-30 minutes at 100C (Perkins, Zwerner and Dias, 1975).

During the summer and fall of 1976, anoxic conditions existed in an area 40 miles wide and 100 miles long starting about 4 miles off the New Jersey coast. It extended south to the mouth of Delaware Bay and out to the depth of 54 to 64 m. Losses of surf clams were calculated to be 53,500 tons; loss of ocean quahogs was not calculated (Forste, 1978).

A second problem common to both species is the fecal coliform contamination due to the Philadelphia dump site located 35 miles off the Maryland coast. This sludge dumping site occupies about 9.5 square miles. Levels of fecal coliform for both species exceeded recommended guidelines. It was calculated that 600,000 bushels of ocean quahogs were influenced.

The surf clam, when exposed to a northern bloom of the toxic dinoflagellate Gonyaulax tamarensis, concentrated paralytic shellfish poison (PSP) and retained it for a period of over one year (Blogoslawski and Stewart, 1978). It is probable that ocean quahogs living in deeper waters would also accumulate this toxin.

Summary

The surf clam and the ocean quahog occupy similar habitats off the East Coast; both have similar problems related to water quality and their environment. Overfishing has occurred for the surf clam. The recent increase in landings for the ocean quahog (in relation to its long life) suggests that this fishery will shortly experience a similar problem.

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HEAT SHOCKING OF OYSTERS

John W. Hazelton

Heat shocking of oysters has been used in the State of North Carolina for several years, especially in the southern section of the state. This method is designed primarily to shuck the long-blade, cluster-type oysters which are otherwise difficult to shuck because of the razor sharp edges of their shell. Heat shocking also makes the shucking of the oysters economically feasible.

The method used for the heat shocking of oysters is commonly referred to as the "hot dip" method. Oysters are transported from the place of catch by tractor trailers and refrigerated Thermo-King trailers. Shell-stock must be pre-containerized in bags, have certification tags attached, and then be placed on pallets in the trailer. The last procedure elevates the oysters off the floor of the trailer by approximately eight inches, thereby permitting air to circulate freely. The temperature should be maintained in the $40^{\circ}F$ range, but preferably at $40^{\circ}F$ to ensure safety.

From the trailer, the oysters are transferred to a refrigerated shell-stock room where they are washed reasonably free of mud and detritus. This is usually accomplished manually by the use of potable water under pressure. Sometimes another method is used; the oysters are placed in a motorized tubular wash machine which cleans the oysters with jets of water as it revolves. Oysters are then placed in one-half bushel baskets.

The shell-stock then enters the heat-shock room, which is separated from the shell-stock room and usually situated between the shucking area and the shell-stock area. The one-half bushel baskets are placed in the hot dip tanks which are constructed of non-corrosive type material for easy cleaning and rapid drainage. The tanks are thermostatically fired with a booster heater, using gas or fuel oil as the source of energy. Each tank normally accomodates 10 one-half bushel baskets (two abreast and five deep on each side).

Removable metal grates are installed at the bottom of the hot dip

tank to elevate the baskets four to six inches, thus preventing sediments and silt from entering the shells of the oyster. The amount of water required in the tank is approximately eight gallons per one-half bushel of oysters. Odors and condensation are generated by the use of booster heaters in the heat shock room. These are vented through exhaust fans installed on the roof or wall.

When the temperature of the water in the tank reaches 145-150°F, the one-half bushel baskets containing oysters are placed in the tank and "shocked" for 3 to 3½ minutes. An accurate thermometer is used to check the temperature of the water in addition to the thermostatically controlled booster heater. A timing device is usually mounted on the wall to ascertain timing. The oysters are shocked only long enough to relax the adductor muscle. The relaxing of this muscle allows the shucker to enter the oyster, usually at the distal end of the hinge. The muscle is then severed and the oyster meat is removed from the shell. Over-shocking of oysters results in the opening of the shells, which is neither desired nor permitted.

Upon completion of shocking, the oysters are immediately removed from the tank and quickly cooled down by running potable water to retard bacterial growth and cooking process. The internal temperature of the oyster at this point is reduced to 100-110°F. After shucking into stainless steel pint containers, the internal temperature at the shucking table is further reduced to 80-90°F.

Since time and temperature are of great importance, additional cooling methods are used. One method is to place shucked oysters in stainless steel containers with ice from approved source. These containers are situated near the skimmer in the packing room. They are emptied into the skimmer and cleansed by running water. Most wells in this area have the water temperature of about 65-70°F.

A water line, usually a three quarter-inch coiled copper tubing approximately 100 feet in length, is installed in the ice room, often under the ice bin. This further reduces the temperature by approximately 10-15°F. After cleansing, shucked oysters are canned, sealed, iced, and placed in the walk-in refrigerated cooler. The internal temperature of the finished product should be 40°F within two hours.

In closing, I would like to say that adequate organization and supervision are essential not only in the "hot dip" method, but in any method you choose to use. With adequate supervision, we can successfully put good safe products on the market for human consumption.

RANGIA CLAM MICROBIOLOGY STUDY

Barney E. Kane

Some of you know the Rangia clam by other names that are not very favorable. The basic reason for studying the Rangia clam is that there seems to be a potential for marketing it. It has some characteristics that are less desirable than other clams; when heat-processed, it has a particularly disagreeable taste. Some of you from Louisiana, Florida and Gulf Coast states are familiar with this clam, but it is a newcomer to us in North Carolina. Due to a range extension or resurgence of population since 1950, it is now abundant in the fresh parts of estuaries all along the Atlantic Seaboard up to Maryland. There are potentials for thousands of bushels of commercial harvest, if we can overcome some of the problems with this organism.

The principal problem is that the clam seems to have a naturally high standard plate count. Interstate shipments of the clam have been seized in the past because of standard plate counts exceeding 500,000 per gram. The State of North Carolina followed up on some of the seizures and found that freshly harvested Rangia clams in fact had a very high count. When freshly harvested under good conditions and shucked aseptically in the lab, most shellfish can be expected to have a standard plate count of about 3,000 per gram. Under ideal conditions, the Rangia clam has counts from 30,000 to 4,000,000 per gram. Standard plate count doesn't seem to be a functional test.

We were asked to study the clam to find out if the high bacterial count presented any particular public health problem. With the assistance of Bob Benton and his staff, we located some sample stations on Albemarle Sound here in North Carolina in both open and closed waters. Our strategy was to examine the clams, their substrates and the water column around the clams at varying sites, and to examine each of these 3 types of samples for fecal coliforms, total coliforms, fecal strep,

and standard plate count. We also screened the clams for bacterial pathogens, particularly salmonella, shigella and vibrio. Out of curiosity, we also looked at staphylococci, although normally not a waterborne disease of shellfish. We split samples and shared them with Dr. Mark Sobsey for viral analysis. Sites 1 and 2 were in approved waters, Site 3 was located near a closed area, and Site 4 was located well into a closed area.

It is of historical interest that Rangia clams were marketed in a variety of forms in the late 1800's. In 1890, they were marketed in Texas as Texas Little Necks. They didn't seem to turn people off with their taste then. They look quite attractive and might be mistaken for little neck clams. But I think you can tell the difference between Rangia clams and little neck clams if you eat them.

The clams collected were put in plastic bags. Polycarbonate soil core tubes washed with a chlorine solution were used to core the bottom sediment. Regular water sample bottles were used to collect the water samples. We made monthly sampling trips to each of the sites to get a year-round pattern and to see if there might be a seasonal variation, particularly if the clams might meet the market standards during one season while in others they did not.

We used standard methods for the analysis of shellfish. For the pathogens, we used the FDA's Bacteriological Analytical Manual for Foods, 1976 edition. We tried not only to screen for the pathogens but also to enumerate them, for we wanted to see how many pathogens there were per gram of sample.

Using aseptic techniques and FDA-recommended procedures, the typical standard plate counts for July were 100,000 organisms per gram in Site 1, 20,000 in Site 2, 32,000 in Site 3, and 150,000 in Site 4. The geometric mean counts for the whole year were 59,000 for Site 1, 66,000 for Site 2, 120,000 for Site 3 and 640,000 for Site 4.

An interesting thing was that, in simulated commercial storage, the dry shellstock refrigerated at $4^{\circ}C$ maintained only about a three-fold increase in standard plate count in 12 days of storage. But if we shucked the clams, there was a three-fold increase in standard plate count in only 5 days. So if the clams started out with an average 110,000 count and had a three-fold increase in 5 days of shucked storage

under ideal storage conditions, they would not meet market standards for SPC.

The total coliform count of the water was very erratic and did not correlate well with the total coliform count of the clam. There were times when the total coliform counts were excessive even in open waters. We found a strong correlation between fecal coliform counts of the clams and fecal coliforms of the water column above them. ($r = .739$ $p<.01$) We found essentially no correlation between the fecal coliform counts of the bottom sediment and those of the clams.

The standard plate counts in the sediments were erratic and routinely fairly low compared to those of the clam. One would think if there is a correlation between the fecal coliform counts of the water column above the clam and those of the clam, then there would be a correlation between the standard plate counts of the water column and those of the clam. But we did not find that to be true. (Correlation of clam SPC with water SPC: $r = 0.05$ $p = .369$).

Rangia clam, like all filter feeders, seems to reflect in kinds of organisms the population of the water over it. But there seems to be a base level population of bacteria residing in the clam that are independent of the fluctuations in the water column above them. Of course, there is a time factor; the water we get for sampling is not the same as the water the clams filtered a few hours before. So we need to follow up on retention times of organisms filtered by the clam from the water column. We found very few indicator organisms in the sediments. Even when the fecal coliforms were high in the water, there were very few fecal coliforms in the substrates.

Of potential pathogens, we did find in the clam Vibrio cholerae. Representative isolates were found to be nonagglutinating vibrios (NAGS) by the Center for Disease Control. Their numbers were fairly low, on the order of 2,000 per gram in summer months to about 10 per gram in winter months. Vibrio parahaemolyticus-like organisms were also higher in the summer months and dropped down in the winter months. Their numbers were generally fairly low (200 per gram in summer and undetectable in winter). We did not use any special techniques such as salt enrichment in isolating V. parahaemolyticus because the salinities

where samples were collected were generally less than 5 ppt. These two potential pathogens were the most abundant, although very low in counts.

On two isolated occasions, we found some staphylococci in the clam. The counts were about 620 per gram, even counting all weakly coagulase-positive Staphylococcus aureus. No enumeration was possible. We did not find any salmonella. In the summer months, there was an outbreak of Achromobacter. Achromobacter violaceum was isolated among others. Interestingly, we found Achromobacter in the clam whenever we found them in the water. In general, the clams obviously pick up microorganisms that are in the water above them. But there is a basic level that is maintained in the clam regardless of the environment, for the number of microorganisms we find in the water, as previously mentioned not correlate with the number of microorganisms in the clam.

In summary, we did not find any bacterial pathogens that we would regard to be significant in the Rangia clam. I wouldn't like Vibrio parahaemolyticus-like organisms being brought into a seafood processing plant without adequate controls. But their number seems to be too low to cause any problems unless the product is mishandled. In the case of salmonella and shigella, it is essentially a situation of no organisms present in the product.

Preliminary studies on the shelflife of Rangia clams are not conclusive. But it looks as though there will be a problem in storing these clams for more than 5 days as a shucked product. They may be stored 12 days or even 21 days if refrigerated in the shell, but that is not the form in which we believe the industry would like to market this product.

An early attempt at pasteurization of the clams seems to be quite successful. First we held the clams in a weak saline solution to get rid of some of the grit and hopefully affect some improvement in their flavor. Then we shucked and pasteurized them (80°C for 1 min. interior temperature). We don't know how long we can keep them that way, for we cut off the experiment at 21 days. But the bacterial counts at 21 days were less than 3,000 per gram when held in refrigerated storage.

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THE USE OF CHLORINE IN FOOD PROCESSING

Richard V. Lechowich

Introduction

The development of the gas chlorinator by the Wallace and Tiernan Company in 1913 has led to an increasing use of chlorine in water treatment. With the use of chlorine, a marked decrease in the incidence of waterborne diseases took place from 1915 to 1955. Public health microbiologists had increasingly encouraged the use of chlorine until recently when the cost of chlorine manufacture, the toxicity of chloramines to marine organisms, and the formation of halogenated organic compounds that appear in drinking water after chlorination have led to the reevaluation of the use of chlorine.

Although chlorine is recognized by microbiologists as a highly active antimicrobial agent with sporocidal properties, caution must be exercised when adding chlorine to water containing organic matter. The chlorine concentration diminishes upon interaction with any form of organic material with a concomitant loss in activity. Thus, a sufficient chlorine residual may not be available for bactericidal action.

There are several alternatives to chlorination that can be used for disinfection. These include ozonation, bromination, iodination, and ultraviolet irradiation. The use of chlorine dioxide possesses many of the advantages of chlorine, such as its oxidizing capacity and it does not form chloramines.

Theories of the Germicidal Action of Chlorine

When an aqueous solution of chlorine comes into contact with microorganisms, death of the microbial cells results if the chlorine concentration is high enough and the contact time is sufficiently long.

It was believed that oxidation of microbial protoplasm caused the death of the bacteria. However, chlorine kills bacteria under conditions that prevent direct oxidation of cell protoplasm by other oxidants.

Chlorination of cell protoplasm that forms toxic chloramines has been suggested as another possible mechanism of destruction. Other hypotheses include the precipitation of bacterial proteins or alteration of the permeability of cell membranes.

The theory that appears most plausible is that chlorine prevents enzyme regeneration and therefore results in inactivation of certain essential enzymes. This theory is supported by the relationship shown between the effect of chlorine on the bacterial cell and the inactivation of certain enzymes necessary for glucose utilization.

The Germicidal Agent in Chlorine Solutions

The undissociated hypochlorous acid (HOCl), produced when chlorine is added to water, is considered to be the germicidal agent, since the rate of death of bacteria is directly proportional to the concentration of undissociated hypochlorous acid. For purposes of chlorination in a food plant, it can be assumed that time required to kill bacteria is an inverse function of undissociated HOCl (Mercer, 1953).

Chlorine gas and the hypochlorites readily produce hypochlorous acid when they are added to water. But when chloramines are added to water, hypochlorous acid is produced very slowly by hydrolysis, which can account for their slower germicidal action.

Factors Influencing the Germicidal Effect of Chlorine

The factors that affect the concentration of hypochlorous acid formed and the subsequent germicidal action are: A.) the chlorine concentration, B.) the pH of the solution, C.) temperature, and D.) concentration and type of organic matter in the water.

A.) The germicidal effect of chlorine should increase as the concentration of chlorine compound added to the water is increased. However, this situation is true only in the case of buffered solutions where conditions of pH, temperature, and organic content are held constant. When only the concentration of chlorine is varied, and all other factors are held constant, a plot of the logarithms of the killing time against the logarithm of the chlorine concentration will approximate a straight line function.

However, data obtained for destruction at low concentrations of chlorine indicate killing times of 10 minutes at 1.2 ppm, 15 minutes

at 0.6 ppm, and more than 30 minutes at 0.3 ppm of chlorine. Thus, in solutions of low chlorine concentration, a greater portion of the germicidal effect of chlorine may be neutralized in side reactions.

When the concentration of available chlorine is increased in unbuffered solutions, the effect on germicidal activity is dependent upon two interrelated factors of: 1) the pH shift toward acidity or alkalinity and 2) the influence of pH shift on the concentration of hypochlorous acid.

B.) pH - The pH of any chlorine solution has a marked influence upon the rate of death of bacteria exposed to the solution. It has been shown that the bactericidal effect of hypochlorite solutions was dependent upon the amount of hypochlorous acid formed and that both the concentration of initial hypochlorite added and the pH were the deciding factors in the activity of the acid. However, pH was the most important single factor influencing the germicidal effect of hypochlorite solutions.

The hydrogen ion concentration determines the fraction of the hypochlorous acid present as the undissociated molecule or as hypochlorite ion. As the hydrogen ion concentration decreases (pH increases), the ionization of hypochlorous acid increases.

The time required for four chlorine compounds to kill 99% of Bacillus macerans spores exposed to solutions having a pH range of 6.0 to 8.0 was studied by Mercer (1953). With equal concentrations of available chlorine and the same pH, no significant difference in sporicidal activity was found between chlorine gas and the hypochlorites. Chloramine required about 10 times as long at each pH level to be equally effective as a sporicide.

C.) Temperature has been shown to be a very important factor on the bactericidal efficiency of chlorine solutions. Charlton and Levine (1937) used Chloramine T solutions containing 2,000 ppm available chlorine with an initial pH of 6.0 at 55°C and found that each 10°C rise in temperature produced a reduction of 82% in the killing time. Other workers concluded that a drop of 10°C required a two-fold increase in exposure time with chlorine and a three- to fourfold increase for chloramine to achieve the equivalent bactericidal action.

In food plant sanitation, the feasibility of elevating the temperature of chlorine solutions to take advantage of this increased

germicidal activity depends on the type of chlorine compound from which the solution is prepared and the purpose for which the solution will be used.

The solubility of chlorine gas added to water changes substantially as the temperature increases from room temperature to sanitizing temperatures. The maximum solubility decreases from 7600 ppm at 20°C to 2200 ppm at 80°C while the 10°C rise from 90° to 100°C shows the loss of solubility of 1200 ppm at 90°C to none at 100°C.

Ordinarily, chlorination of water by dissolving gaseous chlorine is performed in large-scale operations such as product washing or can cooling where the contact time between the bacterial cell and the chlorine is sufficiently long to allow adequate germicidal action at natural water temperature.

Hypochlorite solutions apparently lose little available chlorine at moderately elevated temperatures. No appreciable loss of available chlorine from sodium hypochlorite solutions was found in solutions held at 55°C for 3 hours, and they are recommended as sanitizing agents for food handling and processing equipment when higher temperatures are used.

D.) The presence of different types of organic matter in chlorine solutions causes a marked reduction in germicidal activity. This situation can occur in the chlorination of recirculated can cooling water and water used for fluming raw products. Thus, the free chlorine residuals of these waters must be monitored to retain germicidal activity.

The reaction of chlorine with organic material may be adsorption or chemical combination depending upon the type of organic material present (Mercer 1953). Sugars and starch had little effect on available chlorine, and these substances include monosaccharides, disaccharides such as maltose, lactose, and sucrose, trisaccharides and polyhydric alcohols. Tomato juice addition did substantially reduce chlorine residuals.

Chlorine in the presence of such organic materials as sugars and starches retains the major portion of germicidal activity as shown in Figure 1. Survivor curves for yeast cells in starch or sugar show that yeasts were killed reasonably effectively, while tomato serum produced some reduction in effectiveness and tomato juice addition

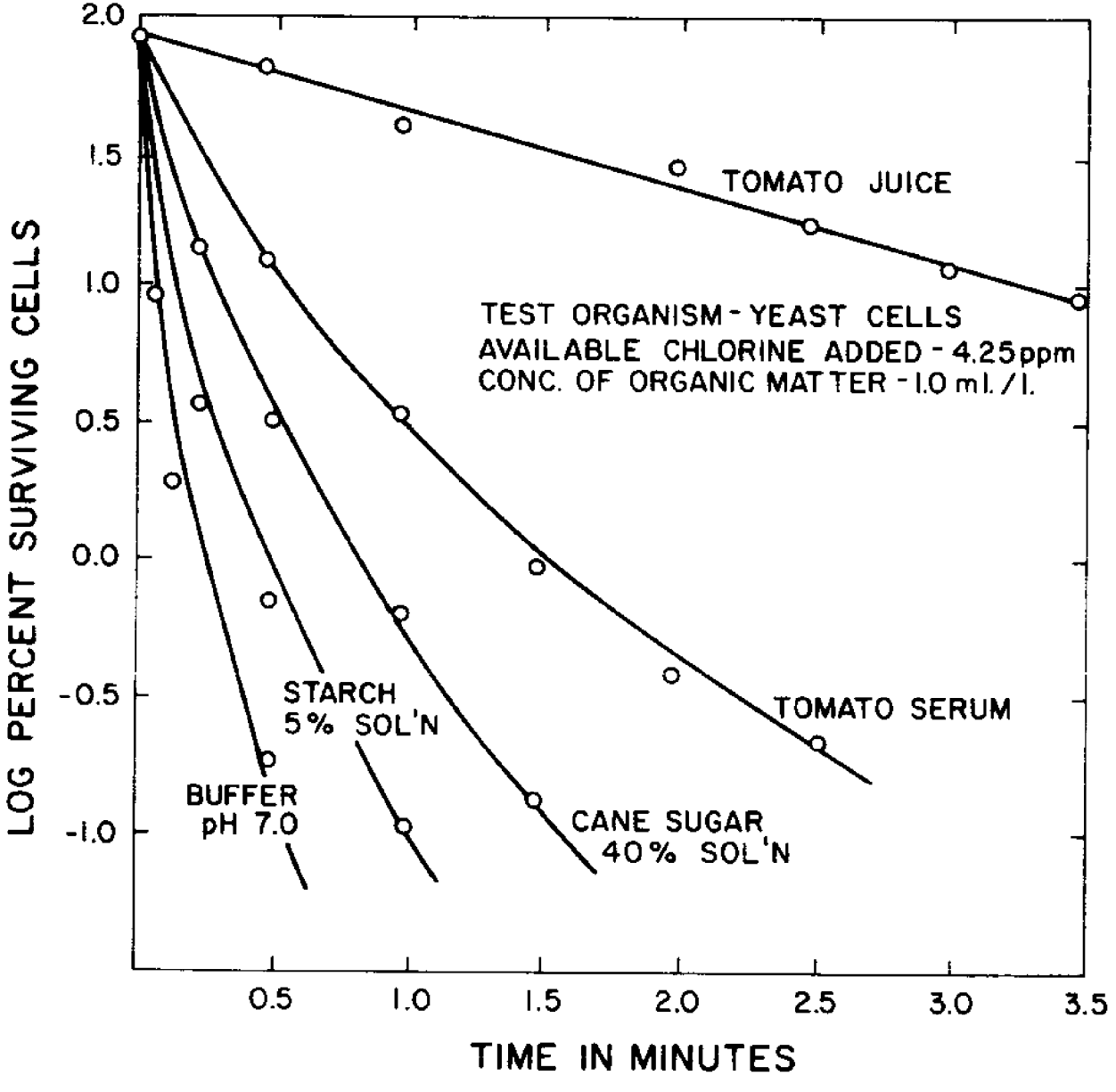

Figure 1. Effect of organic matter on the germicidal activity of chlorine solutions (From Mercer 1955)

produced a substantial reduction in killing effect.

The pH also has an effect on the amount of chlorine lost to organic matter in a solution. In general, as the pH becomes more alkaline, the concentration of available chlorine becomes less.

If the concentration and pH are held constant, hypochlorites and chloramines are as much affected by heterogenous organic material as is gaseous chlorine. Thus, the pH, amount of organic material, and residual chlorine desired must be evaluated for each application.

Bacterial Resistance to Chlorine

In food plant chlorination, the high resistance of bacterial spore forms to killing by chlorine must be taken into consideration. Spores have been found to be 10 to 1100 times more resistant to chlorine than the vegetative cells. Most of the work on the sporicidal effect of chlorine compounds has been done using aerobic spore-forming organisms. Two of the early studies on spore destruction by chlorine compounds dealt with a spore-forming organism isolated by Charlton and Levine (1937) and named Bacillus metiens. A composite of the results of studies by Charlton and Levine (1937) and Rudolph and Levine (1941) are shown in Table 1. These data for calcium hypochlorite indicate the important role of pH on the sporicidal effect of the chlorine. At a pH of 7.3, a 99% reduction in viable spores was obtained in less than 0.33 minutes with 1000 ppm of chlorine, while at a pH of 11.3 with 1000 ppm of chlorine, 70 minutes was required for a 99% reduction.

Charlton and Levine (1937) compared calcium hypochlorite with Chloramine T; their results are also shown in Table 1. The concentrations of Chloramine T in Table 1 are those in the solution as initially prepared. Charlton and Levine (1941) did not determine the amount of free available chlorine (FAC) released from Chloramine T. The FAC concentration was low probably because chloramines release chlorine very slowly. As is evident from data in Table 1, Chloramine T was not effective in killing spores. Even at high pH values (10 to 11.3), calcium hypochlorite was a more effective sporicide than was Chloramine T at low pH values (6.0 to 8.8). Cousins and Allan (1967) reported that 1000 ppm of Chloramine T at pH 6.5 had no sporicidal effect on Bacillus subtilis spores in 4 hours, while 100 ppm of FAC from sodium hypochlorite at pH 8.0 reduced the spore population by 99% in one hour.

Table 1. Effect of Concentration and pH of Chlorine Compounds on the Destruction of *Bacillus metiens* Spores[a].

Compound	pH	Conc. of chlorine[b] (ppm)		Time to kill 99%[c] (min)
		FAC	FAC and CAC	
Calcium hypochlorite (13)	6.0	25		2.5
	7.0	25		3.6
	8.0	25		5.0
	9.0	25		19.5
	10.0	25		80.6
	10.0	100		42.4
	10.0	500		20.6
Calcium hypochlorite (3)	7.3	1000		<0.33
	10.4	100		70.0
	11.3	1000		70.0
Chloramine T (3)	6.0	--[d]	1000	900.0
	6.0	--	2000	324.0
	6.0	--	4000	156.0
	8.7	--	2000	3840.0
	8.8	--	4000	1404.0

[a] *Data adapted from Charlton and Levine and Rudolph and Levine.*

[b] *FAC = Free available chlorine; CAC = combined available chlorine; FAC plus CAC is the total available chlorine.*

[c] *Temperature was at 20°C for calcium hypochlorite test and at 25°C for Chloramine T test.*

[d] *Indicates amount FAC released was not determined.*

These data indicate that the FAC released from sodium or calcium hypochlorite is more effective in killing spores than the combined available chlorine of Chloramine T. Chlorine in the combined form should not be considered for chlorination of cooling water as it is an ineffective sporicide.

Several other papers cited in Odlaug and Pflug (1976) have been published on the chlorine resistance of various species of Bacillus spores. Data from these reports are presented in Table 2. To make the results from the several studies comparable, the time for a 90% reduction in numbers of spores and the concentration of hypochlorous acid were calculated from the available data in each report. The data in Table 2 indicate that, for all spores tested, the sporicidal activity of the solution increased with increasing amounts of hypochlorous acid.

Clostridium Spores

Only a few reports have been published on the resistance of Clostridium spores to chlorine. As a group, the aerobic spore-formers seem to be more resistant than the anaerobic spore-formers.

Dye and Mead (1972) evaluated the effect of chlorine on eight strains of Clostridium spores. Clostridium welchii (perfringens) spores were the most resistant; Clostridium bifermentans and Clostridium caloritolerans spores were the least resistant. Chloramine T at 200 ppm (pH 9) was not very effective in reducing the number of any of the Clostridium spores tested. There was less than a 2-log reduction of any of the eight strains in a 2-hour test period. Also, B. subtilis spores were subjected to 100 ppm chlorine at a pH of 9.8 and found to be considerably more resistant than any of the Clostridium spores tested. Results of Dye and Mead confirm the work of Tonney, et. al. (1930) that Clostridium spores have less resistance to chlorine than Bacillus spores.

Clostridium botulinum organisms in canning plant cooling waters are a public health hazard. The post-processing entry of a single C. botulinum organism into a food container is a serious problem.

Ito, et al. (1968) conducted a thorough study on the effectiveness of commercial germicides on spores of C. botulinum Types A, B, and E. Their results indicate that calcium hypochlorite, sodium

Table 2. Summary of Data for Destruction of *Bacillus* Spores by Chlorine[a]

Organism	Chlorine Compound	Test Temp. (C)	pH	FAC[a] (ppm)	Calculated HOCl (ppm)	Time for 90% reduction (min)
B. cereus	NaOCl	21	6.5	50	43	1.5
			8.0	100	25	2.5
B. cereus	NaOCl	25	7.0	100	75	0.88
			5.2	150	149	0.18
			7.0	150	113	0.40
			8.0	150	38	1.2
B. coagulans	NaOCl	20	4.5	20	20	2.5
			6.8	20	17	6.0
			4.5	10	10	8.5
			6.8	10	8.5	12.0
			7.8	10	3.5	23.0
			4.5	5	5.0	21.0
			6.8	5	4.3	28.0
			7.8	5	1.7	59.0
B. macerans	NaOCl	21	6.0	15	14	4.3
			6.5	15	12.9	4.6
			7.0	15	11.3	6.4
			7.5	15	7.5	10.3
			8.0	15	4.0	21.0
B. metiens	CaOCl	20	6	25	24	1.25
			7	25	19	1.8
			8	25	6	2.5
			10	25	<1	40.3
			10	100	<1	21.2
			10	500	<1	10.3
B. glodbigii (subtilis)	--	22	6.2	1.8-1.9	1.7	22.8
			6.2	.11-.24	.1-.2	255.0
			7.2	2.5-2.6	1.6	20.5
			7.2	.15-.34	.1-.2	270.0
			8.6	23.8-24.8	1.7	22.5
			8.6	1.8-2.3	.13-.16	285.0
			10.5	454	<1	31.7
			10.5	21.9-23.1	<1	330.0
B. stearothermophilus	NaOCl	25	7	1000	750	1.0
			7	2000	1500	.78

[a] *Free available chlorine*

hypochlorite, and gas chlorinated water. Ito, et al. (1968) also evaluated the effect of pH on the germicidal efficiency of calcium hypochlorite. The results indicate that, as pH increases, the rate of destruction decreases. The results are not surprising since chlorine is more effective as a sporicidal agent at acid pH values where hypochlorous acid predominates.

The effect of the temperature of the calcium hypochlorite solution on time for a 99.99% kill of C. botulinum Types A, B, and E spores was also investigated by Ito and co-workers. The time for a 99.99% kill decreased with increasing temperature; the kill-time at 25°C was only 0.3 to 0.5 of the kill-time at 15°C.

Ito, et al. (1968) also reported that organic debris, such as peptone, will combine with the free available chlorine reducing the amount of free chlorine in the solution, ultimately to the point where it is ineffective. Therefore, in a commercial application such as a cooling canal, chlorine must be added continuously to maintain the desired level of free available chlorine.

Data from Ito, et al. for C. botulinum Type A and Type E show that 2 ppm of HOCl will reduce a population of C. botulinum spores by 90% in 2 to 3 minutes, whereas 30 to 50 minutes are required for a 90% reduction in the number of Bacillus spores.

An objective of Odlaug and Pflug's (1976) work was not only to review research regarding sporicidal effect of chlorine but to develop data to predict spore destruction as a function of chlorine level. With the exception of the data of Cerf, et al. (1973) for B. stearothermophilus, the data appear to form an overall pattern. If the data for B. stearothermophilus are disregarded, the logarithm of the time for a 90% reduction for Bacillus and Clostridium spores at the low effective HOCl concentration is a linear function of the logarithm of the HOCl concentration. As the HOCl concentration increases to where the time for a 90% reduction is 1 to 2 minutes, the rate of destruction increases.

When chlorine compounds are added to water so there is free available chlorine present, the solution is both bactericidal and sporicidal. Vegetative bacterial cells are killed more easily than spores; and Clostridium spores are killed more easily than Bacillus spores. The lethal effect of the chlorine in solution increases with:

(a) an increase in the free chlorine concentration in the solution, (b) a decrease in pH, and (c) an increase in temperature.

The relative microbiological quality of the water in the canning plant cooling system will be a function of the quality of the water that is added to the system. Quality factors are: the amount and source of soil and organic matter that are added to the water, pH, temperature, and chlorine level. It is anticipated that the predominant flora will be resistant Bacillus spores when free chlorine levels of 2 to 5 ppm with a pH in the range of 7 to 7.5 are maintained in the cooling water.

The public health hazard from the post-process leakage of C. botulinum spores into cans of low acid foods should be extremely small if the cooling water is properly chlorinated, the pH level is controlled, and the addition of soil or any other outside source of C. botulinum spores is eliminated. Since C. botulinum is not likely to multiply in cooking water or in a cooling canal containing chlorinated water, only the introduction of large numbers of C. botulinum spores into improperly chlorinated cooling water will create a public health hazard.

Sanitizing or Germicidal Materials Used

Chlorine in the presence of moisture is one of the most reactive elements known. It attacks many kinds of metals and has a great affinity for most organic materials.

Generally, the chlorine used for food plant sanitation is an aqueous solution containing active chlorine from one of the three commercial sources: a) liquid elemental chlorine, b) hypochlorites, or c) organic chloramine compounds.

Chlorine gas is generally considered to be the best source for in-plant chlorination where large volumes of water are to be chlorinated to 5 to 7 ppm. It is the most inexpensive source of chlorine on the basis of pounds of chlorine available. The main disadvantage is the initial high cost of chlorination equipment, but the lower cost of gaseous chlorine offsets the equipment cost in large volume uses.

Hypochlorites

Calcium hypochlorite, $Ca(OCl)_2$, containing from 30 to 60% available chlorine, is available commercially. Sodium hypochlorite (NaOCl) solutions are available at 10 to 18% available chlorine for commercial uses. Household bleach contains 2 to 6% available chlorine. These solutions are used without further dilution for chlorinating water supplies or is a 5% solution for sanitizing purposes.

Calcium and sodium hypochlorites lose chlorine upon storage, and moisture, heat and light increase the rate at which chlorine is lost. No hypochlorite can be chemically modified to be both highly stable and rapidly germicidal. Hypochlorites have the following disadvantages:

1) increased salt deposition when added to hard waters;
2) the amount of chlorine added as hypochlorite is more difficult to control than gaseous chlorine addition;
3) on the basis of available chlorine content, hypochlorites are more expensive than chlorine gas.

Hypochlorites have advantages as chlorine sources when only small amounts are needed as in non-overflow can cooling systems, hot applications during clean-up, and dripping chlorine solutions on belts and other equipment.

Chloramine Compounds

Chloramine T was first produced in 1916 by reacting p-toluene sulfonamide with sodium hypochlorite. Chloramine T is a white crystalline powder soluble in water at room temperature. Saturated aqueous solutions contain about 15% available chlorine.

Dichlorodimethyl hydantoin is another chloramine that is soluble in water up to concentrations to 1000 ppm available chlorine. Aqueous solutions are slightly acidic in contrast to the alkalinity of hypochlorites and Chloramine T.

Chloramines are the most expensive form of available chlorine and are not recommended for chlorination of in-plant water because of their slow germicidal action. However, the slow release of chlorine is an advantage where a long contact time is possible.

The chloramines formed in chlorination exhibit about 1/50 to 1/100th the disinfecting property of hypochlorous acid, and dichloramine is approximately two times as effective as monochloramine.

The chloramines present a great toxicity hazard to aquatic life. Unlike chloramines, bromamines are short-lived and pose much less of a hazard to aquatic life.

Chlorine Dioxide (ClO_2)

The most important aspect of chlorine dioxide as a disinfectant is probably its oxidizing capacity. Chlorine dioxide does not form HOCl in water and thus differs from chlorine. However, it is seldom reduced any further while hypochlorous acid is routinely reduced to chlorine, and is thus considered the better oxidant.

Studies of the virucidal and sporicidal activity of chlorine dioxide done in the late 1940's showed that chlorine dioxide was equal to or more effective than chlorine as a disinfectant in the pH range of 6 to 10. In acidic water, chlorine was slightly more effective, but in alkaline water, chlorine dioxide was markedly superior. Chlorine dioxide was also a satisfactory disinfectant when added to water with low organic content.

Chlorine dioxide was shown to be effective against Eberthella typhosa, Shigella dysenteriae, and Salmonella paratyphi B, while E. coli was more resistant. Chlorine dioxide was more effective at lower temperatures under alkaline conditions.

Figure 2 (McGhee, 1976) shows a comparison of the bactericidal effects of chlorine and chlorine dioxide on E. coli at different temperatures and pH with 5 minute contact. The effect of chlorine dioxide on three bacterial spore formers was also evaluated. Results indicate that chlorine dioxide is a significantly better sporicide than chlorine when either dosage of residual is considered, both in demand and demand free water. Ridenour (1949) theorized that chlorine and chlorine dioxide act in the same manner but that chlorine dioxide must be able to use more of its oxidizing capacity on the spores than is indicated to be available by the orthotolidine (OTA) test. The advantage chlorine dioxide has over chlorine is greater against spores than against vegetative cells. This, the authors explain, is also due to the fact that the spore coat is more reactive with chlorine dioxide than with chlorine.

The only work investigating the virucidal activity of chlorine dioxide has been with poliovirus. Hettche and Ehlbeck (1953) reported

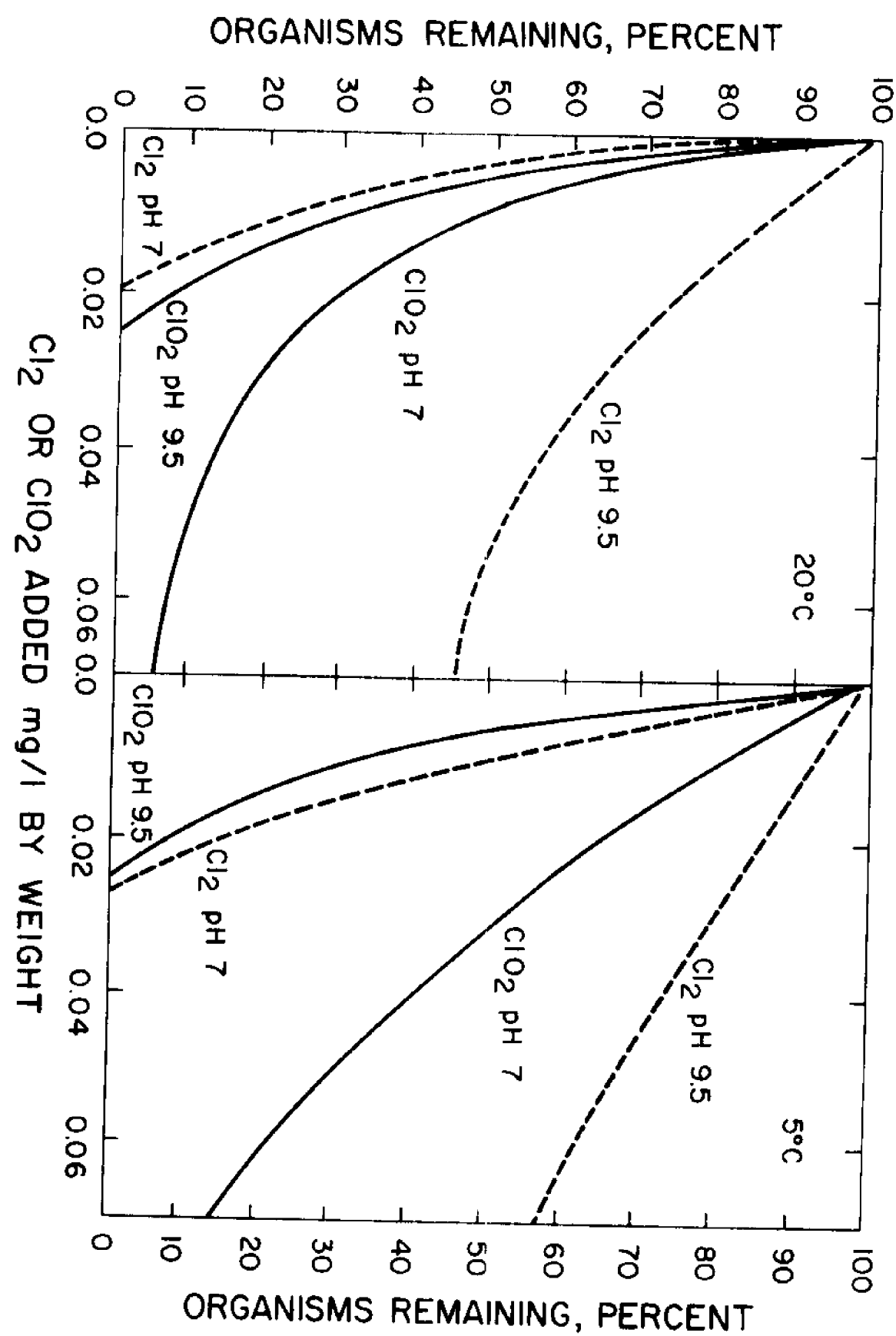

Figure 2. Comparison of the bactericidal effects of Cl_2 and ClO_2 on E. coli at different temperatures and pH with five minute contact (from Woodfin 1975).

that chlorine dioxide was more effective in deactivating the poliovirus than either chlorine or ozone. Ridenour and Ingols (1946) reported that deactivation of virus particles depended on the redox potential created within a system, and this effective potential (as measured by the nonspecific OTA test) was independent of the material used to generate it. Ozone, chlorine, and chlorine dioxide were all used and an OTA residual of approximately 0.1 mg/l was required to inactivate poliovirus with a contact time of 30 minutes. The data indicate that, on a weight applied basis, ClO_2 was 50% more effective in a system that contained 0.15 mg/l NH_4-N and 15 mg/l organic-N.

Chlorine dioxide becomes much more effective than chlorine with increasing temperature. For 99% kill, 0.25 mg/l of chlorine dioxide was required to obtain this lethality at the following temperatures:

0.25 mg/l	
5°C	110 seconds
10°C	74 seconds
20°C	41 seconds
30°C	15 seconds

A five fold increase in time is thus required to produce the same lethality when the temperature is lowered from 30° to 10°C.

The possible mechanism by which chlorine dioxide kills vegetative cells was reported by Bernarde (1967); due to the almost immediate disinfection, the mechanism must involve a primary cell function such as protein synthesis. The precise mechanism has not been elucidated, but chlorine dioxide probably does not react sufficiently with amino acids to prevent their incorporation into proteins.

Thus, chlorine dioxide should continue to be investigated as a disinfectant, since it is a good oxidizing agent, but does not react with organics to form chlorine hydrocarbons or with ammonia to form chloramines. It is also more effective at higher pH levels and temperatures.

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SAFE DRINKING WATER ACT AS RELATED TO SHELLFISH AND CRUSTACEA PLANTS

Nancy Manley

I understand that, last year, general requirements of the Safe Drinking Water Act were discussed here. This year, I would like to talk about how the Act affects noncommunity water systems, of which the shellfish processing plant is one.

The Safe Drinking Water Act allows for various limits to be set for different contaminants and for monitoring requirements. The noncommunity water system is tested for three contaminants: bacteria, nitrate and turbidity. Basically, the criteria are the same as those set by the old 1962 Public Health Service Standards.

The noncommunity water system must be monitored for bacteria once each calendar quarter that the system is in operation. One sample is taken to determine whether the system exceeds the bacterial limit of one coliform per 100 milliliters. If it does, more samples are taken. If the routine sample is positive, the system must determine the source of contamination, correct the situation and notify the public. I will talk about public notification later, because it is different for the noncommunity water system.

The nitrate limit is 10 mg per liter. Many question why it has been changed from 45 mg/l to 10 mg/l. It has not been changed; it is now based on the nitrogen content of nitrate, whereas it was based on the weight of nitrate before. The Act states that a sample will be taken by June, 1979, and thereafter as the state designates. Most states take water samples every three or five years. The Environmental Protection Agency requires it every three years, unless a water system is suspected of exceeding or being close to the limit.

If the sample taken contains more than 10 mg nitrogen per liter, a second sample must be taken within one week. The average of the two samples is used to determine whether or not the system exceeds the limit. If it exceeds the limit, the system must notify the public and correct the problem. Generally, the system has to find a new

source of water or pour in a lot of treatment. Because there have been a lot of comments from noncommunity water systems on the treatment involved, some people have proposed changing the regulations.

Analysis for turbidity is done only on those systems that use surface water such as lakes and streams. The turbidity test must be done daily during the time the system is in operation. For most water systems, the limit is one turbidity unit for the monthly average and 5 turbidity units for the two-day average. However, the state or the EPA -- whichever has jurisdiction -- is allowed to change the limit for the monthly average up to 5 turbidity units, if the system can prove that 5 turbidity units do not influence disinfection, does not influence the amount of residual in the system, and does not interfere with the analysis for coliform bacteria. Most systems generally comply with this regulation if they have some kind of treatment in addition to chlorination such as filtration.

Whenever the system takes routine samples, it must report the results to the state or the EPA within 40 days. Whenever a sample exceeds the limit, the system must report it within 48 hours. Water systems also have to maintain records: bacteriological records for 5 years and chemical records for 10 years. The record of any violation, any information on the violation and any correction that was made to the water system must be maintained for 3 years. The record of sanitary surveys conducted by the state must be kept for 10 years. The record of variances or exemptions issued, if any, must be kept for 5 years. These records must be made available to the state or EPA for inspection.

Now getting back to public notification, the Act is very general on public notification by noncommunity water systems. The regulations state that a noncommunity water system will give public notification as determined by the state. If a noncommunity water system exceeds the nitrate limit, most states tell the system to put signs up over the water faucets or something like that. The same goes for the EPA. Some states may require more detailed notifications in newspapers. But I think in most cases, the systems will just placard the area and perhaps put the notification in the employees' newsletter if there is one.

Variance or exemption is a key word in the Safe Drinking Water Act; it allows the water systems to exceed the limit but not actually be in violation of the Act or subject to penalties by the state or the EPA. Theoretically, a variance could be indefinite, but very few will be issued for the current regulations. I don't think any noncommunity system will anticipate being issued a variance or exemption just because of the contaminants for which it is being monitored.

The exemption allows a water system to exceed a limit for a specified period of time. The schedule is attached to the exemption allowing a certain time for designing, construction and so on. In the Act itself, the Congress has given the time limit of January 1, 1981, to end all exemptions coming under the interim regulations for most systems, and January 1, 1983, for those systems that joined a regional water system. If the changes to the regulations that are being proposed now take effect, it is unlikely that any noncommunity water system would need an exemption.

The plant water system is not subject to the Safe Drinking Water Act, if it is connected to a city's system and is actually just storing the water, or if it is servicing only the production process with its water. Generally, it is the main water systems that come under the Act.

There are also siting requirements in the Act. However, they are so general that most water systems comply with them by chance. For instance, you don't put your water system in an earthquake zone, you don't put it in a flood plain unless it is an intake to a surface plant, things like that.

For over two years, changes in the bacteriological and turbidity regulations have been proposed and instigated by many states. It is difficult for me to say when they will take effect. The EPA headquarters says that the proposed changes will be published soon and that, within the EPA regions, there are some changes which are out for review right now.

One other proposal which I heard of just recently concerns a change in the nitrate limit. It would allow some noncommunity systems to go up to 20 mg nitrate per liter. If a system exceeds the limit, it must give public notification such as posting signs above the faucet. High

levels of nitrate can cause what is called the blue baby syndrome to very small children. Children can get very ill and even die from it. But since most processing plants do not have little children around, it is being proposed that these systems be allowed to have higher nitrate levels.

STANDARDIZATION AND EVALUATION OF STATE SHELLFISH PROGRAMS

George Morrison

I appreciate the opportunity to attend this year's Interstate Seafood Seminar and discuss with you the evaluation and standardization of state programs and the Food and Drug Administration's role in this aspect of the National Shellfish Sanitation Program (NSSP). Before I get to the specifics of my subject, I believe it profitable to provide some background information for those of you who are unfamiliar with the nature and purpose of the NSSP.

The program was developed over 53 years ago to stop the interstate spread of shellfish-associated diseases and to permit the continued safe use of all edible species of molluscan shellfish (oysters, clams and mussels). No other shellfish such as crustacea (shrimp, crabs, etc.) are covered by this program. It is a cooperative federal-state-industry program which provides sanitary control over all aspects of shellfish production, harvesting, processing and interstate shipment.

Under this cooperative arrangement, the states have primary responsibility for the development and enforcement of sanitary controls over their respective shellfish industry. The Food and Drug Administration provides training and technical assistance to the states, conducts research necessary for development or improvement of standards and criteria, and annually evaluates state programs. The industry maintains sanitary processing facilities and purchases shellfish only from sources approved by the states.

To provide industry, health authorities and the consumer a reliable guide for safe sources of shellfish, the FDA publishes a monthly list of shellfish shippers certified by the states. The list may at times contain the names of over 1400 certified dealers from 25 states and three foreign countries participating in the program. You may be interested to know that the October list contains two firms in Hawaii. Hawaii was accepted into the program on August 14, 1978.

In evaluating state programs, the FDA Regional Shellfish Specialists are currently guided by Compliance Programs issued annually by Shellfish Sanitation Branch Headquarters. Compliance Programs are internal documents developed in accordance with standardized FDA administrative procedures. The shellfish document incorporates the basic provisions contained in Part III of the National Shellfish Sanitation Program Manual of Operations. However, reporting procedures have been modified to permit a continuous flow of current evaluation data to the headquarters and the states. The new reporting procedure immediately alerts both parties to problems as they are found, and encourages early corrective action. Comprehensive annual reporting as outlined in Part III of the Manual provides information of limited value because reports are usually submitted 6 to 12 months after field investigations are completed.

Numerical ratings are no longer given. Each state program element is judged satisfactory or unsatisfactory based on compliance with the NSSP criteria and effectiveness of the operation. For example, growing areas considerations include: timeliness and completeness of files; validity and agreement of files with observed field conditions; thoroughness and frequency of sanitary surveys, hydrographic studies, and water quality determinations; accuracy of growing area classifications based on analysis of water quality and hydrographic and survey data; and adherence to NSSP microbiological water quality standards.

Patrol is assessed on sufficiency of staff and suitability of equipment to patrol prohibited areas; frequency and thoroughness of patrol; ability to deter or apprehend violators; records of arrests and convictions; adequacy of prohibited area posting; and absence of obvious indications that violations could occur.

During the FY-1977 evaluation period, most state program elements were found to be in agreement with standards and criteria of the NSSP. However, 15 states had significant deficiencies in one or more program elements. These deficiencies may be summarized as follows:

- Failure to officially identify and manage areas that should be conditionally approved -- areas that are in fact periodically subject to rapid water quality degradation.
- Failure to update and follow conditional area management plans.

- Outdated and/or insufficient sanitary survey data -- hydrographic, bacteriological and pollution source information.
- Failure to recognize or consider public health significance of non-point sources of pollution.
- Failure to promptly close conditional areas after excessive rainfall, plant failures, etc., and reluctance to close additional areas when survey data indicate that approved area classification criteria are no longer being met.
- Patrol personnel insufficient and disorganized or uncoordinated.
- Patrol equipment inadequate and unsuited to areas assigned.
- Surveillance inopportune and ineffectual - blatant violations are frequently observed.
- Apprehension and prosecution ineffective deterrants to illegal harvesting and marketing.
- Inadequate training of new personnel.

Many of the deficiencies identified were amenable to immediate correction, others are of long term resolution and may require state action at the executive or legislative level. Some conditions could be considered grounds for withdrawal of endorsement in accordance with Manual provisions, but this sanction is not legally available to FDA.

In 1972 an opinion by the FDA Office of General Counsel concluded that withdrawal of a state's endorsement was not founded upon procedures described in the Administrative Procedures Act, U.S.C. 553, and the sanction probably would not be sustained by the courts. Consequently, FDA has not attempted to force correction in this manner since that time. With loss of this sanction, the Food and Drug Administration has no direct regulatory means of assuring state compliance with National Shellfish Sanitation requirements.

In June, 1975, the Agency proposed comprehensive Federal regulations which incorporated all aspects of shellfish sanitation presently covered by the NSSP. Although the federal-state-industry responsibilities were to continue, the proposed regulations provided for direct FDA enforcement actions when a state failed to effect needed corrective measures. During the comment period, the proposed regulations received much publicity and adverse comments. Because there was considerable misunderstanding about

the provisions in the proposal, the Agency is now considering a totally new approach.

The FDA continues to provide comprehensive evaluation and documentation of state programs, but must rely on the integrity and ability of responsible state control authorities to provide adequate programs and enforcement, i.e., voluntary federal compliance. However, when a critical aspect of a shellfish industry operation is found to be in substantial violation of the Federal Food, Drug, and Cosmetic Act or to pose an imminent hazard to the public health, the FDA will consider taking regulatory action under the Act.

We have observed that, over a period of years or even a few months, the status of a state's program may deteriorate from complete compliance with NSSP recommendations to seriously low levels of performance. Causative factors vary but, in general, the problems common to most states are:

- Uninformed administrations and regulations of shellfish control activities to a low priority state program.
- Budget and staffing limitations.
- Administrative difficulties due to multiple agency involvements, decentralization, etc.
- Reorganization, lack of administrative continuity, delayed budgeting and loss of trained staff.
- Inflationary impact and increased competition for available funds.
- Socio-economic pressure against additional closures of valuable productive areas.

Both federal and state shellfish control agencies have always faced difficult financial and technological situations in program administration, and the full measure of public health protection possible under the framework of the NSSP is not always realized. However, it must be recognized that, in spite of these constraints and the ever increasing pollution of our productive estuarine areas by biological, chemical and natural marine contaminants, the consumer has been afforded a high degree of protection throughout the 53-year history of the program. Moreover, the states have substantially met and are meeting their obligation to provide sanitary control over the shellfish industry.

LATEST DEVELOPMENT IN CRAB MEAT MECHANIZATION

Theodore S. Reinke

I would like to tell you a little bit about the machine called "Quik-Pik" and show you some of the mechanical principles. Envision the crankshaft of an automobile with only a 3/16 of a degree offset, being driven at high speed. Originally, this was vibrated at a supersonic level to extract meat from crabs. We had to discontinue this approach, for when we applied vibrations at that supersonic level, we wound up with mush.

The machine itself is the size of a big household refrigerator and weighs about a ton. It is constructed of two units with a gantry unit holding a 25-horsepower motor. The vibration of the machine does not affect the motor which is loosely connected by the rubber adjustment.

We take a crab, clean it with its back removed, turn it upside down, put it in a holding device and shake it. We vibrate it so fast that the meat comes out. This holding device moves up and down, oscillating with a total amplitude of 3/8 of an inch. There are slots in the shaking mechanism and the tray that fits into them. The tray is made with sockets shaped like inverted pyramids. The pyramids are angled so precisely to correlate to the slope of the back of the crab with its shell removed that, no matter what the size of the crab, it fits properly.

So a small crab or a big crab will be held satisfactorily in this device. When loaded, you have about 25 crabs on such a tray. Above, you have a rubber diaphragm. As you get ready to load the crab tray in the slots in the shaking device, this rubber diaphragm automatically sucks in so that the tray slides along with it. After the tray is pushed in, the operator turns on the switch on the wall, with the bag inflating automatically and holding these crab bodies in their respective sockets. The rubber bag compensates for the inequities of the crab, whether a little crab or a big one. After about five seconds to permit full pressure on the rubber bag, the mechanism starts vibrating for about four seconds.

When you try to vibrate something that is approximately 20 inches wide, you have extreme metal fatigue. Apparently the most effective removal technique we have had is a strictly vertical up-and-down movement. We found that we could not build the machinery or equipment to handle many crabs at one time without causing such tension of the metal that we couldn't live with it. Thus the present Quik-Pik uses an orbital rather than a vertical oscillation. The maximum operation we can get and still control the metal was about 4,500 oscillations or vibrations.

We also have a machine which has a strictly vertical motion. But it can only handle one or two crabs at a time because we cannot get a sufficiently broad expanse of metal without having all kinds of structural problems.

The machine Quik-Pik does work. Right now in the plants in Maryland, it is taking off somewhere between 100 and 200 pounds of crabmeat an hour. I think the best rate we have achieved on it is 178 pounds an hour. It does not take off any lump meat. All the meat is of the same grade and is relatively bone-free, almost entirely bone-free when the machine is operated properly.

However, we are facing a number of problems. Some of you are aware of the publicity we got in the spring of 1978. That is the reason this machine is now being operated at three of the plants. We want to put it in the field and bang it around to see what the problems are. We want to correct the problems before we make the machine available to the industry. It will probably be made available to the industry next spring on a franchising and royalty-per-pound basis.

One of the problems is preparing the crab body for the machine. It is a hand operation so far. We have tried to develop some machinery where crabs could be stripped, cleaned and be ready for the machine. I would say we achieved about 80% effectiveness which is too low. You must have above 90 to 95% effectiveness. So we have a hand operation, though not necessarily a skilled one.

We have an even greater problem with bacteria in crab bodies. A sponge crab usually never gets cooked completely because there is an extremely dense collection of half a million eggs at the bottom of the crab. Even cooking at 250°F for 8 to 10 minutes sometimes does

not penetrate it. If that crab body is allowed to lie, squeezed or touched in any way, the juice can run off and contaminate the whole group of crabs. While trying to develop a cleaning machine, we found that you have almost the same problem without having any sponge crabs.

When you start to wash the crabs on a bulk basis trying to minimize manual contact, we do have a situation where one out of ten or twenty has not had a complete bacterial kill. You put the crab in the wrong washer or in the washer where there are a lot of crabs together, and you have contaminated a whole host of crabs.

This is what happened on the older Reinke bobbing machine. We had a long hood, a fast moving brush and a water jet that washed out the cavity. By the time it's run for an hour or two, there is quite a buildup of crud in the interior of the tunnel. Bacteria multiply and every crab going through there can be contaminated.

We are facing up to this problem of potential contamination of crabs in the heat treatment method. After the crab bodies are cleaned, whether by hand or machine, we are running them through steam before they are placed into the Quik-Pik. This does two things: it kills bacteria, thus controlling contamination; and it does make the extraction of the meat simpler because it loosens collagen -- the gluing agent that causes the meat to adhere to the shell, and because it makes the shell softer, thus preventing chipping of the shell.

We are also trying to use dry heat to minimize the addition of moisture in crabmeat during processing. We have a problem with the water content of crabmeat picked by this machine. When you extract crabmeat, you rupture some cells and get a buildup of water. Also the crabmeat tends to peel, looking wetter than it really is. For this reason, we had to give up the spinner machine, one of the machines we had developed in the past.

However, there is another angle we must look at should the use of the Quik-Pik become widespread. Most of the waste from the industry is now being dehydrated in simple dehydration operations and sold as crabmeal. Most of these operations are antiquated, the technology obsolete. But the industry, as it is now, cannot afford to update it with the present market price of crabmeal. And the new Quik-Pik machine will worsen the problem because it actually takes out approximately 15-20%

more meat out of the crab than hand picking. All the meat is taken out of the body cavity, and there is very little protein left to dehydrate. So the industry has to address itself to what it is going to do with the loss of revenue from crab waste.

There are a few chemical companies interested in converting crab shells into chitin and chitosan. Nothing has really happened yet, but possibly something may develop and the chemical industry might convert this waste into a valuable commodity. If this does not happen, however, our industry is shortly going to be in trouble. We are talking about maybe two, three, or four years.

We have one other problem. To stabilize the machine, there are a couple of wings that come out on either side of the vibrating unit. They hit a rubber snubber which abruptly dampens and controls excessive vibration of the machine. And this bumping effect helps the extraction of crabmeat. There is a certain amount of moisture in the machine coming out of the crabs, particularly from crab bodies which have been held in the cooler after cleaning. Moisture condenses on these bodies when brought into ambient room temperature. During the operation of the Quik-Pik, some vapor comes out of the machine. The rubber snubber acts like a bellows and blows the vapor all over the place, spreading possible contamination. So we are now in the process of changing the position of the rubber bumpers for easier disinfection and cleaning.

HEAT SHOCK METHOD OF PREPARATION OF OYSTERS FOR SHUCKING: STEAM TUNNEL METHOD

Clayton L. Rudolph

In Virginia, we have been using the heat shock method of preparing oysters for shucking since about the middle 1960's. We did pilot studies in 1967 and 1968 with the hot dipping method being used widely in North Carolina. Prior to that, a study was conducted in South Carolina on clustered oysters which the shuckers could not shuck cold, and a paper was presented on that particular problem at the Fifth National Shellfish Sanitation Workshop held in November, 1964. Our conclusion in Virginia during that time was that "hot dipping" was a feasible method of shucking oysters, if the packers followed sanitation rules specified by our bureau, and if the processing tanks were kept clean throughout the operation. Since 1968, we have had a few experiments using both the hot dip method and the steam tunnel method of heat shocking oysters. We have arrived at a conclusion that, in Virginia, the steam tunnel method of heat shocking is the better of the two.

In the steam tunnel operation, many methods are used and the tunnels are constructed of various materials; plywood, aluminum, stainless steel, or cast iron, in lengths of 12 to 21 feet. They are either V-shaped on the bottom or square to pass through shell oysters in baskets or in conveyor buckets that have been subjected to steam from a pipe or pipes. Pipes can be galvanized, aluminum, stainless steel, or cast iron. They may or may not be equipped with steam jets that may or may not be fabricated of pipe nipples, perforated pipe or pipes, and have an entrance and an exit with some sort of flaps made of rubber or plastic strips to keep the steam inside. Instead of flaps, the tunnel may have swinging type doors that are forced open by the entrance of a wire basket filled with oysters and then close as the basket goes by.

Steam tunnel temperatures are controlled by thermo-regulators which have a probe located near the center of the tunnel. There is usually a cut-off switch to control the flow rate of the oysters. When the

shucking benches become overloaded with oysters, the steam tunnel temperature drops so that oysters caught in the tunnel do not become overly steamed or subjected to too much heat. The optimum temperature in the tunnel is in the 170°F range, and the oysters are subjected to steam treatment for approximately two minutes. Depending on the length of the tunnel, exhaust hoods or vents are placed at either end or in the middle of the steam tunnel to vent excess steam.

A bacteriological comparison was made between the cold shucked oysters and the steam tunnel shucked oysters. The oysters that were put through the steam tunnel had lower bacterial counts than cold shucked oysters, especially when they were five to seven days old when shucked. It is my opinion that, in Virginia, the steam tunnel type of heat shucking of oysters can be a reliable and economical asset to the packer.

In each and every one of the steam tunnel operations, we find that there is a cleanability problem within the steam tunnel. All steam tunnels should be made with a bottom-hinged opening so that the inside of the tunnel can be hosed down and cleaned out on a daily basis. Pieces of shell, mud, mussels and other extraneous material that build up in the tunnel turn septic within one day if not cleaned up properly. The steam tunnel operations we observed had no cleanability possibilities.

It is recommended that clean oyster shells be put through the steam tunnel rather than the muddy shellstock going through at the present time, because live oysters feed on the mud and the condensed steam when forced open.

Another recommendation is to ice down the shucking buckets in which the oysters, warmed from the passage through the steam tunnel, are shucked. This cooling, in addition to the normal pre-cooling operation, will greatly improve the overall bacteriological picture. I also think that the steel-belted conveyor is probably the best conveyor because, with it, the amount of oysters going onto the shucking table can be controlled and no one oyster will be caught in the steam tunnel and exposed to excessive heat.

It has been observed that, of the shell oysters in the wire basket proceeding through the steam tunnel, the ones in the middle of the pack do not receive their due amount of steam heat as do the ones on the outer perimeters. A new basket or conveyor bucket should be designed to

alleviate this problem. It has also been observed that, due to the moisture in the tunnel, the lubricant used on the monorails or conveyance system sometimes drops off and falls into the now gaping oysters. A special lubricant such as Thermolube -- a versatile, white lubricant approved for food service -- is recommended for use throughout the conveyance system to prevent oil dropping.

MODELING THE EFFECTS OF STORM WATER RUNOFF ON WATER QUALITY

V.O. Shanholtz, M.D. Smolen and B.B. Ross

I. Introduction

Mathematical Modeling

Modeling is a technique for analyzing large complicated systems by constructing smaller systems that reflect only the characteristics of interest. With the rapid development of computers and computer technology during the past 20 years, it has become possible to model the hydrologic processes of a watershed as a series of interacting equations inside the computer. Mathematical watershed models have generally been developed using one of two approaches: the 'systems' approach and the 'parametric' approach. In the systems approach the watershed is considered to be a black box in which rainfall input and streamflow output are related only through decision theory, systems analysis or operations research methods that need have no direct relation to the physical watershed system. In the parametric approach, on the other hand, the rainfall input is normally operated on by empirical or deterministic relationships that attempt to describe specific physical processes such as infiltration, evaporation, overland flow, and channel flow. Thus each process is related to the streamflow by some function of the related watershed parameter.

In parametric modeling, a conceptual framework (road map of interactions) is first developed to link all the known significant system components. The components then are described (modeled) by either theoretical or empirical equations. In this way the individual parameters, at least in concept, have physical meaning and can be studied by laboratory and/or field experiments.

Recent concern for environmental pollution has provided the impetus for using modeling techniques to relate land use practices to downstream water quality. Since pollutant transport is predominantly a hydrologic

process, there has been a major effort to interface material transport submodels with existing parametric hydrologic models. This effort has brought to light an awareness that the values assigned to hydrologic parameters of a watershed vary widely within a single watershed. Thus, it is necessary to consider the spatial characteristics of the watershed such as soil boundaries, local topography, and land use, to account for the resulting water quality.

Nonpoint Pollution

Legally, sources of pollution are classified as point source--those originating from a pipe or clearly defined overflow, or as nonpoint, which includes all other sources that cannot be precisely located. During the initial phases of cleaning up our nation's waters, efforts focused on controlling point sources. As point source pollution decreased, it became apparent that runoff from land areas, nonpoint pollution, was responsible for much of the existing poor water quality. Among the many nonpoint pollutants are sediment, pesticides, nutrients, microbes, heat, radioactivity, salinity, heavy metals, and acid. Many of these originate in both the urban and rural environments through land-based activity such as transportation, construction, cropping, timber harvesting, mining, and waste disposal. The one aspect common to all nonpoint pollution is that it is primarily transported to streams and estuaries by the runoff process.

Since nonpoint pollution is tied so closely to the hydrologic system, any effort toward controlling it must consider the soil-water interface and the hydrologic transport system. Here hydrologic modeling can have its greatest benefit. The hydrologic model can be used to reduce the very complex natural watershed system to a more easily observable system. The model user could then create a rational plan of attack for abatement of nonpoint pollution. First, it would be possible to isolate areas of the watershed where critical pollutional potential exists. Then the transport system could be examined to determine whether pollution arising at the specified location reaches the receiving stream, lake or estuary or is deposited or transformed en route. Finally, the modeler could test pollution abatement schemes to be sure that the public receives the best possible pollution abatement for each dollar spent.

Department of Agricultural Engineering Program

The Department of Agricultural Engineering hydrology program was initiated in the late 1950's as a contributing part of a Southern Regional Research effort (S-53) to identify and classify factors affecting runoff from upland agricultural watersheds. This program was concluded in 1975 and a new Southern Regional Research effort was initiated to develop hydrologic and water quality models for upland watersheds (S-108). The data base accumulated by the S-53 project was to be used extensively in the S-108 effort. An Environmental Quality program was initiated at the Southern Piedmont Research and Continuing Education Center in support of S-108.

Our modeling efforts have dealt solely with upland areas with complex landuse. Our primary objective has been keyed to the development of modeling techniques to predict the quantity and/or quality of water entering receiving streams from upland agricultural lands. We have not been involved with modeling in stream processes. The modeling concept is readily adaptable to the inclusion of such processes, but monetary and manpower constraints have simply prevented any effort in this direction at this time.

This discussion, therefore, will focus on what we have accomplished and where we plan to go from here. Hopefully, this will provide some insight into current concepts of modeling with the vision of how they can best be used as a tool to analyze water related problems that involve agriculture.

II. Some Approaches to Nutrient and Sediment Modeling

Soil loss and the resulting production of sediment has been one of the greatest concerns of agriculture. The loss of agricultural soil constitutes not only the loss of a portion of our nation's resources, but also a major pollutant in waterways and estuaries. This sediment carries with it many of the nutritive and toxic chemicals which may upset the balance of downstream ecosystmes.

One of the most widely used models to estimate soil loss from agricultural fields is the Universal Soil Loss Equation (Wischmeier and Smith, 1961). This is a simple empirical equation derived from statistical techniques that relate rainfall intensity, cropping

practice, slope, slope length, and soil erodability to the annual soil loss per unit area. The model was primarily developed for use in designing conservation cropping practices to keep agricultural soils in the field. Since this empirical relation is based on data from all parts of the country, includes many crop-years, and is easy to use, it is recommended for assessing the extent of nonpoint pollution (McElroy, et al., 1976). However, the soil loss equation does not predict how much soil reaches streams or rivers. Therefore, there is no known mathematical relationship to provide the proper linkage between USLE predictions and actual sediment yields.

More recent efforts have adapted the USLE to run as part of a parametric hydrologic model (e.g., Negev, 1967; Crawford and Donigian, 1973, 1976). For this purpose the USLE was adapted to predict soil loss from individual storm events. The USLE was changed so that soil loss is a function of runoff rather than rainfall intensity. The problem remains, however; the relationship between soil loss and sediment yield must be obtained by calibrating or guessing. In a modified universal soil loss equation model, Williams (1975) incorporated a deposition function based on particle size and peak flow rate. This modification provides an improved means of predicting transport of soil particles from fields through the stream system. By considering particle size, Williams model allows the model user to look at the smaller, clay particles which may be enriched with nutrients or pesticides.

At the more detailed level, several workers have developed sediment transport models which consider the erosion-deposition relations as a function of surface flow conditions. A sediment model developed by Smith (1976) determines soil detachment as a function of overland flow velocity, and transport and deposition are related to the total transport capacity and unit stream power. By a similar procedure, Kuh and Reddell (1977) and Beasely, et al. (1977) developed two-dimensional sediment transport models. All three of these sediment models have been shown effective in predicting the relationship between areas of high erosion and the resulting sediment load to streams. In addition, the two-dimensional erosion deposition models reflect unique characteristics of sub-watershed areas. This spatial responsiveness, however, is achieved at excessive costs of computer time and storage capacity.

In the VPI & SU Agricultural Engineering Department, we are using similar equations for predicting sediment detachment and transport, but the use of Finite Element numerical techniques allows considerable savings in computer time and storage capacity. At the same time, the Finite Element approach provides a more exact spatial representation of subwatershed areas. Further discussion of the Finite Element modeling procedures with application to stormwater and sediment yield prediction on ungaged areas will be presented in a later section of this report.

Chemical transport modeling is considerably more complex than sediment transport modeling. The chemicals of greatest concern from agricultural areas are nitrogen, phosphorus, herbicides, and insecticides. An appropriate model must consider temporal variation in chemical sources as well as spatial variations. This is important because agricultural chemicals are applied to fields on a seasonal basis or in response to specific emergency problems. Also decomposition and interconversion of chemical species is highly dependent on the conditions that affect biological activity: temperature, moisture content, availability of oxygen, and the presence of organic energy sources.

Conceptual models for nutrient loss (Frere, 1975) and pesticide loss (Bailey, et al., 1974 and Bruce, et al., 1975) have been proposed. Application of these models to a nonhomogenous watershed requires the use of a hydrologic model that is spatially responsive. Clearly the same biological and physical effects are not active at all locations in a watershed. The model must be capable of distinguishing changes in chemical availability that occur in both well drained and poorly drained field areas, in marshy areas, under trees or in open sunlight. The Finite Element Hydrologic model with which we are working has the capability of incorporating these conceptual models such that different locations would respond uniquely. The effluent water quality would thus be a composite of runoff from the distinct subareas of the watershed. In-stream processes can also be incorporated to reflect the water quality changes as runoff from one subarea passes over or through another where different conditions exist.

At present, we are only beginning to develop the rate equations and coefficients to adapt the biochemical submodels to the Finite Element Hydrologic Model. Considerable effort will be focused on this aspect for the next three years.

III. A Spatially Responsive Model for Upland Watersheds

Considerable progress has been made in the Department of Agricultural Engineering, VPI & SU, toward the development of a Finite Element Spatially Responsive Storm Hydrograph Model (FESRSHM). This model has shown promise for predicting storm runoff from ungaged watersheds (Ross, et al., 1978; Ross, 1978), simulating the effect of landuse change on flood flows of the South River (Ross, et al., 1976) and tracing flows from subwatersheds areas (Smolen, et al., 1977).

The most significant advantage of this concept is that many aspects of the natural watershed system can be incorporated to answer specific questions about the area's response to perturbation. Thus, the system properly constructed can be used with data at varying levels of resolution and can be used to analyze the water quantity and/or quality response from single farm units, entire basins, or the effect of single farm units on a larger watershed when the area is subjected to a given rainfall distribution.

The finite element numerical method was used as the basis for the development of a spatially responsive model structure. A fundamental concept in finite element analysis is that most complex systems can be sub-divided to form some minimal number of subsets, which can be analyzed independently and the results collected to form the total system response. This concept provides a ready mechanism for routing surface flow, provided that a reliable procedure can be developed to predict rainfall excess and to predict reinfiltration as flows move across subsequent elements.

The above procedure provides for tremendous flexibility for the incorporation of data for the purpose of generating synthetic hydrographs. All available data can be incorporated so that the area's spatiotemporal integrity can be maintained. A new model structure is not necessary to do either micro- or macro-level modeling.

Spatial Variability

Spatial variability is incorporated into the FESRSHM to improve estimates of the spatiotemporal variations inherent in rainfall excess and the time distribution of runoff. Two discretization structures are

required to represent the spatial and temporal distribution of rainfall excess and the routing of the excess water through the drainage system.

Rainfall Excess

A procedure was designed by Li (1975) and Li, et al. (1977) to improve estimates of rainfall excess by considering the spatial variability in soils and the spatiotemporal variability of landuse. Basically the area is subdivided into unique combinations (hydrologic response units) based on soil mapping units and landuse data, with rainfall excess being calculated for each response unit by a soil moisture algorithm. An empirical approximation was used to describe infiltration in lieu of the partial differential equations of vertical unsaturated flow. The primary component of the soil moisture model was the simulation of infiltration with the Holtan equation (Holtan, 1961).

The procedure for describing hydrologic response units (HRUS) within a given watershed is illustrated by Figures 1, 2 and 3. Figures 1 and 2 represent typical soil and landuse maps, respectively. These maps, after proper scale registration, are combined to form the HRU map (Section A-B, Figure 3).

It becomes readily apparent that a grouping such as shown in Figure 3 can result in a large data matrix. The maximum number of unique combinations is equal to the product of the number of soil mapping units (Figure 1) and the number of landuse categories (Figure 2). The actual number of HRUS, however, can be much larger because a given HRU can occur at several locations within the drainage area.

Flow Routing

To provide the best spatiotemporal representation of flow by the finite element method, the watershed was subdivided into finite-sized elements where each was a function of those watershed physical factors that affect flow timing such as roughness and slope. The procedure is briefly described in the following section.

A watershed consists of many sub-sheds unless a field or unit source drainage system is being analyzed. To maintain flexibility, the model structure was designed so that a drainage system with multistream orders could be routinely analyzed. Since the one dimensional approximation is assumed and the concept of line elements results, element boundaries can be

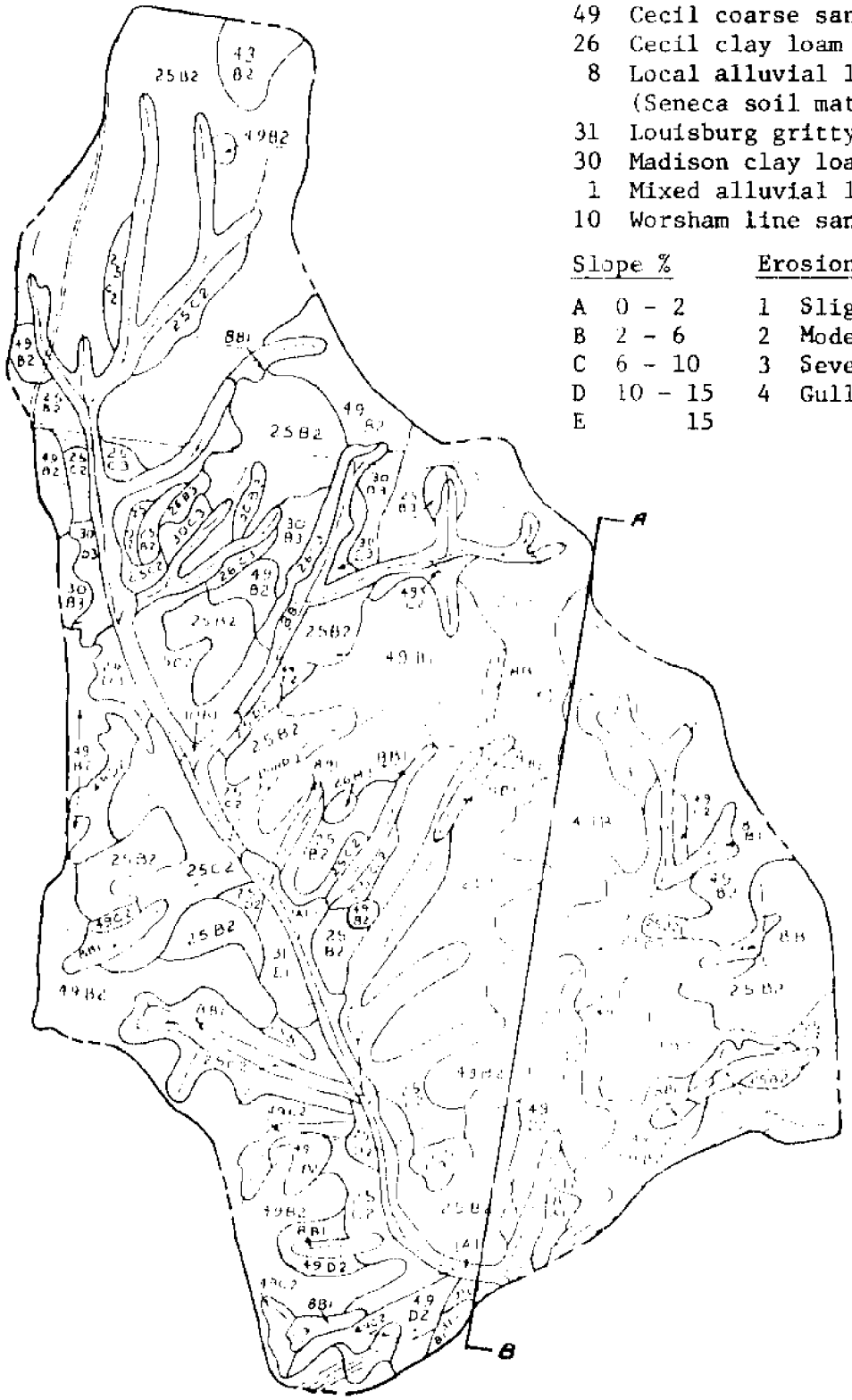

Figure 1. Soils map for Rocky Run Branch Watershed, Brunswick County, Virginia

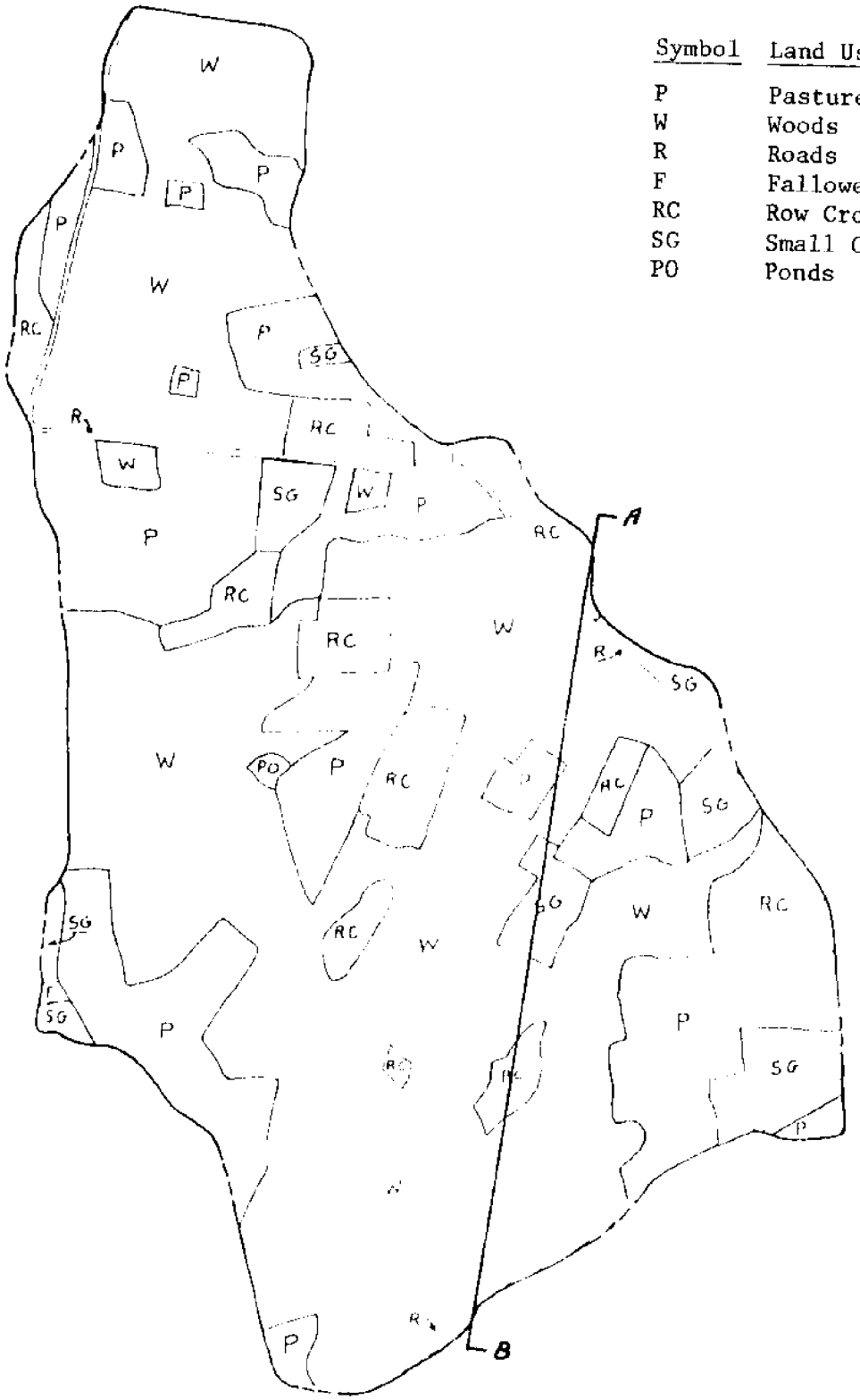

Figure 2. Landuse map for Rocky Run Branch Watershed, Brunswick County, Virginia

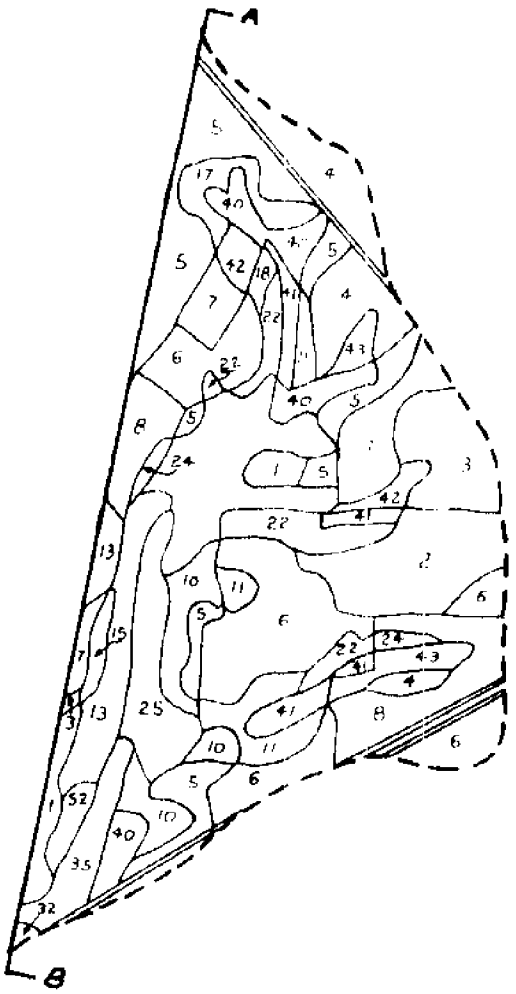

Figure 3. Partial hydrologic response unit map for Rocky Run Branch Watershed, Brunswick County, Virginia

irregular. This is permissible because element areas are employed solely for the purpose of obtaining lateral inflow into the elements. With this flexibility, the element boundaries can be established so that they conform to the natural topographic boundaries. Sub-sheds (tributary drainage systems) can be easily identified from a contour map. These sub-sheds can also be separated into unit source drainage areas (strips) and the strips then can be subdivided into finite-sized elements. A typical example is given by Figure 4.

The division of unit source areas or strips into elements provides additional flexibility for incorporating spatial variation (Note that without subdivision a strip is also defined as an element). After the element structure has been refined to represent the topographic system, the modeler has the option to refine the discretization of elements to the configuration that will provide the best solution to his problem. For example, if the modeler must determine the fate of nutrient applications on a particular crop, then the crop must be isolated to form one or more elements so that flows emanating from that point can be separated from those originating from adjacent areas.

Creating a Spatially Responsive Data Base

The first prerequisite to the application of a spatially responsive modeling system requires the creation of a data base that will adequately represent the spatiotemporal character of the area being investigated. A number of watershed characteristics vary spatiotemporally and their variation must be described for proper evaluation of the impact of each on the problem being addressed.

The distribution of landuse is particularily important since the proximity of a given cropping system to well defined drainage channels will play a significant role in the magnitude of sediment and nutrients entering the receiving stream. The orientation of agricultural cropping systems with respect to slope and drainage channels can significantly alter the area's hydrologic response. This is particularily true for small storm events. In general, the effect of landuse change on storm water runoff tends to decrease as storm size increases.

Surface retention storage varies significantly from one area to another. Values exceeding 1.5 inches may not be uncommon. The effect

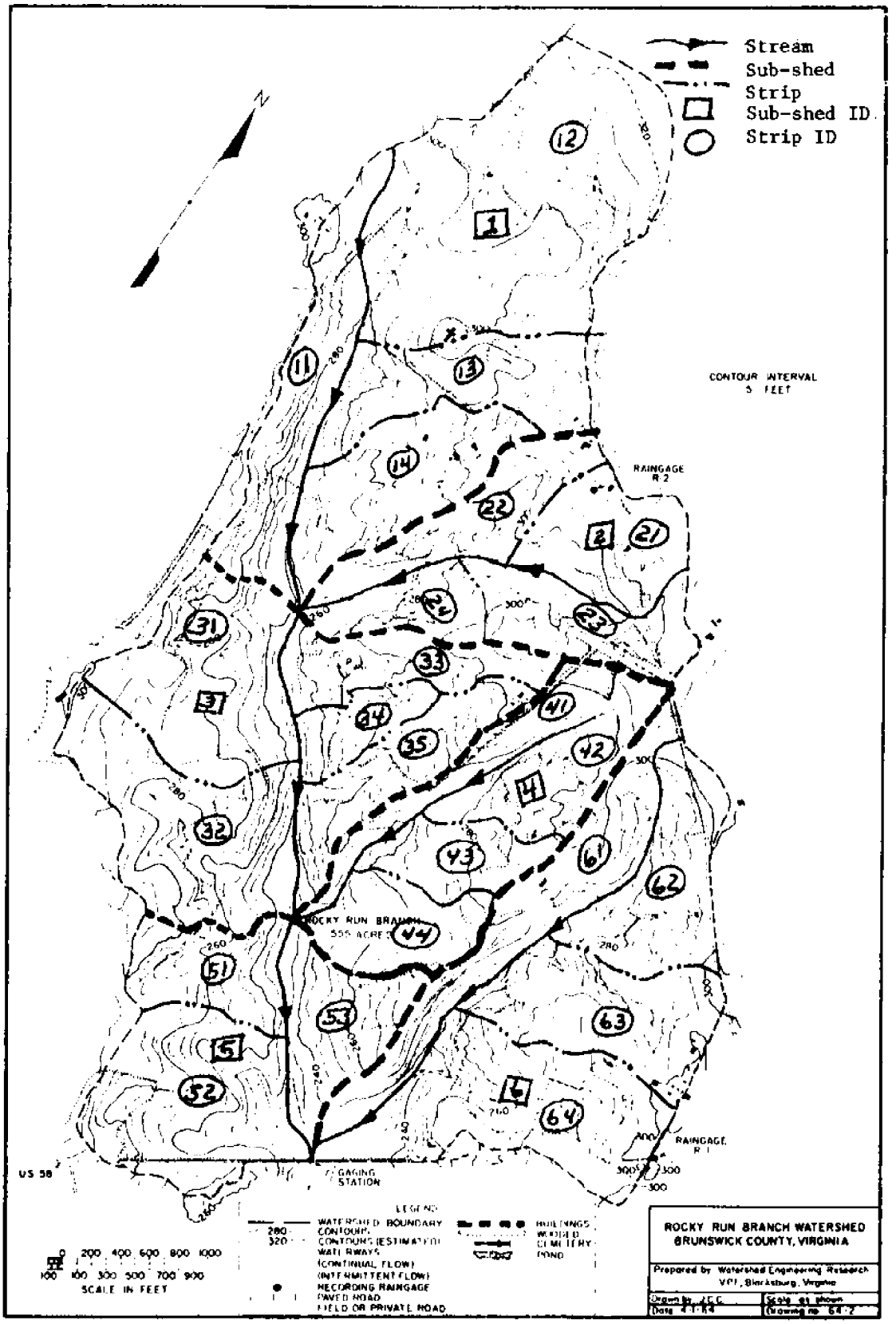

Figure 4. Rocky Run Branch Watershed, Brunswick County, Virginia

of such areas on storm water runoff and the associated impact on the movement and concentration of agricultural nutrients can be significant.

Soils vary greatly in their hydraulic characteristics, therefore their hydrologic response often varies greatly. These variations are tempered somewhat by soil cover conditions, landuse patterns and management practices. Wet land areas, localized channelization, drainage projects, farm ponds, etc., can all impact the quantity and quality of water moved from a specific area.

The preparation of a data base to include spatiotemporal variations is perhaps the most time-consuming and often challenging task that confronts a modeler (user). As previously noted, the FESRSHM is structured to provide the user with a high degree of flexibility for the inclusion of spatial detail to provide answers to a specific question. The major task that faces the user, however, is deciding on the level of discretization (i.e. spatial variability) that must be included to get a reliable simulation at the desired level of predictability (accuracy).

The problem is complicated because of the general lack of objective criteria to define precisely what detail is necessary for most applications. We have not, unfortunately, been able to define an exact line of demarcation. No single set of criteria appears to be usable. Rather, the separation point is a function of an incredibly complex set of factors, which not only includes physical properties of the drainage system but economic constraints. The reader is referred to Li, et al. (1977), Ross, et al. (1978) and Ross (1978) for additional detail on discretization and the effect of different levels of aggregation on simulation.

IV. Application to Upland Watershed

Predicting Stormwater Runoff

To illustrate the use of the FESRSHM in simulating stormwater runoff from natural watersheds, three experimental watersheds with complex landuse were selected that range in size from 183 to 1058 acres. Streamflow measurements were obtained with continuous water level recorders. The control section was a Virginia V-notch weir located in an existing highway culvert (Burford and Lillard, 1963). At least two continuous recording raingages were located within the boundaries of all watersheds. Detailed soils, landuse and topographic information was collected on all areas. Additional detail is given by Wilson, et al. (1975).

The data matrices for spatially varying watershed characteristics were created for each watershed following guidelines given by Ross, et al. (1978). These data coupled with appropriate rainfall distributions were used to obtain the results given in Figures 5-8. These comparisons represent flood flow conditions. Thiessen weighted rainfall was plotted for the sake of simplicity, although the actual distributions were used in all simulations.

The ungaged context (that is, no parameter optimization or data manipulations were performed to obtain near perfect flow matches) was used for all simulations. The results are considered by the authors to be good to excellent, particularily since they were obtained by simply compiling the data base and executing the model. The underestimate of the second peak in Figure 6 is attributed to a poor representation of the magnitude and distribution of rainfall in the upper reach of the watershed. Both recording raingages were located on a line parallel with the watershed outlet. Therefore no measure of actual rainfall was available for Pony Mountain (upper reach watershed).

All comparisons could be improved somewhat by optimization of specific model parameters. However, with inherent errors in the input data matrix of unknown magnitude due to variations in soil boundaries, hydraulic properties, etc. and an average error of approximately plus or minus five percent for excellent quality streamflow records, such an exercise would appear purely academic. The reader is referred to Ross (1978) for many more comparisons.

Predicting Wash Load

The potential use of the FESRSHM as a tool to predict sediment from upland areas (wash load) is presented by a numerical example utilizing an approach suggested by Beasley, et al. (1977). Detachment by raindrop impact is assumed to be a function of cropping management factor (C), soil erosivity factor (K), area (A) and the square of rainfall intensity (I). The detachment of sediment due to overland flow was assumed to be a direct function of C, K, A, slope (S) and lateral flow (q). The total soil available for transport at any given time was assumed to be the sum of these two detachment processes. The soil transported by overland flow is determined as the difference between the flow's transport capacity and the soil available for transport from the previous computations.

The procedure is illustrated by computing the wash load from Powells Creek Watershed for storm event 5-31-62. The appropriate C and K values for each landuse and soil type were determined from Soil Conservation Handbook for Virginia (SCS, 1973). The simulated discharge hydrograph and sediment graph at the watershed outlet for these conditions is shown in Figure 9. The peak sediment concentration reached 1087 mg/l. This is considered a reasonable estimate since 8 percent of this watershed was in corn.

Management Effects

Three examples are given to illustrate how management alternatives may be evaluated. The first example is changing the tillage practice of corn production from conventional turn plow to no-tillage. The second example demonstrates how a small area when changed from wooded to fallow can significantly alter the sediment yield. Finally, a typical contour strip crop sequence of corn and hay demonstrates the effectiveness of this management practice to reduce sediment loss.

Tillage Practice

All areas in which corn was grown following the conventional tillage practice were changed to no-tillage. The results are also shown in Figure 9 and represent a significant reduction in the sediment load.

Wooded area changed to fallow

The effect of clearing a wooded strip for cultivation is illustrated in Figure 10. The peak sediment concentration (2360 mg/l) and volume were increased by 129 percent and 109 percent, respectively. Flow characteristics were only slightly changed. However, a significant effect was noted in the wash load despite an areal change that represented only 8 percent of the watershed.

Strip Crop Sequence of Corn and Hay

A typical strip crop agricultural practice and the discretization to properly define the elements in the overland flow strip is given in Figure 11. A series of land slopes were selected to represent a recommended farming practice for minimizing soil loss when row crops are grown on hilly terrain. For the purpose of this example, a rectangular flow strip sub-divided into equal-sized elements was assumed. A hypothetical

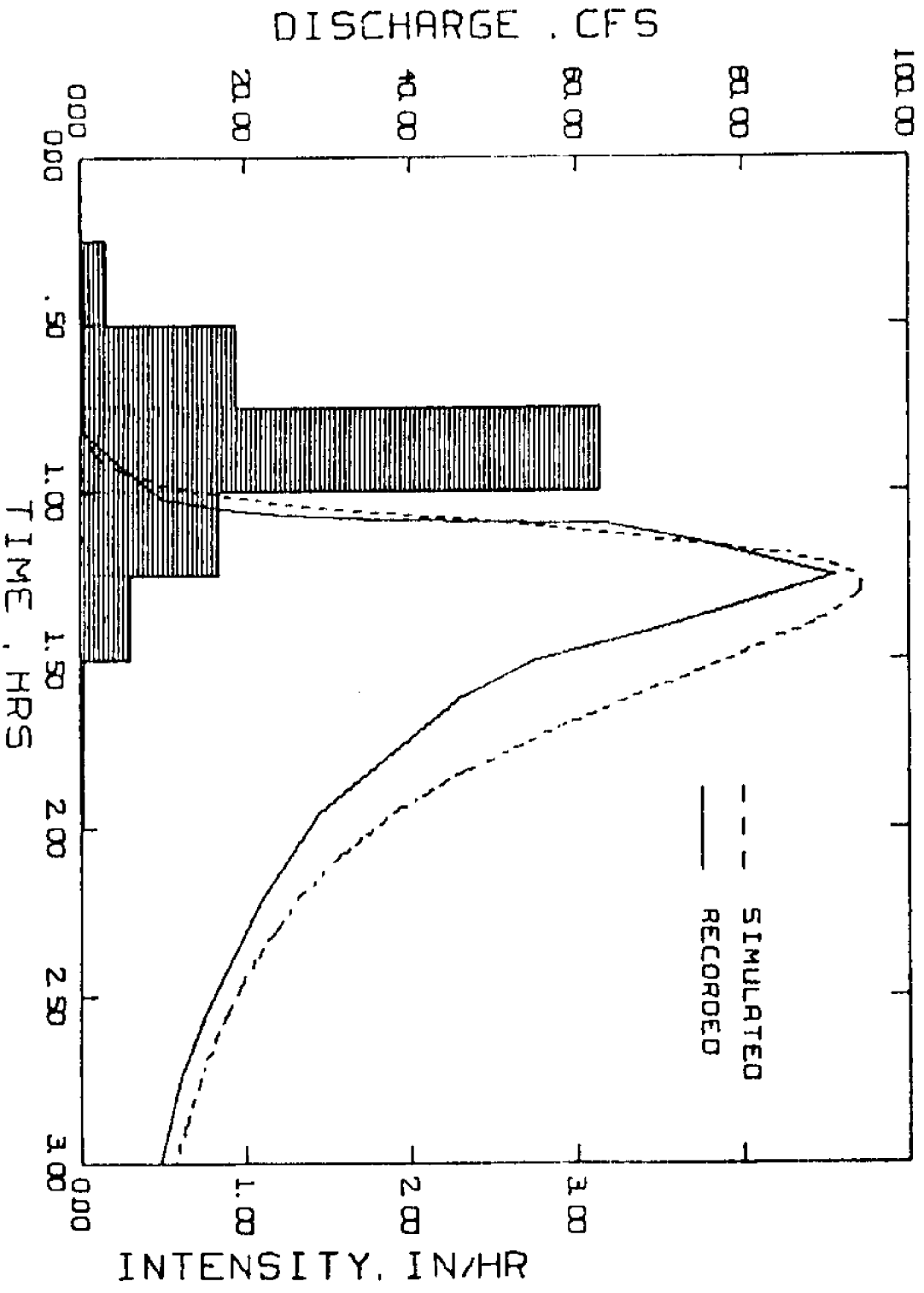

Figure 5. Comparison of simulated and recorded discharge for storm 6-24-58 Pony Mountain Watershed, Culpepper County, Virginia

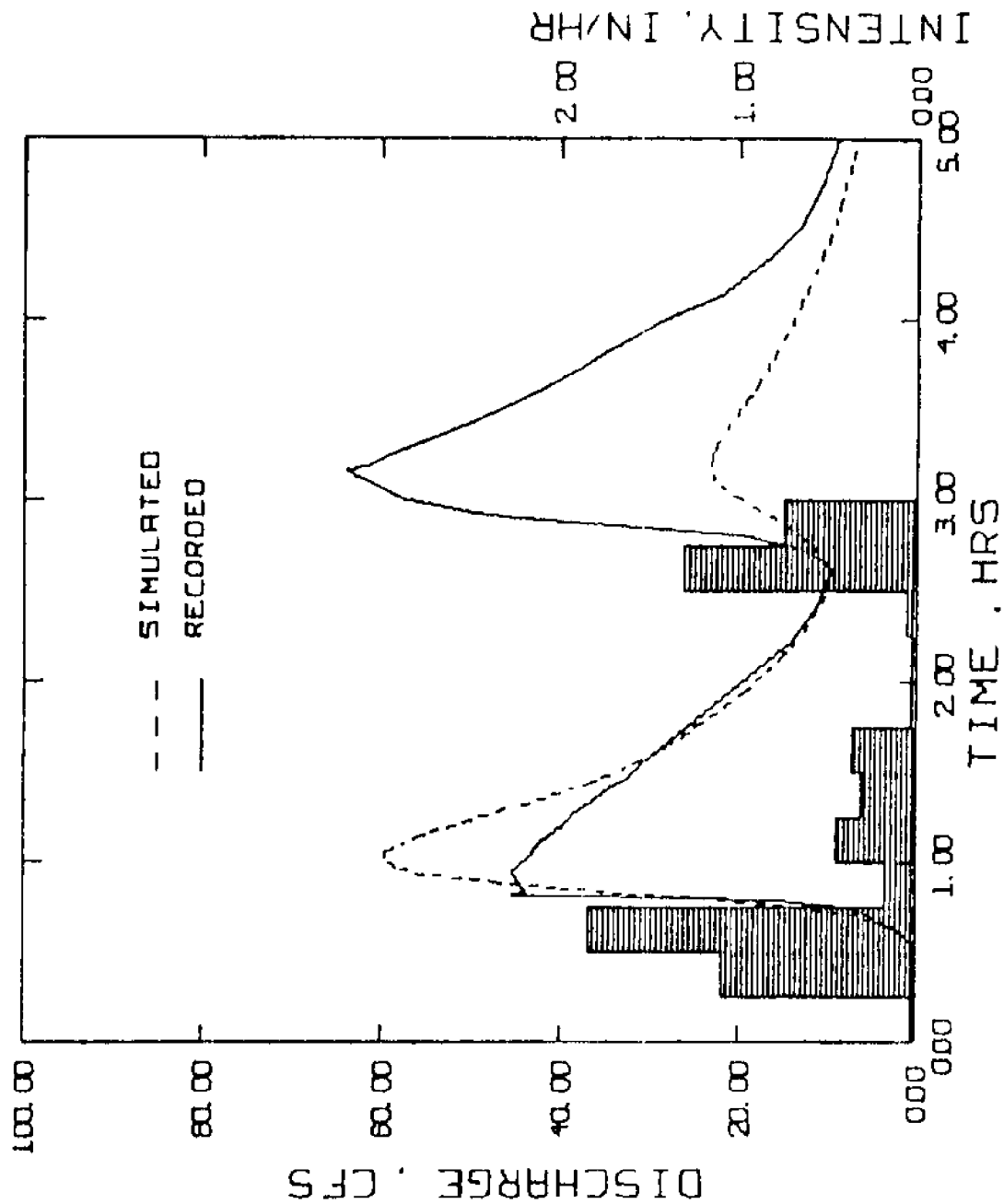

Figure 6. Comparison of simulated and recorded discharge for storm 9-19-60 Pony Mountain Branch Watershed, Culpepper County, Virginia

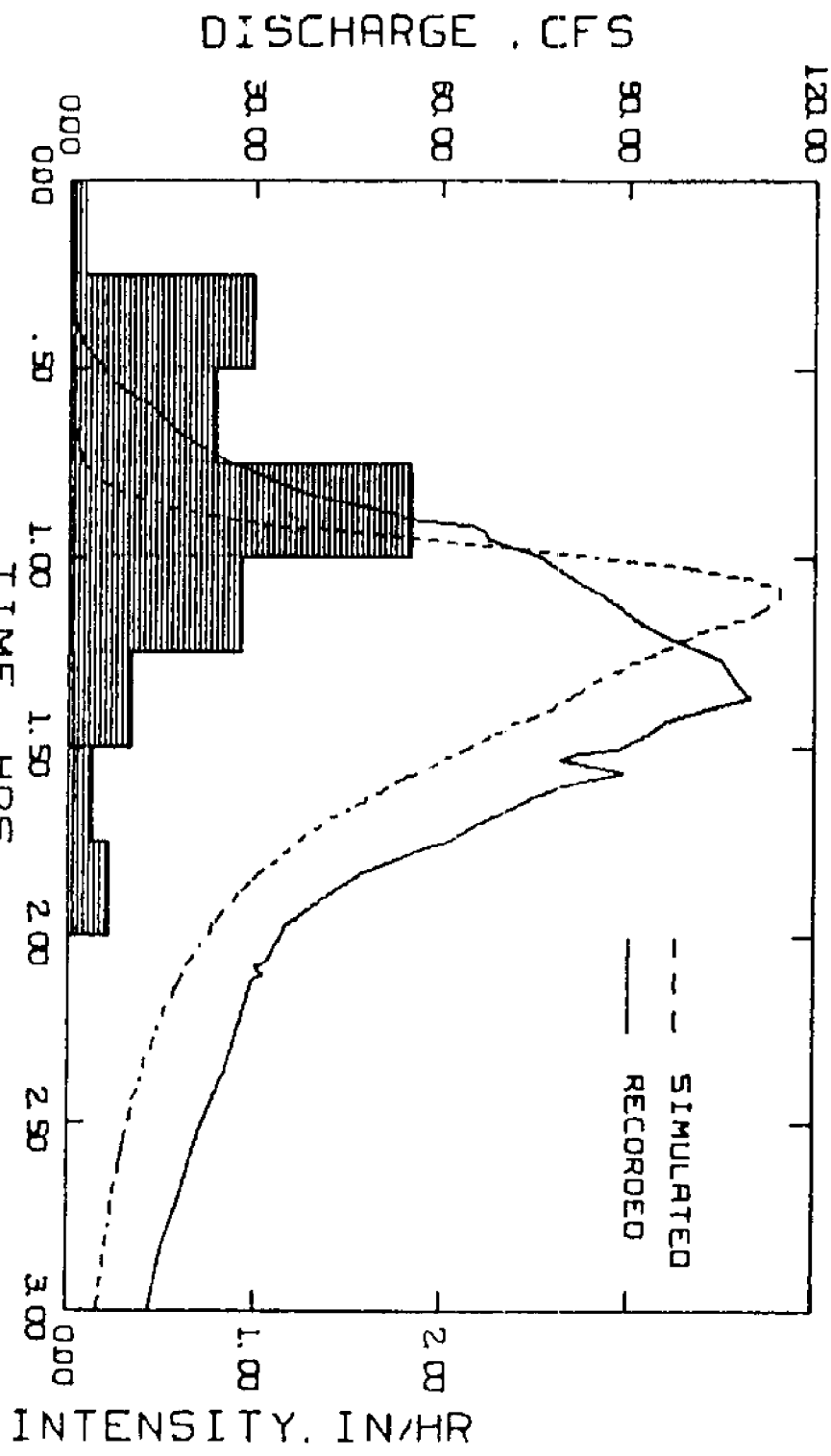

Figure 7. Comparison of simulated and recorded discharge for storm 10-10-59 Powells Creek Watershed, Halifax County, Virginia

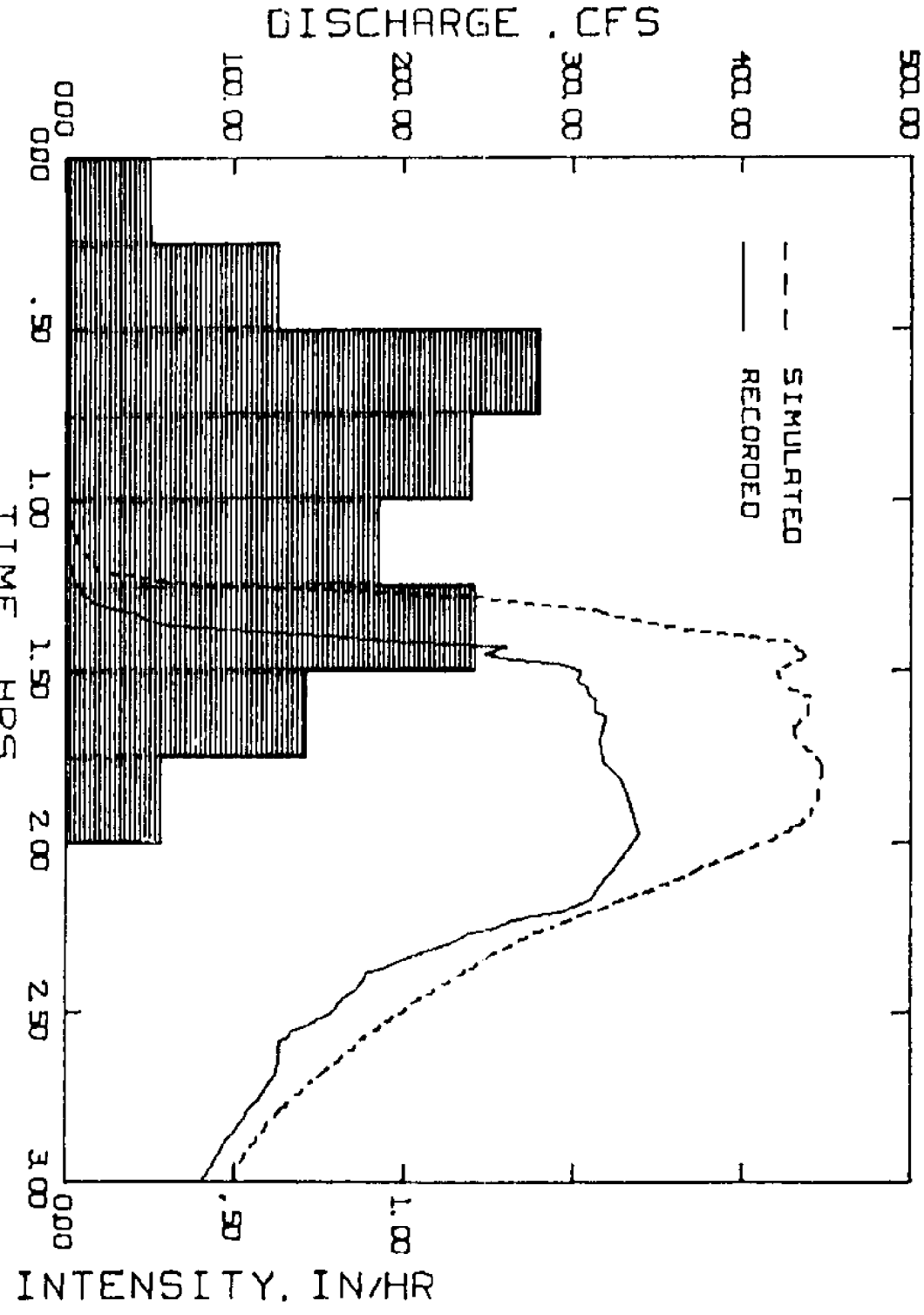

Figure 8. Comparison of simulated and recorded discharge for storm 8-4-74 Chestnut Branch Watershed, Bedford County, Virginia

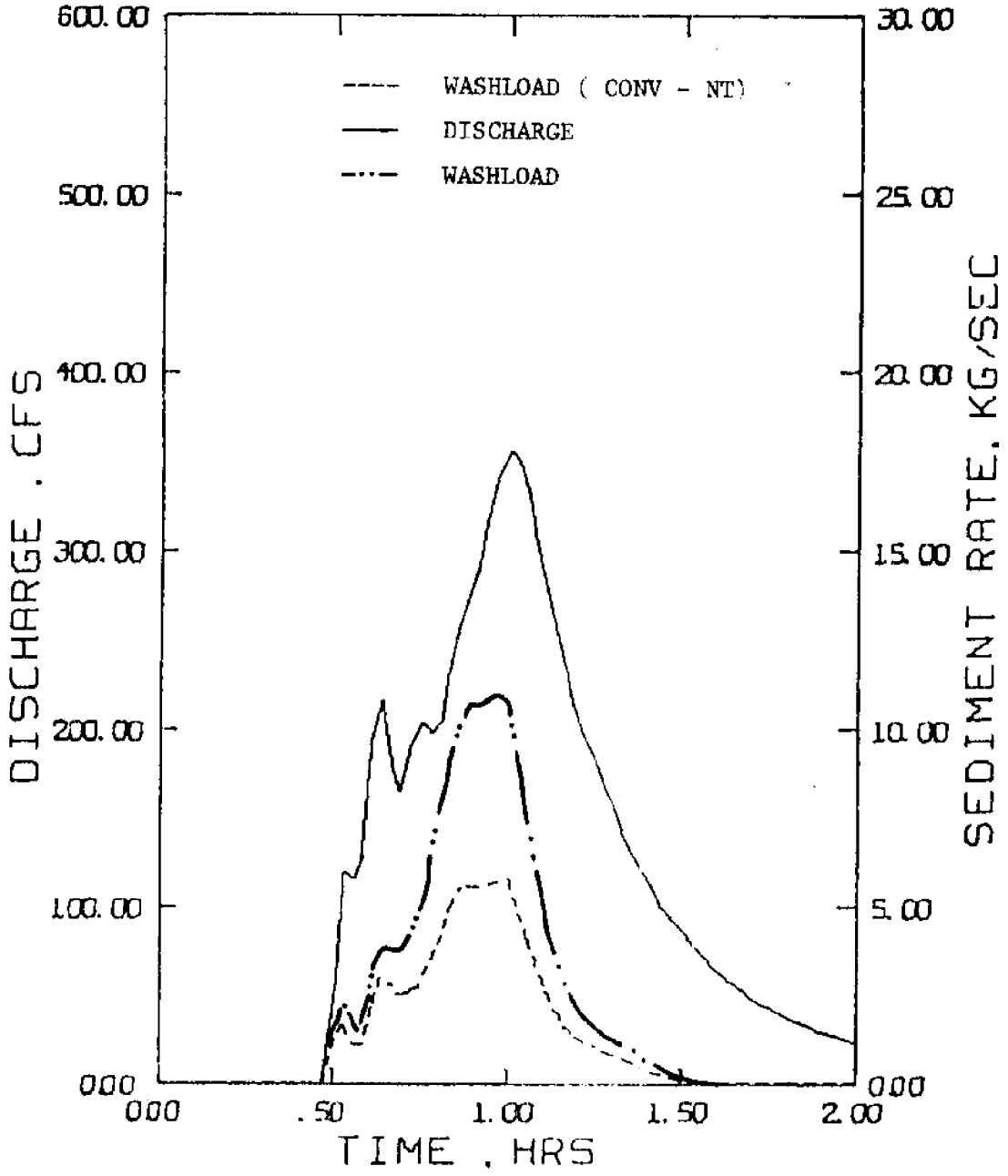

Figure 9. Prediction of wash load from Powells Creek Watershed, Halifax County, Virginia, when 8% of the area changed from conventional to non-tillage corn production

storm of 1-inch per hour for a duration of two hours was applied and the FESRSHM was used to obtain the results given in Figure 11. The sequence of erosion and deposition is given for each landuse. A total of 193 tons of soil from an area of 57.5 acres entered the stream.

An alternative land management practice was illustrated by reversing the cropping pattern in the last two strips. The total sediment entering the stream was reduced by 66 tons due to this change. Despite the absence of recorded data to verify the accuracy of the wash load predictions, the values certainly appear reasonable.

V. Summary

Recent concern for environmental pollution has provided the impetus for using modeling techniques to relate landuse practices to downstream water quality. Since pollutant transport is predominantly a hydrologic process, a major effort has focused on interfacing material transport sub-models to existing parametric models. This effort has brought to light an awareness that the values assigned to hydrologic parameters of a watershed can vary widely within a drainage area. Thus, it is necessary to consider the spatial characteristics of the watershed. The rapid development of computer technology and data acquisition systems has made possible a more detailed consideration of the spatiotemporal variations of such factors as soil boundaries, local topography, landuse and rainfall.

Several approaches to nutrient and sediment modeling were summarized. These varied from sophisticated nutrient balance models to simple loading function techniques.

Considerable progress has been made in the Department of Agricultural Engineering to develop a spatially responsive hydrologic model for use in all phases of agricultural soil and water management. The most significant advantage of this concept is that many aspects of the natural watershed system can be incorporated to answer specific questions about a watershed's response to some man-made change. In addition, all available data can be utilized to its fullest advantage to insure that the spatiotemporal integrity of the system is maintained. A new modeling structure is not required to do either micro or macro level modeling.

The spatially responsive hydrologic model was developed for the purpose of providing reliable predictions of storm water discharge on ungaged

watersheds. Several examples were presented and these results were considered to be good to excellent, particularily when considered in the ungaged context. Additional examples for other watersheds located in Virginia are given by Ross (1978). The water quantity model is not a static system and will be modified as the state of the art for the description of spatial variability is improved.

Utilizing concepts developed by Beasley, et al. (1977), several examples were given to illustrate the use of the model for predicting wash load from upland watersheds. The model was used to demonstrate how several management practices effected the wash load that entered the stream. The peak concentration of sediment was reduced from 1087 mg/l to 577 mg/l when 8 percent of the watershed area was changed from conventional tillage to the no-tillage method of corn production. The clearing of an area covering only 8 percent of the total watershed area of 183 acres increased the peak sediment concentration from 1087 mg/l to 2360 mg/l. The placement of a grass strip in place of corn at the bottom strip (at stream channel) of a 57.5 acre strip cropped slope reduced the volume of erosion entering the stream by 66 tons for a hypothetical storm with a duration of 2 hours and an intensity of 1-inch per hour.

The model, to date, has not been adapted to nutrient transport, nor has the sediment detachment and transport process received a rigorous treatment. During the next two to three years, both nutrient transport and sediment modeling will receive high priority in our research program.

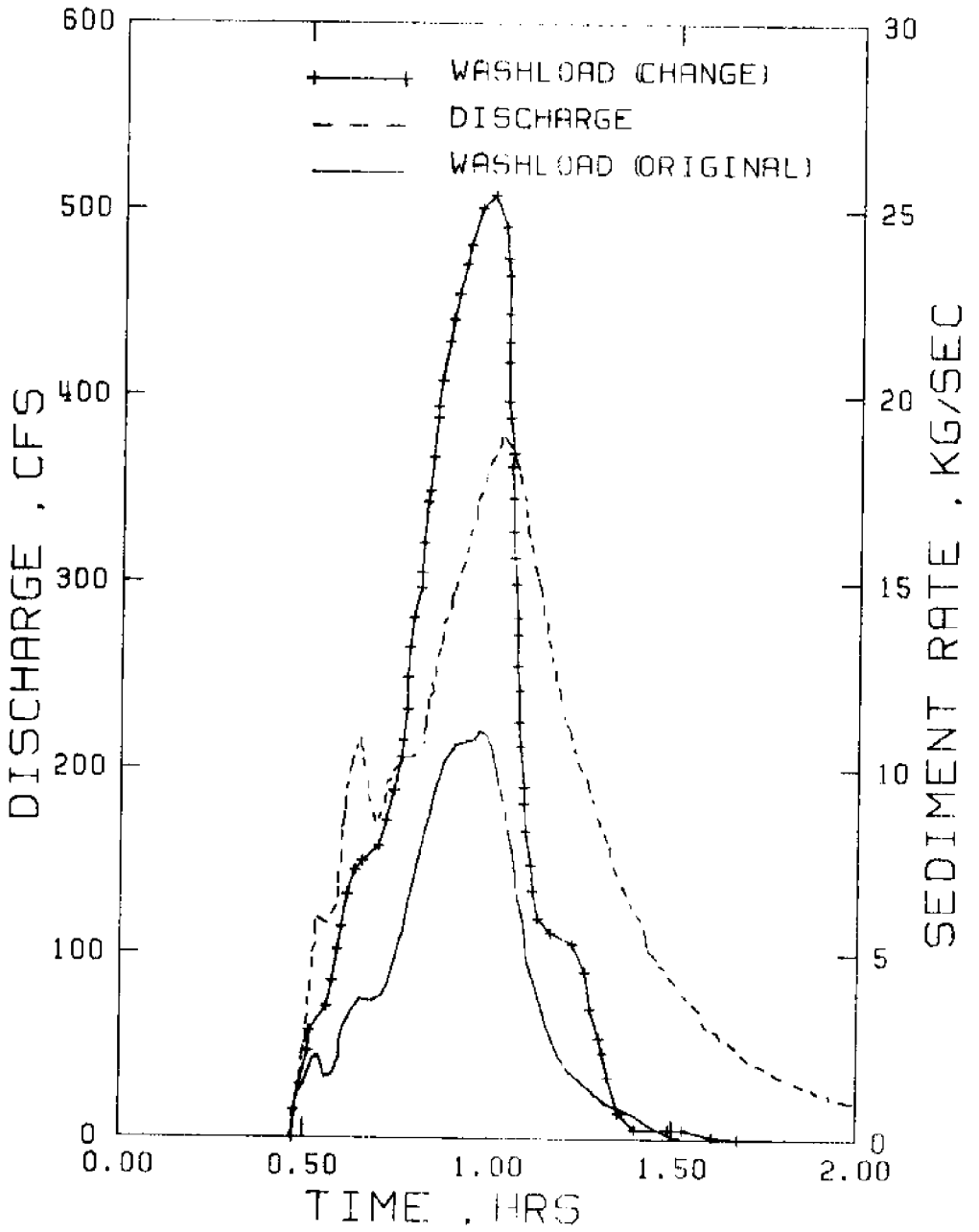

Figure 10. Predicted sediment yield when wooded area cleared to fallow condition

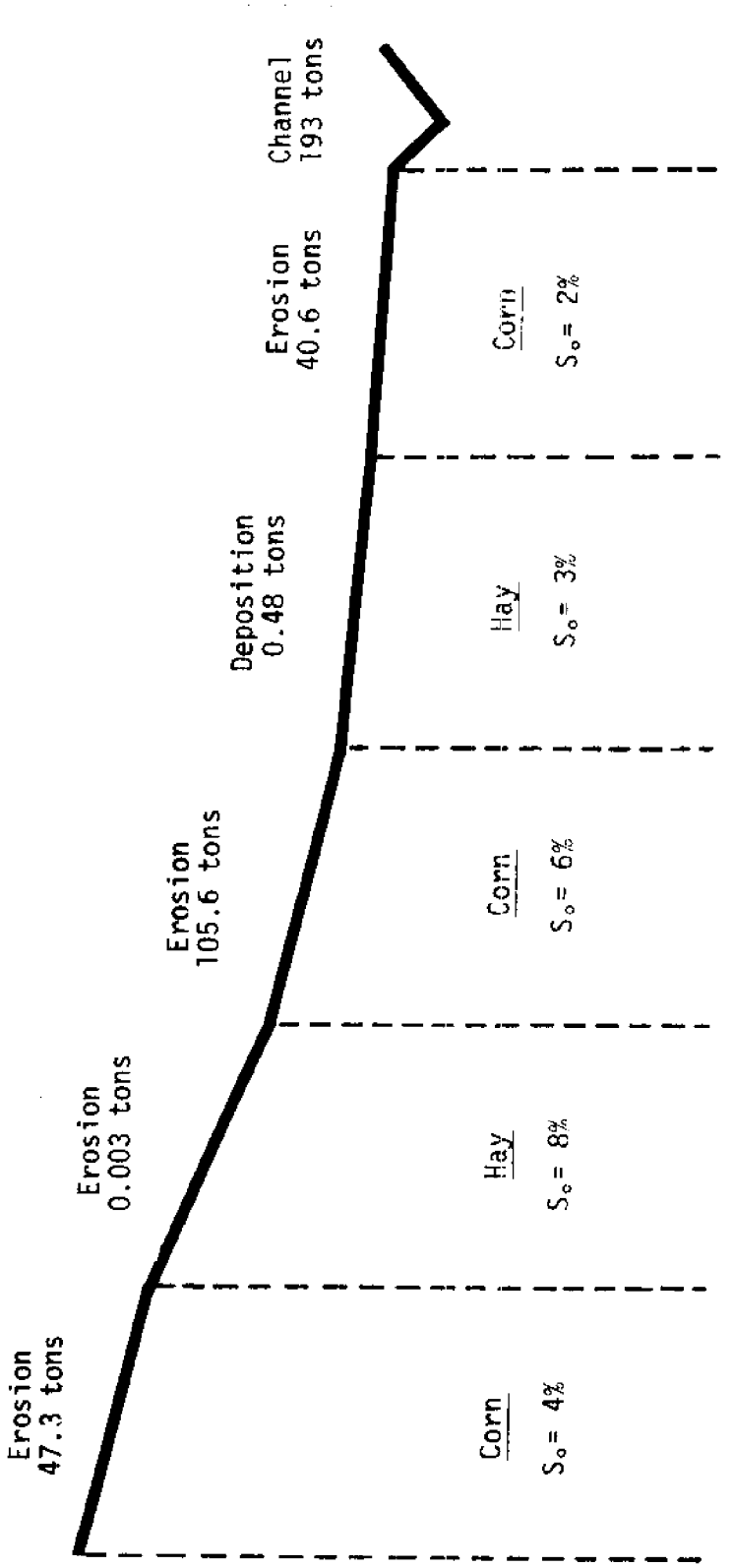

Figure 11. Sediment detachment and deposition from a strip crop practice

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USE OF THE COWNOSE RAY AS A FOOD SOURCE

Sam D. Thomas

With the establishment of a 200-mile economic resource zone, the seafood industry entered an era of new technology. As a seafood technologist and member of the industry, I feel we have a two-fold concern: Conservation and management of marine resources, and proper utilization of these resources.

We need to concentrate on the promotion of underutilized species of fish. "Underutilized species" are those which are found in relative abundance but are not being harvested. Fishermen do not want to go out for an underutilized species because its market is of such a low economic return that it does not justify a specific fishery. Although there is little demand for underutilized species in domestic market, there is a demand for them or similar species in many European and other foreign markets. This is the case with the cownose ray.

The eating of skates and rays is not new. Many European countries are importing skate wings, with a very small portion coming from the U.S. The skates exported from the U.S. are probably incidental catches of some other type of fishery. With proper marketing and handling, the cownose ray can become a new major export item.

The cownose ray, referred to as whipparee by many fishermen along our coast, has a range from South America to Chesapeake Bay. There may be two groups of cownose ray: the one that stays mainly along the Atlantic Coast, and the one that goes up into the Gulf of Mexico on a clockwise migration pattern, then back down to South America.

The cownose ray has long been a nuisance along our coast. Oystermen and clammers of the Chesapeake Bay area have been plagued by these animals, for they come into the bay in large numbers and devour tremendous amounts of shellfish. Sea Grant studies in Virginia have estimated losses of over $30,000 in one oyster garden, and losses to one clammer in excess of $100,000. The shellfishermen were at wit's end, willing to do anything to rid themselves of this menace. Some

people suggested the use of nets around their oyster gardens and shellfish beds to keep the rays out. They also suggested poisoning.

In the spring, the rays come into North Carolina waters in tremendous schools. They have been spotted in schools a quarter of a mile wide. Considering each animal is probably less than a meter across its wingtip, weighing anywhere from 25 to 30 pounds, a quarter mile-wide school with rays swimming wingtip to wingtip represents a tremendous quantity of fish. They come into the shallows of North Carolina and feed mainly on the scallops which they find in beds of eel grass. They are capable not only of wiping out an entire scallop bed in a matter of hours but also of uprooting the eel grass by violent activities of their wings, quite often destroying an eel grass bed.

The rays have also been the cause of anguish to millions of long-haul operators and netters. The netter sometimes accidentally traps rays in the nets. Having no reason to bring them in, he has to turn them loose by turning loose the rest of his catch.

Complaints from fishermen stirred up enough interest for researchers to begin studying rays as a possible new food supply. One project, "The Study of Utilization of North Carolina Skates and Rays" by Drs. Tyre Lanier and Steven Otwell of the North Carolina State University, Food Science Department, ended in June 1978. As the project progressed, they realized that they should direct more attention to the cownose ray than other rays and skates, mainly because of the former's abundance in North Carolina waters and the relative ease of harvesting it in large numbers.

The results of the project included the composition: the meat of the cownose ray was very high in protein -- 19 to 20%, and very low in fat -- less than 2%. It contained approximately 0.5% urea. The urea content has caused some problems in the utilization of sharks and has been a great concern to researchers of elasmobranch fishes. Even though the cownose ray has a relatively low urea content compared with other elasmobranchs, it still requires pre-soaking before cooking. A mild vinegar solution was used to soak the ray meat for about 35 to 40 minutes.

The results of taste tests of the ray were most favorable. Many of the panels rated it better than most other seafoods they had eaten and replied that it had an oyster or scallop flavor. Personally, I thought it had more of an oyster flavor. The texture is somewhat like pork chop, tougher than most fishery products. Dr. Otwell prepared ray dishes for the fishermen at Harker's Island and received some very high ratings. Some fishermen even suggested recipes of their own and said that they planned to freeze some meat for future use. Those of you who are familiar with the Harker's Island fishermen would know that it is quite a positive attitude coming from them.

In April, the rays started to show up in Core Sound, North Carolina. On April 24, a longhaul crew from Harker's Island with the assistance of Dr. Robinson of the National Marine Fisheries Service encircled a large school of rays and caught a very small portion of it. This large school was estimated to be somewhere in the neighborhood of 75,000 to 100,000 pounds. A very small portion of it, about 10,000 pounds, was caught. Of that, only 2,000 pounds were kept for the project use.

The 2,000 pounds of cownose ray were taken back to the pilot plant at the Seafood Lab in Morehead City. Each fish was weighed, measured, and determined for sex. Pectoral fins, which we call wings, were removed and were found to have a yield of around 42%. This is the part that is actually used as a food. The liver of the animals accounted for 2 to 3% of weight. The wings were packaged and frozen at -20°C. Storage at -20°C for one month yielded a very good product. A longer storage study was not possible to conduct within the duration of this project.

For taste panel evaluation, the wings were thawed rapidly in cold running water, and the skin was removed carefully by skinning or by hot water blanching. In the case of blanching, the wing was dipped into a large vat of boiling water for about 40 seconds, then skinned. The skin peeled off very easily. But blanching left the exterior meat partially cooked, which might be objectionable. Baked wing was not very favorably received, but fried wing with corn meal coating received a high rating.

At the Seafood Lab, we conducted some tests of our own using a group of Carteret County homemakers. They come in, work with new pro-

ducts and new ideas, and give comments on them. They tested several recipes of cownose ray, and I think the most interesting recipe was mock scallops.

I am sure that most of you have wondered if the meat of skates and rays is actually used in scallop recipes. Maybe you have eaten it, thinking it was scallops. It may be possible to disguise some skates as scallops, but I think it is impossible to pass cownose ray as scallops because of the dark color and firm texture. But we found that the cownose ray was a good substitute for scallops in casserole recipes. Four recipes tested were evaluated by a taste panel to be of the highest average ratings for any product. Some of the panel wanted to know where and when they would be able to buy it on the market.

There is a lot of positive response to the utilization of cownose rays. But before any fishery develops a great deal, we need more research and more marketing. We know most European markets prefer the white flesh of skates. But we don't know if they would accept the much darker meat of the cownose ray, or in what form they want it; whole wings, the skin on or off, filleted or not? These are some of the things we need to look into for marketing cownose ray. To support marketing, we need technology. We need to evaluate processing methods, packaging techniques, and more storage tests for proximate composition and product development.

Finally, we know very little about the biology of the cownose ray. We need to know what the maximum sustainable yield is. This is going to require a great deal of work on population assessment, reproduction rate and other biological aspects of the ray. It would appear that, without proper management of the cownose ray fishery, we could wipe out the whole population very easily.

Vibrio parahaemolyticus UPDATE

Robert M. Twedt

The incidence of food poisoning in Japan due to Vibrio parahaemolyticus is high. The number of reported cases has ranged from approximately 5,000 to 20,000, representing approximately 50 to 70% of all reported cases of food poisoning due to bacterial causes. In 1974, approximately 8,000 cases were reported (14).

Outside of Japan, V. parahaemolyticus has been isolated from seafoods and marine sources from many areas including Asia, Far East, South Pacific, North America, and Western Europe. The bacterium has been identified in water samples from the Atlantic and Pacific Oceans, the Gulf of Mexico, and the Baltic, North, Mediterranean, and Black Seas (14). Undoubtedly, V. parahaemolyticus is an organism universally distributed in the marine environments of the world (13).

The isolation of V. parahaemolyticus in North America was first reported by Baross and Liston in 1968 (2). They recovered the organism from the waters, sediments, and shellfish in the Puget Sound region, and later (3) from the waters, sediments, and finfish of Washington coastal waters. In quick succession, this organism was found in moribund blue crabs (12), processed blue crab meat from the Chesapeake Bay (8), and in Gulf coast shrimp along the Texas coast (27). The first Canadian isolation of V. parahaemolyticus was reported in 1971 from Canadian Atlantic shellfish (22, 23). American workers found that the estuarine waters and shellfish of New Hampshire contained V. parahaemolyticus (4).

This rapidly accumulating evidence indicated the universal presence of V. parahaemolyticus in the waters, sediments, and seafoods of the Pacific, Gulf, and Atlantic coastlines of the U.S. and Canada. It also suggested the possibility of a serious health hazard, in the light of Japanese experience with this organism. This expectation was realized shortly thereafter by the epidemic events which began in 1971 in the U.S. In the summer of that year, a succession of four foodborne out-

breaks of V. parahaemolyticus occurred in Maryland following serving of steamed crab and crab salad. This and 13 subsequent episodes were the first documented examples (14) of parahaemolyticus food poisoning in the U.S. in which the causative bacterium was recovered from both patients and contaminated foods (16).

The importance of obtaining stools, rectal swabs, or vomitus specimens in addition to specimens of suspect foods during outbreaks cannot be overemphasized. In all likelihood, patient specimens will contain the pathogenic strains of V. parahaemolyticus that have been shown to be responsible for the outbreaks. Such specimens must be obtained at the earliest opportunity because the carrier state is short-lived (3 to 7 days).

A minimum of 10 characters are required to identify the genus Vibrio and 15 to identify V. parahaemolyticus (1, 9). Some authors are apt to further reduce these minimal identifying criteria for the sake of brevity and to avoid the vagaries of the flagellar stain or the difficulties in determining the guanine to cytosine ratio.

Some Japanese investigators feel that a lesser number of identifying criteria for V. parahaemolyticus are required when the organism has been recovered from human stools rather than from seafoods. Sakazaki (19) lists the required criteria as follows (Table 1).

> TST agar alkaline slant, acid butt; negative gas from glucose; negative acid from sucrose and negative hydrogen sulfide; lysine decarboxylase positive,; growth in 8% NaCl peptone water; oxidase positive; gram negative rod; V. cholerae antisera 01 negative; human pathogenicity positive.

Presumably these biochemical criteria effectively separate V. parahaemolyticus from the enteric forms that are likely to be encountered such as the Enterobacteriaceae, Aeromonas, Plesiomonas, Pseudomonas, V. cholerae, and V. alginolyticus.

V. parahaemolyticus is effectively separated from related marine vibrios and from its most common competitor, V. alginolyticus, by means of the Voges-Proskauer reaction and sucrose reactions, and is differentiated from V. anguillarum, the fish pathogen, by most of the reactions listed in Table 2.

V. parahaemolyticus possesses three antigenic components; H, O, and K. The H or flagellar antigen was found to be common to all strains

Table 1. V. parahaemolyticus Identifying Characteristics (after Sakazaki)

Test	Response
Gram stain	Gram negative
Morphology	Curved/straight rods
Motility	+
TSI	K/A. H_2S(-), Gas (-)
Hugh-Liefson glucose	Fermentation, (+), Gas (-)
Cytochrome oxidase	+
Arginine dihydrolase	-
Lysine decarboxylase	+
Gelatin	+
Halophilism (NaCl)	6%, 8%, (+)
	0%, 10%, (-)
Growth at 42°C	+
Voges-Proskauer	-
Indole	+
Cellobiose	-
Sucrose	-
Maltose	+
Mannitol	+
Trehalose	+

Table 2. Separation of V. parahaemolyticus from Related Marine Vibrios

Species	TCBS	43C	NaCl 8%	NaCl 10%	Lysine decarboxylase	V.P.	Sucrose
V. parahaemolyticus	+	+	+	-	+	-	-
V. alginolyticus	+	+	+	+	+	+	+
V. anguillarum	-	-	-	-	-	-	-

of V. parahaemolyticus and to V. alginolyticus as well, thereby rendering this component of little value for serotyping (20).

The K antigen is a capsular, polysaccharidal antigen and may be removed from the bacterial body by heating. The K antigens, which coat the organism, prevent agglutination with homologous O antiserum in the living state. The O or somatic antigen is a thermostable antigen, which is resistant to 50% alcohol and 1 N HCl treatment. Since the K antigen masks the O antigen in living cultures, it is necessary to subject the cells to heating to 100°C for 1 to 2 hr to obtain O agglutination (18). Based on agglutination reactions, using adsorbed sera, 12 O groups and 54 K types are recognized in the current antigenic schema for V. parahaemolyticus (32) (Table 3).

Table 3. Antigenic Schema of V. parahaemolyticus

O Group	K Type
1	1, 25, 26, 32, 38, 41, 56, 58
2	3, 28
3	4*, 5, 6, 7, 29, 30*, 31, 33 37, 43, 45, 48, 54, 57, 59
4	4*, 8, 9, 10, 11, 12, 13, 34, 42, 49, 53, 55
5	15, 17, 30*, 47
6	18, 46
7	19
8	20, 21, 22, 39
9	23, 44
10	24
11	36, 40, 50, 51
12	52
Total - 12	54 K types/56 serotypes

Absent = K 2, 14, 16, 27, 35

* K-types appearing in more than O group.

Serological tests of themselves are not diagnostic of V. parahaemolyticus because of the interreactivity with many other marine organisms. We have found that V. alginolyticus, for example, reacts actively with both parahaemolyticus O and K antisera. Further, it must be recognized that since the current antigenic schema was based on patient strain isolates only, many marine strains obtained from seafoods are untypable. The Committee on Serotyping of V. parahaemolyticus (7) currently holds to the position of enlarging the antigenic schema only by the addition of new human serotypes.

An analysis of V. parahaemolyticus serotypes was conducted by the Food and Drug Administration, Division of Microbiology, on a total of 750 epidemic and environmental samples during the period 1969-1972. Approximately 30 different marine species including fish and shellfish were positive for V. parahaemolyticus. No particular serotypes were exclusively associated with any marine species. We found 11 odd serotypic combinations from seafood isolates that do not conform to the antigenic schema proposed by the Typing Committee. Whether this represents a true geographic variation in the serotypes of this bacterium as Zen-Yogi, et al. (33) suggested remains to be determined.

The routine serological identification of V. parahaemolyticus can be based solely on the K antigenic analysis. In practice, an unknown isolate is first tested in polyvalent K group antisera. A positive test is then followed by testing in the monovalent K antisera to determine the specific serotype. Since almost all of the K types are associated with only a single O group, the determination of the K group establishes the O group more or less automatically.

An analysis of the recovery dilutions of all positive seafood samples for the 4 years showed that only 14% of the positive recoveries occurred at a level of 1,000 org/gm of seafood or higher. The remaining 86% of positive recoveries occurred at a level of 100 org/gm of seafood or lower. The total positive dilutions may be represented as follows: 100 org/gm - 16%; 10 org/gm - 31%; 1 org/gm - 39%. These data confirm similar observations made on oysters sampled from Galveston Bay (21). Evidently, under natural conditions, V. parahaemolyticus appears to occur in counts of 100 organisms or less per gram of seafood in the majority of instances.

The recovery of V. parahaemolyticus from seafoods is generally associated with the summer months when the marine waters are warm enough to support the growth of this organism. The monthly frequency of V. parahaemolyticus recovery determined from a composite of 307 isolates during the years 1969 to 1971 is shown in Figure 1.

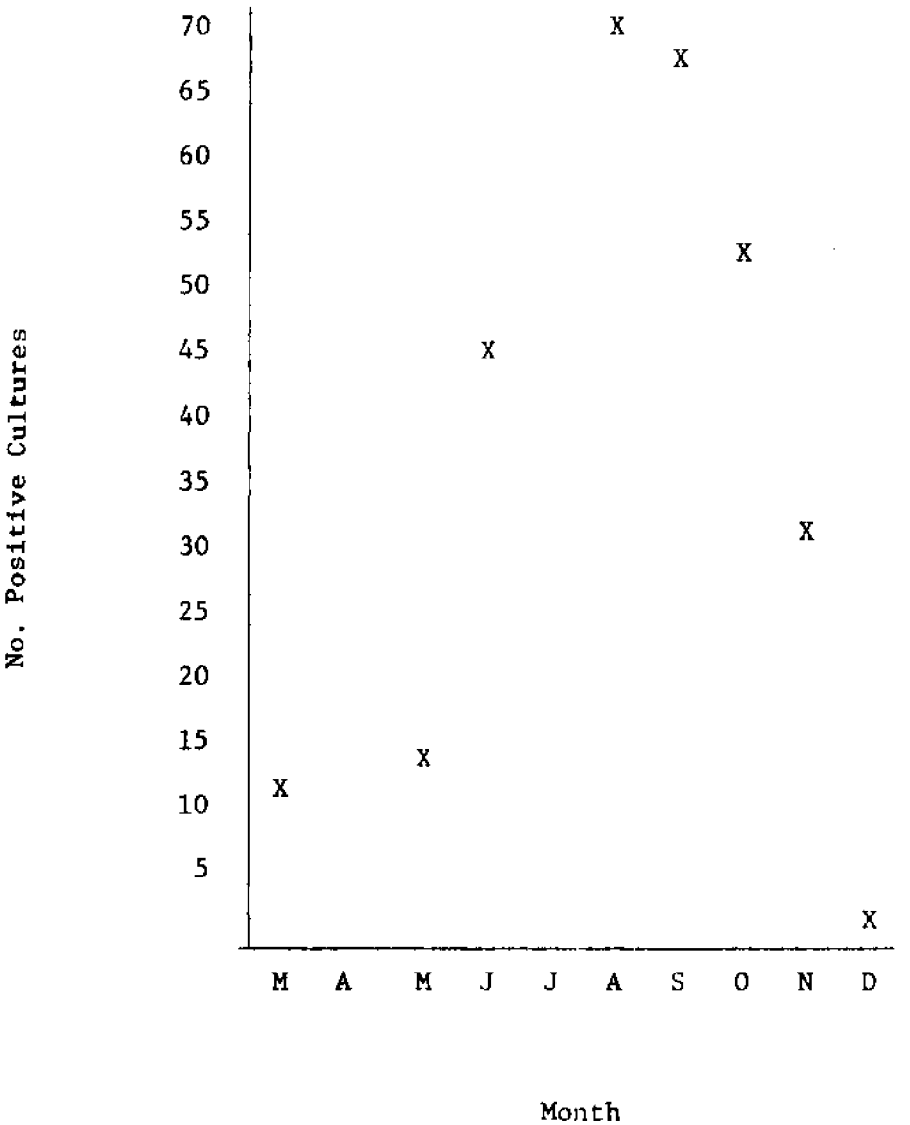

Figure 1. Monthly recovery of V. parahaemolyticus from seafoods - 1969-1971

Maximal recoveries of this organism from seafoods occurred in the months of July, August and September. These findings confirm the data of Miyamoto, et al. (15) for Tokyo Bay waters, Baross and Liston (3) for Washington State coastal waters and Kaneko and Colwell (10) for the Chesapeake Bay area. They are at variance with the observations of Thompson, Vanderzant, and Ray (21), who reported no seasonal distribution of V. parahaemolyticus in oysters from Galveston Bay. In explanation, they supposed that the relatively high water temperature in the Gulf of Mexico failed to cause the drastic reduction in V. parahaemolyticus count described by the data of Figure 1. The lowest temperature recorded in these waters in February was 11.6°C, still above the minimum temperature shown by Kaneko and Colwell (10) to be required for the growth of V. parahaemolyticus in the natural environment.

In an effort to differentiate enteropathogenic strains from non-enteropathogenic strains, Kato, et al. (11) showed that strains of V. parahaemolyticus obtained from stools of enteric patients were hemolytic on a special high salt blood agar, whereas strains of V. parahaemolyticus derived from seafoods and marine waters were not hemolytic on this agar. This finding was confirmed by other workers (14). Later on, this special high salt blood agar was modified by Wagatsuma (29). The special hemolytic reaction was referred to as the Kanagawa phenomenon, after the Kanagawa Prefectural Public Health Laboratory study group that described it.

The close association between the source of V. parahaemolyticus isolates and their hemolytic activity as established by Japanese investigators has been solidly confirmed in the examination of hundreds of epidemic and environmental isolates by FDA, Division of Microbiology. All Kanagawa-positive strains were isolated from stool cultures; until very recently, not a single seafood isolate was positive.

Work conducted in FDA-Bacterial Physiology Branch laboratories has established that a given strain's hemolytic ability is directly related to its capability to produce reactivity in the rabbit skin and ileal loop tests (25, 26) (Table 4). It is paradoxical that the hemolytic enteropathogenic strains predominant in patients' stools are only rarely discovered in raw sea fish and shellfish, or in implicated food. Speculative explanations that have been proposed suggest that:

Table 4. Rabbit Ileal Loop Reactivity of Live Cell Suspensions of Vibrio parahaemolyticus Strains

No. Strains	Source	Kanagawa Type	Times Tested	Times Positive	Reactivity
25	Human	+	163	118	23/25[a]
5	Human	-	25	5	1/5
5	Marine	-	31	8	1/5

[a]Number of positive strains/number of strains tested.

(a) the rare Kanagawa-positive enteropathogenic strain present among any non-virulent varieties on sea fish (30) is selectively enriched within the living host, colonizes the host's intestinal tract, multiplies, and produces toxin, resulting in disease, or: (b) there is an in vivo exchange of extracellular genetic material coding for toxin production between enteropathogenic strains (perhaps even of another species) and Kanagawa-negative Vibrio.

The second alternative explanation was eliminated by collaborative studies done by Zink and Vanderzant at Texas A&M University and ourselves. Our results showed the absence of cryptic plasmids that were associated either with Kanagawa hemolysin or pathogenic activity by several in vivo animal models. The first alternative explanation has yet to be disproved, but our present evidence, drawn from the rabbit ileal loop model (unpublished data), suggests that parahaemolyticus disease is the consequence of a large inoculum ($ILD_{50} = 2 \times 10^6$). In terms of human public health epidemiology, parahaemolyticus disease results from the multiplication of virulent strains to an infective dose in mishandled food prior to consumption.

To investigate the possible existence of a toxic factor associated with the cell structure, we (5) compared loop reactivity of various preparations from hemolytic strains isolated from patients' stools (Table 5). As expected, broth cultures produced severe dilatation 31 out of 40 times tested (78%). The percent positive reactivity of culture supernatants was 20%, of lysates, 63%, of resuspended lysates, 50%, and of lysate supernatants, 0%. These results indicated that a toxic

Table 5. Rabbit Ileal Loop Reactivity of Various Preparations from Broth Cultures of Kanagawa-Positive Vibrio parahaemolyticus Strains

Strain	Broth Cultures	Culture Supernates	Lysates	Resuspended Lysates	Lysate Supernates
550	9/10[a]	3/10	9/10	6/10	0/10
551	8/10	2/10	5/10	5/10	0/10
552	6/10	2/10	5/10	4/10	0/10
553	8/10	1/10	7/10	5/10	0/10
Total Positive	31/40	8/40	26/40	20/40	0/40
% Positive	78	20	63	50	0

[a]Number of times positive/number of times tested.

factor present in broth cultures of live cells was present minimally, if at all, in the culture supernatant. Furthermore, the factor seemed to be associated primarily with the lysate particles.

We further investigated lysates from cells grown on BHI agar (5) (Table 6). Lysates in phosphate buffer saline (PBS) were uniformly reactive; after overnight dialysis in distilled water, they were inactive. The particulate toxicity was not lost completely because, when the dialysands were centrifuged and resuspended in PBS to their original volume, most of the particulate reactivity was restored in each case. The dialysates were inactive until concentrated 10-fold (maximum NaCl 1.5%).

These data suggested that the toxic principle associated with the lysate particles was dissociable and active in the absence of the particles themselves, though not in the absence of a minimal saline concentration.

During this period of investigation, Japanese workers, proceeding on the assumption that the enteropathogenicity of Kanagawa-positive strains results from biologic effects of the Kanagawa hemolysin, have attempted to isolate and characterize that factor.

Several workers have isolated and purified a thermostable direct hemolysin from culture filtrates of Kanagawa-positive strains (14). The purified hemolysin has been shown to be a toxic protein of 45,000

Table 6. Rabbit Ileal Loop Reactivity of Various Preparations From Lysates of Kanagawa-Positive Vibrio parahaemolyticus Strains Grown on BHI Agar

Strain	Lysates	Lysate Dialysand	Resuspended Dialysand	Lysate Dialysate	Concentrated Dialysate
553	3/3[a]	0/3	2/3	0/3	2/3
9337	3/3	0/3	3/3	0/3	0/3
11590	3/3	0/3	2/3	0/3	3/3

[a]Number of times positive/number of times tested.

daltons that exhibits a mean lethal dose for 20g-mice by intraperitoneal inoculation of 1.5 µg, a minimal cutaneous response dose in guinea pig skin of 2.5 µg, and a minimal ileal loop dilatation dose of 200 µg. Cardiotoxicity of the purified hemolysin has been demonstrated in rats and in cultured mouse heart cells (14).

We tested various preparations from V. parahaemolyticus strains on Wagatsuma agar (5) (Table 7). Lysates from Kanagawa-positive cultures were hemolytic. Whereas lysate supernatants also showed hemolysin, filtration through 0.45 µm membranes removed this reactivity.

Table 7. Kanagawa-Type Hemolysis by Cultures and Various Preparations From Lysates of Vibrio parahaemolyticus Grown on BHI Agar

Strain	Cultures	Lysates	Lysate Supernates	Lysate Filtrates
393	-	-	-	-
557	-	±	-	-
553	+	+	+	-
554	+	+	+	-
9337	+	+	+	-

Two principal lines of evidence support a direct relationship of the toxic particulate factor with the purified hemolysin. (a) There is a close association between the ability of broth cultures or cell

suspensions of a V. parahaemolyticus isolate to dilate the ligated rabbit ileum and the strain's ability to produce Kanagawa hemolysin. (b) Non-filterable lysate particles of Kanagawa-positive cells produce rabbit loop dilatation and also hemolyze Wagatsuma agar. Lysates of Kanagawa negative cells are unreactive in both cases.

We cannot determine from our data whether the association between particles and hemolysin is a consequence of preparation or because the particles are the source of hemolysin synthesis.

Reports from several laboratories, including our own (6, 17, 24, 28, 31, 34), have suggested that V. parahaemolyticus strains can invade host tissue, causing wound and generalized infections. We utilized immunofluorescence to determine whether strains of virulent V. parahaemolyticus isolated from gastroenteritis patients were capable of invading layers of intestinal tissue in the rabbit ileum (unpublished data). Specific tagged rabbit antisera were developed against strains isolated from shellfish or from symptomatic humans. Fresh frozen ileal tissue sections from animals tested with both hemolytic and non-hemolytic Vibrio strains were examined.

Figure 2 shows a transverse section through villi of a control ileal loop inoculated with sterile broth. The staining of this tissue with specific anti-K sera tagged with isothiocyanate served as a negative control. There is no indication of tissue or bacterial fluorescence.

Figure 3 shows an oblique section through villi of a loop which had been inoculated with a strongly hemolytic patient isolate of V. parahaemolyticus that proved to be highly invasive and destructive. The lamina propria has been damaged by the presence or action (elaboration of hemolysin) of V. parahaemolyticus. Such massive destruction was not observed in strains which were either not as invasive or non-hemolytic. All invasive strains tested could penetrate to the muscularis externa of the intestinal tract.

In a hematoxylin and eosin section of the muscularis externa of tissue not exposed to Vibrio, normal tissue architecture is observed (Figure 4).

In Figure 5 the action of an invasive Vibrio is shown. The section taken from tissue that was macroscopically friable shows total

loss of normal architecture. Similar H and E slides were used in conjunction with consecutive fluorescent sections as additional evidence for the establishment of foci of infection. This evidence was manifest as greater number of polymorphonuclear leukocytes than in control sections.

Results of our studies indicate at this time that V. parahaemolyticus is an organism that elaborates a cytotoxic hemolysin and is capable of penetrating host tissue defenses. These virulent factors are not vigorous enough to allow establishment of infection unless a rather large infecting dose is ingested by the host. The epidemiology of parahaemolyticus disease suggests food vector source with no involvement of host-to-host transfer and little danger from the short-lived carrier state.

Appropriate and reasonable food handling techniques are probably sufficient to prevent infection from V. parahaemolyticus.

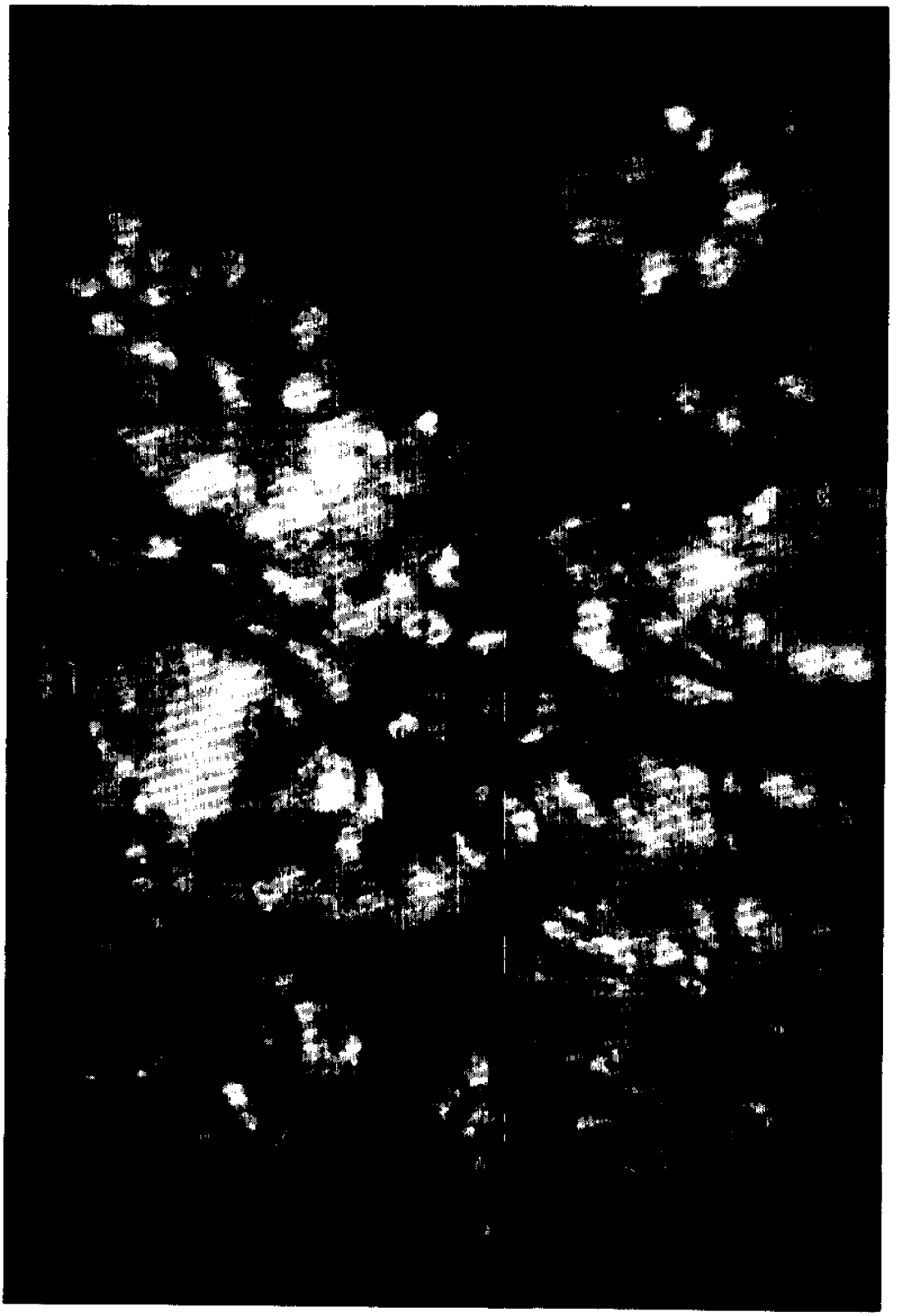

Fig. 2. A transverse frozen section of villi from a rabbit ileal loop injected with sterile broth. Fluorescence 192X.

Fig. 3. An oblique frozen section of a villus from a rabbit ileal loop injected with Vibrio parahaemolyticus strain 553 (patient isolate). Fluorescence 192X.

Fig. 4. Section of muscularis externa from a rabbit ileal loop injected with sterile broth. H and E 192X.

Fig. 5. Section of muscularis externa from a rabbit ileal loop injected with Vibrio parahaemolyticus strain 553. H and E 192X.

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the material to be scooped up. The material was deposited either on board the craft or on the adjacent bank. The spoon and bag was probably introduced into Europe by the Phoenicians or the Romans.(2) This plant ruled supreme until around 1400 AD when significant improvements began to appear in Holland. John Huston, in his book "Hydraulic Dredges", relates that remarkably similar versions of this dredge still work in southern England today.

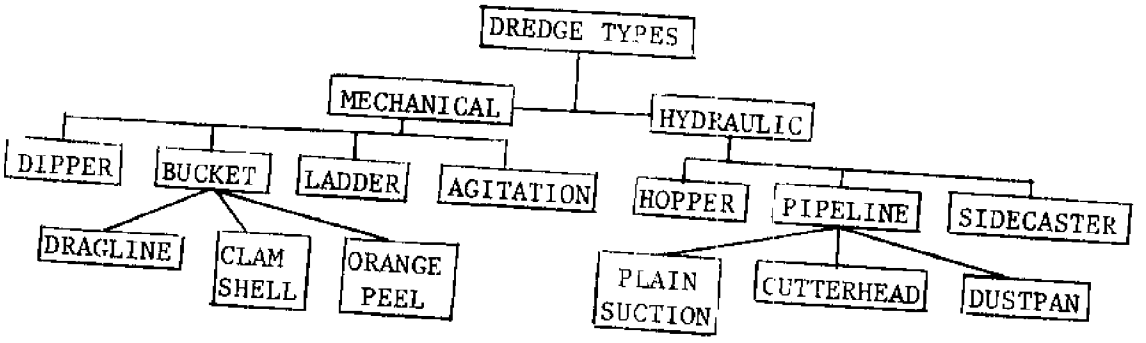


Figure 1

Agitation dredging is perhaps the oldest form of dredging. It is recorded, prior to the earthly ministry of Christ, that weighted tree trunks or crude rakes were dragged by boats down the Indus and Euphrates Rivers.(3) The agitated river deposits were carried downstream by river currents. When Abraham, then Abram, left the gulf town of Ur of the Chaldeans, he perhaps witnessed this effort on the Euphrates. That dredging was required is attested to by the fact that, in Abraham's time--about 1300 BC, Ur was on the Persian Gulf. Today it is--rather, its remains are--about 150 miles upriver from the Gulf. In 1435 AD, elaborate sailing vessels equipped with spike-tooth harrows appeared in Holland and were used extensively until 1800. Agitation dredging is still performed today, but on a drastically reduced scale.

The direct anscestor of the modern mechanical dredge did not appear until around 1600. The endless chain-of-buckets dredge or the "Mud Mill" was first operated by a man-powered treadmill. It was also known as "Noah's Ark", probably because by 1620, horse-drawn treadmills were in use. The material was dumped into a nearby scow. By 1793, some "Mud Mills" were large enough to have five horses aboard, two pulling and

three resting. The advent of the steam engine and its adaptation to dredging eventually rendered the "Mud Mill" obsolete.(4)

The late 19th century saw the nearly simultaneous development of the hydraulic dredge in Europe and the United States. In 1855, Nathaniel H. Lebby designed what is considered by many to be the first successful hydraulic dredge in the United States, if not the world.(5) The plant was used in 1857 to remove about 35,000 cubic yards from a bar at Charleston, South Carolina. It averaged 328 cubic yards a day.

So there are basically two types of dredges -- Mechanical and Hydraulic. The sub-types are:

Dipper Dredge (see Figure 2). Merely a power shovel operated from a barge. It is an effective dredging plant which can dig its own flotation in almost any material, at near in-situ density. On the forward hull, there are two spuds to anchor the dredge. A kicking spud, located in the stern, is used to move the dredge ahead. The dredging depth is limited to the boom length, 65 feet being about the maximum. This plant is almost exclusively American.

Ladder Dredge (see Figure 3). The direct descendant of the "Mud Mill", it is probably the oldest productive dredge in general use today. In the United States, these are used almost exclusively in mining operations. But in Europe, they are by far the most popular and used extensively. The ladder dredge utilizes five or more anchors, rather than spuds, for mooring and is consequently a constant hindrance to navigation.

Bucket Dredge (see Figure 4). This category includes several types of dredging receptacles. The dragline scrapes material off the bottom. The clam shell and the orange peel are grab-type buckets that bite bottom material and lift it to a conveyance mode. The clam shell is used for mud and the orange peel for loose rock or other hard material. These plants are used extensively around dock and piers where they can excavate close to structures without structural damage. The bucket dredge is particularly suited where there are obstructions and trash. Dredging to depths of 100 feet is not uncommon.

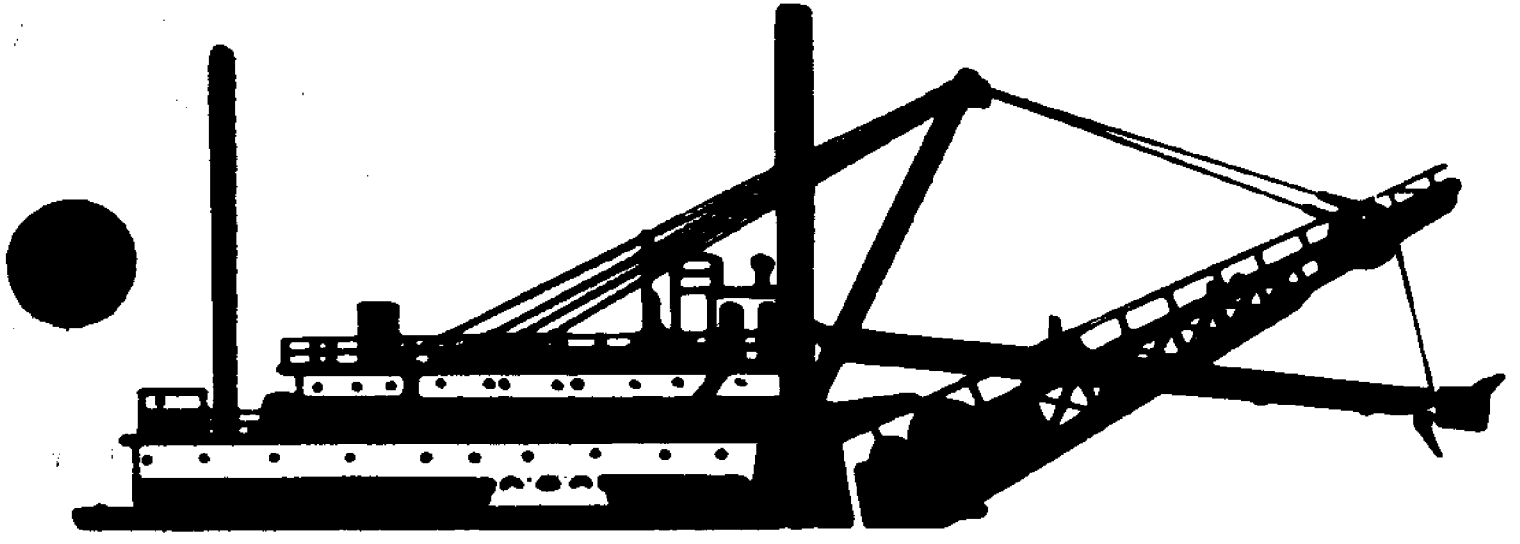
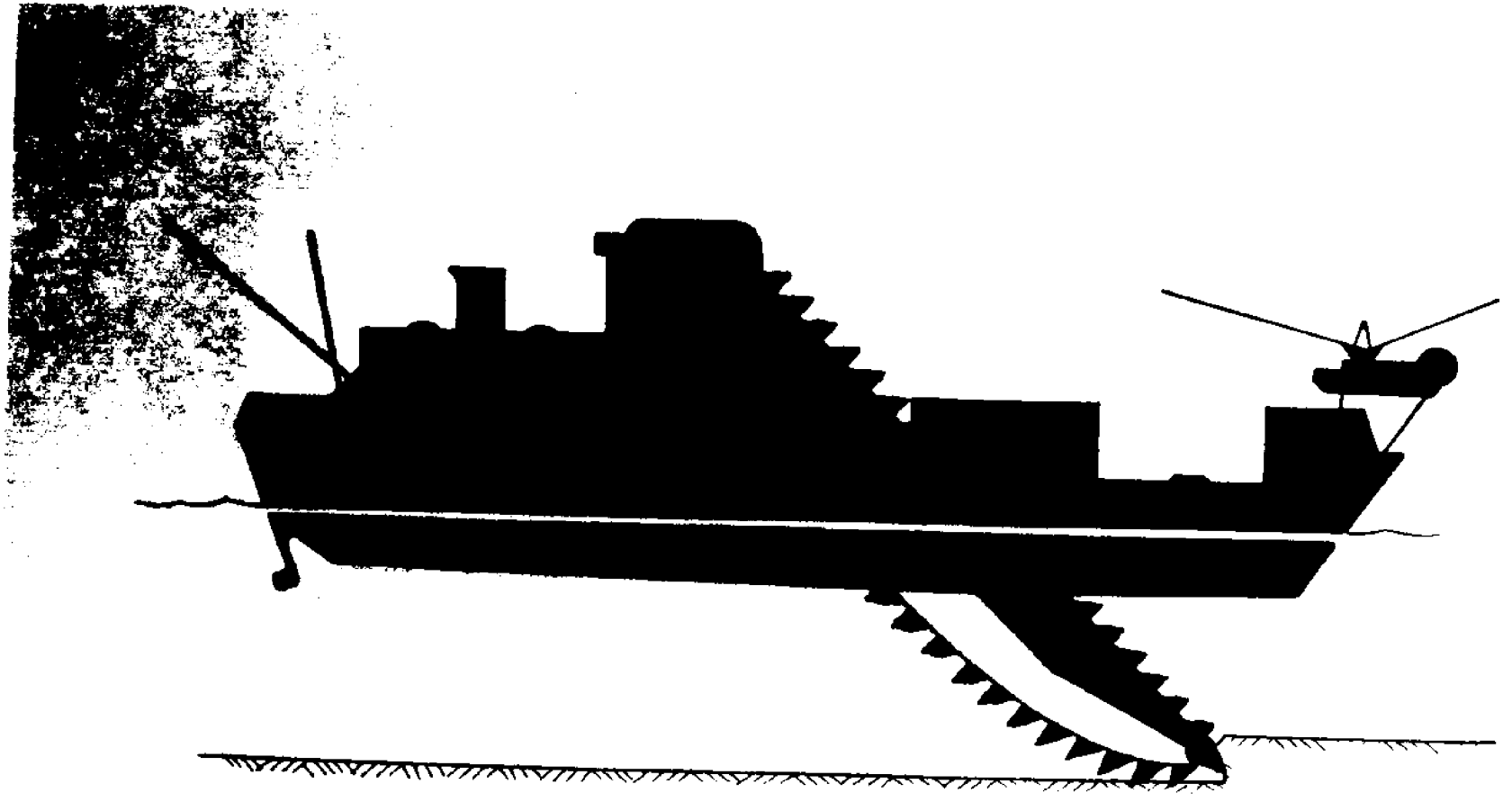


Figure 2. Dipper dredge



Ladder Dredge

Figure 3. Ladder dredge

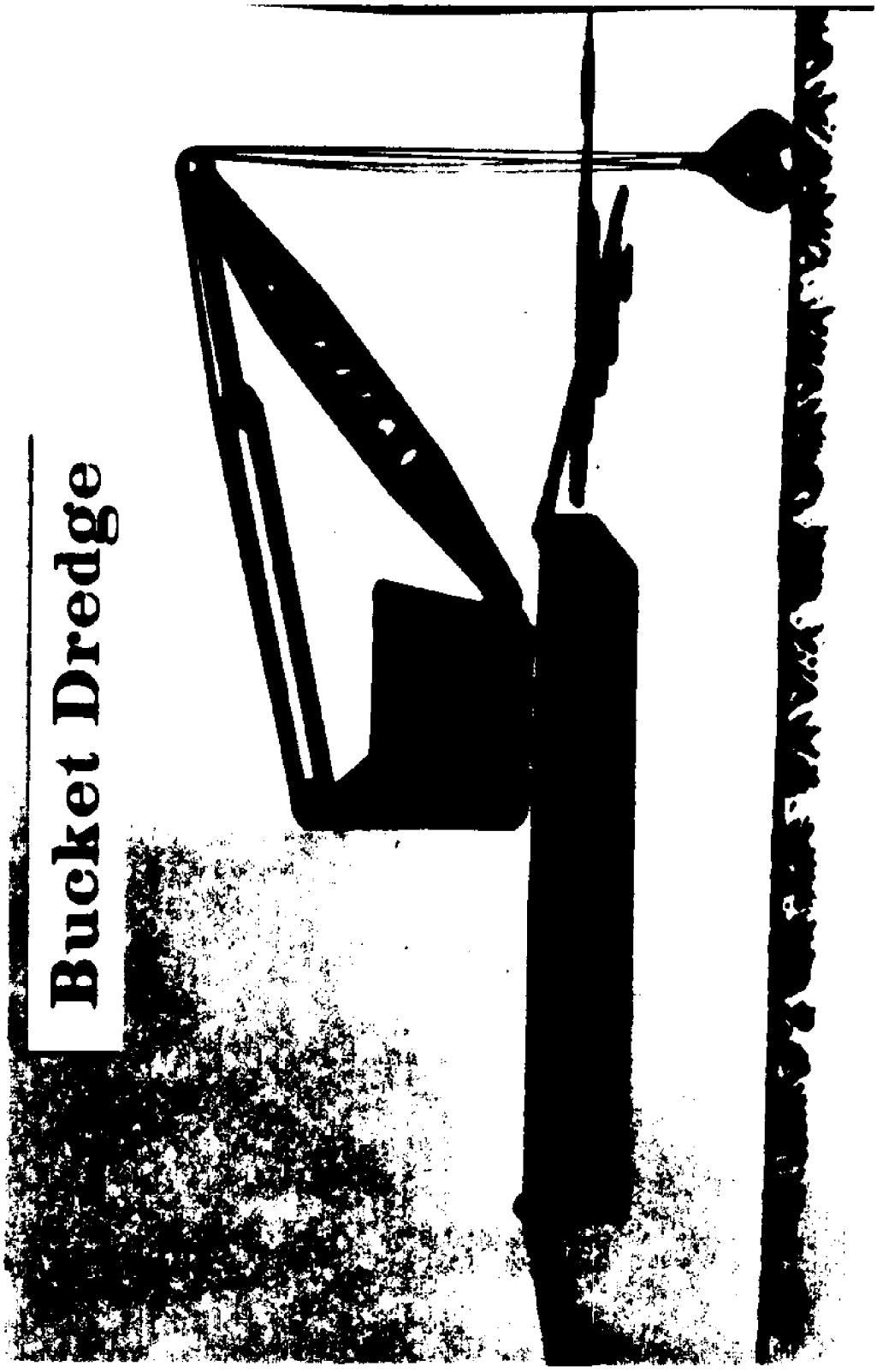


Figure 4. Bucket dredge

Dredging must be viewed as a total function involving the pick-up, transporting, and depositing of material. Mechanical plants specialize in the pick-up phase. They deposit the material in some type of conveyance mode, perhaps dump scows or conveyor belts, for transport to the dump site. Their outstanding features are that they can operate in cramped quarters and excavate at near in-situ densities. Hydraulic dredges, on the other hand, perform the total function -- they pick up, transport, and deposit, but not without some serious concessions, particularly in today's environment-conscious society.

All hydraulic dredges have one thing in common: they employ a centrifugal pump discharge into the hole of the dredge itself, overboard, ashore, or into a barge. Since centrifugal pumps are used, these plants have to add dilution water to form a dredge slurry -- as much as nine parts water to one part solids. Therein lies the major disadvantage of hydraulic plants. The diluion water complicates disposal operations and often generates undesirable turbidity levels.

Hopper dredges (see Figure 5) are generally regular ocean-going ships, equipped with drag arms and built-in bins or hoppers. The capacity of the hopper dredge varies from 500 to 8000 cubic yards. With the ship underway, the drags are lowered, pumps activated, and the bins filled. Once filled, the dredging stops, and the material is transported to the dump site. This is the main disadvantage of the hopper; it has to stop dredging to transport the material. The ESSAYONS only has bottom dump capabilities, and must use overboard disposals. Some hopper dredges, such as the Goethals, has both bottom dump and pump-out capabilities. The pump-out capability gives added disposal flexibility so that confined, upland disposal areas can be used. Other hoppers have sidecaster capabilities as well. Also, a number of dredges are solely sidecasters. Hoppers, in general, are suited for dredging in exposed waters.

Today's hydraulic dredge is an unparalleled excavating plant. When sufficient water and adequate disposal area are available, it has no economic competitor. The pipeline cutter-head is the most versatile and popular dredge in the world today. It is, however, susceptible to wave action.

The pipeline dredge (see Figure 6) is generally referred to by the size of its discharge line which varies from 6 to 30 inches. The larger

the pipeline, the more productive the plant. The dredge is comprised of a ladder, cutterhead, suction pipe, cutter motor, pump, engine, spuds, A and H frames, hoist machinery, spud frames, hull, house, lever room, and anchor booms.

Most dredgers agree that two things are critical; the pump and the cutterhead. The dredge swings with the aid of anchors and advances by stepping forward on the spuds. One spud is called the digging spud, the other the setting spud. The dredge pivots on the digging spud, the cutter cuts into and under the material. It swings back for advance on the setting spud, then cuts again on the digging spud. The dredge does not generally cut on the return unless the material is very soft. The reflection of the cutterhead tends to walk on hard material rather than excavate.

In the Norfolk district, our primary environmental concern is oysters because of their economic importance to the particular locality and the state. Oyster producing areas get preferential treatment. Dredging jobs are planned with this in mind. Our first contact is the Virginia Marine Resources Commission. With the aid of their maps, the locations of leased and public grounds are shown on our plans relative to the dredging areas. Grounds are located in the field, if needed. The dredging layout, the route of the dredge pipeline, and the disposal area configurations are influenced strongly by oyster ground locations.

Special turbidity control devices are considered; turbidity curtains may be installed, pipeline joints are bagged - this involves sealing joints with burlap - to prevent leaks, sometimes trestles are constructed to elevate the pipeline above the oyster ground. While attractive in theory, trestles have proven to be detrimental rather than beneficial. Susceptible to wind and wave action, the trestles cause more damage to shallow oyster beds than the pipeline bottom-placed across the bed. Every effort is made to schedule the dredging during environmentally preferred periods. In oyster producing areas, upland disposal is strongly preferred. Overboard disposal is only considered as a last-resort alternative.

All of the above are included in the process of developing what we believe to be our best dredging procedure. The proposed dredging is then coordinated with state and federal environmental agencies and

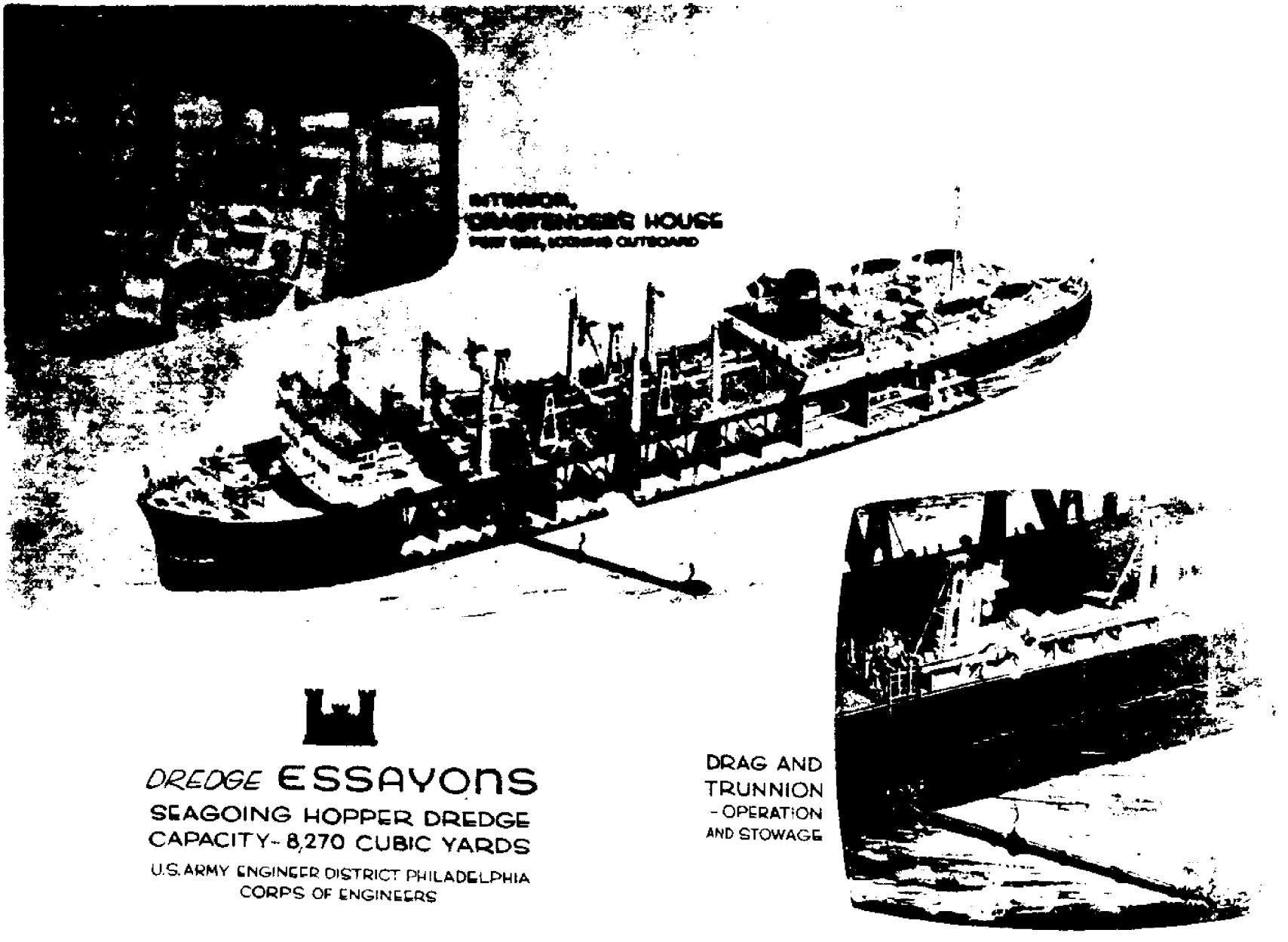


Figure 5. Hopper dredge

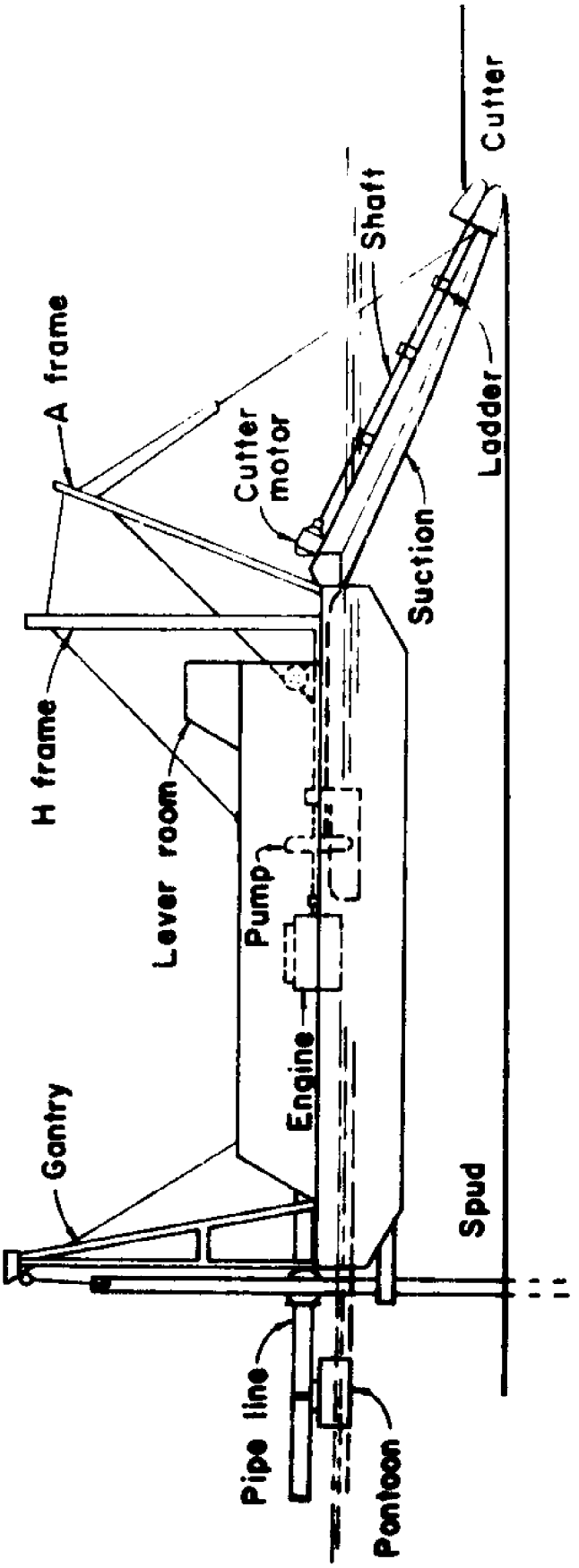


Figure 6. Pipeline dredge

frequently modified to reflect safeguards they consider necessary. The Norfolk District has found the agencies to be reasonable and most willing to work within our accommodative ability. Now the most important factor, by far, enters the picture; namely, the contractor's operational skill and professionalism.

There are some operational techniques that contractors should use, and generally do use, and which, I believe, promote a clean operation. In the Norfolk District, the majority of the dredging near oyster areas is in small navigation projects. The channels are generally less than 10 feet deep and less than 100 feet wide. The grounds most impacted by the dredging are those on shallow tidal flats adjacent to the dredge cut. Most of the damage to these grounds are caused by the plant attendant to the dredge, not by the dredge per se. I strongly suspect that anchor booms significantly reduce secondary plant effects.

The dredging area should be adequately controlled and marked, both horizontally and vertically. Adequate control minimizes overdredging. The dredge should be operated by an experienced leverman, knowledgeable in spudding and maximizing slurry density. Hydraulic dredges generally transport nine parts water into the disposal area with every one part of solids. Since most problems associated with disposal areas are attributable to the water, the solids concentration should be maximized at all times. The contractors should clean up behind themselves. There is no excuse for the work areas to be left littered with trash, empty oil drums, abandoned pipeline, and assorted debris.

In closing, I would like to say a few words in favor of--perhaps, even in defense of--the dredging industry. We should remember that dredging is a unique profession. It is not like building a building, or pouring concrete. Dredging is always "plus or minus" at best. Given the large, clumsy, too often archaic equipment, it is a folly to demand exactness. Some overdredging and operational latitude is to be expected. I believe that the industry is responding positively to the environmental challenges and that it can meet any reasonable demand.

We in regulatory authority have a great responsibility in this matter. As I see it, the industry asks only one thing of us. They ask us to be reasonable in our demand and not to expect "pie in the sky"

results that they cannot deliver. Balanced professionalism is the key - professionalism on both sides of the issue. Can dredging cohabit with oyster? I believe it can.

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4. Huston, p. 5.

5. Huston, p. 11.

ANIMAL WASTE RUN-OFF INTO OR IN PROXIMITY TO SHELLFISH WATERS VIRGINIA'S MANAGEMENT PLAN

Gerald T. Yagel

I. Introduction

Estuarine tributaries of the Chesapeake Bay are valuable producers of the American oyster Crassostrea virginica. A study recently published by the Virginia Institute of Marine Science revealed that Virginia was the most important producer of this species of oyster in the nation during the early part of this century and even until the 1950's. Earlier records indicate that in 1875, annual production had increased to 17 million bushels and may have reached 20 million bushels or more per year in the period between 1875 and 1885. From 1931 to 1960, the annual production decreased but was still high, and the average production during that period for Virginia ranged from about 1.3 to 3.5 million bushels, prior to the decline caused by the oyster disease MSX.

By authority of the State Water Control Law, the Virginia State Water Control Board is charged with the responsibility to:

1. Protect existing high quality of State waters and restore all other State waters to such a condition of quality that any such waters will permit all reasonable public uses and will support the propagation and growth of all aquatic life, including game fish, which might reasonably be expected to inhabit them,
2. Safeguard the clean waters of the State from pollution,
3. Prevent any increase in pollution, and
4. Reduce existing pollution in order to provide for the health, safety and welfare of the citizens of the Commonwealth.

Pollution sources identified have created, in the past, the necessity for condemnations of valuable shellfish growing waters under the classification of "Prohibited" which prohibits the direct marketing of shellfish.

Since the enactment of the Virginia State Water Control Law on July 1, 1946, the Board has been engaged in intensive programs to insure the construction of adequate waste treatment facilities throughout the

Commonwealth. In Tidewater, Virginia, the Board has proposed to reduce the number of acres of estuarine waters that are condemned of direct marketing of shellfish. Although domestic sewage and waste from large industrial operations are probably more familiar to the layman because of the publicity given such causes of pollution, there are many other types of pollution sources, such as marinas and other types of onshore fecal generating waste sources, which adversely affect shellfish water quality.

Animal waste resulting from certain agricultural operations also poses a serious threat to the quality of the Commonwealth's shellfish growing waters and to the social and economic benefits derived from the shellfish industry. One of the culprits of this type of waste is large female hogs, affectionately known as "sweetlips." The indiscriminate behavior of "sweetlips" creates a definite waste problem, especially when that behavior generates waste which flows directly into Virginia's estuarine waters.

II. Historical Activities by the Board in Animal Waste Management (July 1, 1946 through January 1, 1972)

The Board and its staff have been aware, since its inception, that concentrated animal feeding operations which discharge waste into surface waters have created water quality degradation. When these operations have been brought to the Board's attention, corrective measures were sought and required under appropriate sections of the State Water Control Law. Technical advice and assistance in these instances have been made available over the years by the VPI&SU Extension Service. Their expertise, a result of research and other studies, has made it possible for the operator to secure corrections without an inordinate amount of capital expense.

However, prior to 1972, no massive animal waste correction program existed for all farms in the Commonwealth. The problems were corrected on a case-by-case basis for those that were obviously causing water quality problems. No extensive "search and correct" program was initiated. Although many small programs were in existence, they were unknown to the Board and its staff.

III. 1972 Confrontation with the U.S. Food & Drug Administration (FDA)

In early 1972, the Board was informed by the State Department of Health, Bureau of Shellfish Sanitation, that the Food & Drug Administration was very unhappy with Virginia's shellfish sanitation program, and that animal waste sources uncorrected near tidal waters were a significant point of concern. As a result of emergency funding from the Virginia General Assembly, the Board was provided with a considerable increase in staff, equipment and other ancillary needs. In March of 1972, the staff embarked upon an intensive animal waste pollution abatement program. That program has continued and has become highly refined since 1972.

IV. Legal Basis for the Board's Agricultural Waste Management Program

A. Provisions of the State Water Control Law as Amended

The Board's authority to control animal waste, even though some wastes are not directly discharged into estuarine waters, has been derived from two segments of the State Water Control Law. The law empowers the Board with sufficient authority to regulate the handling, treatment, storage, disposal, and any other actions connected with animal waste when State waters may be contaminated or the quality of shellfish growing waters is threatened. The law applies to all animal operators who handle, store, distribute or produce animal waste or cause that waste to be handled, stored, distributed or produced from any establishment or farm.

B. Federal Water Pollution Control Act Amendments of 1972 (PL 92-500)

The objective of the Federal Water Pollution Control Act Amendments of 1972 (PL 92-500) is to restore the chemical, physical and biological integrity of the nation's waters. To achieve this objective, the law sets a national goal of no discharge of any pollutants into navigable waters of these United States by 1985. Section 402 of that Act sets forth requirements for point source discharges and a permitting system under the National Pollution Discharge Elimination System (NPDES). Agricultural waste is included in that permitting system, if that waste is being discharged in discreet and significant amounts.

In the rules and regulations published by the U.S. Environmental

Protection Agency (EPA), a concentrated animal feeding operation is defined as an animal feeding operation where animals had been or will be stabled or confined and fed or maintained for a total of 45 days or more in any 12 month period and containing more than 1,000 animal units. One animal unit is equivalent to one slaughter and feeder cattle. Animal unit equivalency for other species of animals vary depending on the species.

C. The Board's Adopted Strategy for Animal Waste No-Discharge Certificate

Prior to EPA's promulgation of these rules and regulations, the State Water Control Board already had an ongoing animal waste management program which exceeded or was at least equal to EPA's requirements. The administration of that program is entitled "A State No-Discharge Certificate Program" which, in essence, requires that under certain conditions there must be no discharge of animal waste into State waters. Certificates are required for operations which have a potential or actual discharge of waste in liquid or semi-liquid form and, less commonly, in solid form. These certificates are legal documents and violation of any provision contained can result in enforcement action.

V. Animal Waste Sources and Animal Waste Management Procedures

A. Concentrated Animal Feeding Operations

1. EPA Requirements. For concentrated animal feeding, EPA requires an NPDES permit for all operations which confine 1,000 animal units or more and may result in a discharge during a rainfall event that is expected to occur at a 25-year frequency in a 24-hour period. Those operations that are designed to contain waste and would discharge only in the event of a 25-year 24-hour storm are exempted from the EPA requirement of the NPDES permit.

2. Board's No-discharge Certificate Program. As stated earlier, the Board's management program is equal to, and in many cases, more stringent than EPA's requirements. The Board's recently adopted strategy is to continue the State program that is at least as restrictive as EPA's for several reasons, which are as follows:

a. No discharge of pollutants virtually eliminates all possibility of water pollution of shellfish waters except in times of prolonged and unusually severe precipitation.

b. Detailed record keeping and reporting, generally associated with NPDES permits, are not required by the State no-discharge certificates. This makes the entire program easier to understand and more economically feasible for the small-scale producer/operator.

c. State no-discharge certificates are easy to handle administratively since involvement with the EPA in the issuance process is eliminated.

d. The relative infrequency with which animal wastes are collected and disposed of does not make the waste readily amenable to conventional treatment to a degree that will allow a discharge.

e. No waste water treatment operator is generally required for no-discharge operations.

f. And in no-discharge mode of operation, animal wastes are recycled to the land, thereby conserving a valuable and useful resource and almost guaranteeing that the waste from these concentrated feed lots will not reach shellfish waters.

There are many types of waste containment facilities which a farm operator could utilize following the receiving of design assistance from the experts of the agricultural agencies. The most prevalent type of facility utilized in Virginia is the anaerobic lagoon. If properly designed, allowing for 60 days extra storage rainfall in excess of evaporation, an additional capacity for the 25-year 24-hour rainfall event and one foot of freeboard, these facilities will operate satisfactorily without malfunction as long as proper maintenance is employed. When the one foot of freeboard is reached, the state certificate requires the owner to dispose of the contents on to cultivated land. As long as good land application practices are employed, as recommended by the experts of the agricultural agencies, carry-over of fecal waste into adjacent streams can be virtually eliminated. This includes immediate incorporation into the soil of the lagoon excess.

B. Non-Point Source (Unconfined) Animal Operations

Non-point source animal pollution from unconfined animal production sites is a second category of specific concern to FDA and Virginia's shellfish control authority, Bureau of Shellfish Sanitation. The most common change in stream water quality caused by these types of operation following rainfall and pollutant transfer to contiguous waters---often the only change that can be definitely identified---is elevated counts of indicator bacteria. One particular study found that it is difficult

or impossible to distinguish between pollutants from farm animal production units in an unconfined pasture-grazing type of operation and natural pollutants in the receiving stream. EPA has concluded that control of pollutants from unconfined animal production units may be to no avail unless other pollutant sources naturally occurring in the watershed are controlled as well.

If Virginia's Animal Waste Management Plan is to continue to be successful in allaying the concerns of the shellfish authorities about these types of operations, some form of model method must be established to help eliminate pollutant losses from these sources. No such model exists at present for unconfined animal production systems. Since the prediction of increases of bacteriological concentrations in shellfish waters caused by these operations is not possible within the present state of technology, good general management practices by the farm owner are the only immediate tools available to remove the question of animal waste contaminating shellfish.

Guidance regarding the significance of these types of operation on shellfish water quality has repeatedly been sought from the FDA. All manuals, publications, strategies, design criteria and other materials utilized by the Board in its Animal Waste Management Program were made available to FDA for their review in the hope that some definitive guidance would be established. Further, EPA and FDA have been requested formally to coordinate their animal waste control guidelines as they relate to shellfish water quality and animal raising operations. To date, responses received have been unacceptable and noncommittal.

It is difficult to require stringent corrective measures that impose a severe economic burden on all unconfined animal production operations. There is no assurance of success or of concurrence of a corrective program as it relates to shellfish water quality from the FDA. Thus, the inability of EPA and FDA to agree on acceptable animal pollution control requirements adjacent to shellfish growing waters is one major problem nagging our staff and Board. If we are to protect the bacteriological quality of shellfish growing waters in accordance with existing statutes, we must at least have some assurance that corrective actions taken will achieve positive results for the protection of the shellfish industry.

It is recognized that the movement of pollutants from unconfined animal production units to shellfish waters is governed by numerous complex and variable hydrological and management factors which are yet to be codified. This is probably one of the reasons FDA and EPA are reluctant to establish firm guidelines for these types of operations that would be considered sufficient to protect the marketability of shellfish. Indeed, this problem must be addressed to animal waste control programs of all shellfish producing states.

1. Studies of Diffuse Waste Generated Adjacent to Shellfish Growing Waters

Our agency is presently committed to developing designs of several non-point source studies on selected critical shellfish watersheds. The purpose of these studies is to develop some form of a mathematical model that would serve as a reliable predictive tool and provide case-by-case guidance on these types of animal waste operations.

2. Bacteriological Indicator Organisms

Field and/or laboratory procedures need to be developed which can differentiate between human and farm animal organisms found in estuarine waters. Our personnel are aware that much work is needed on this problem. But, before final rules and regulations are promulgated, clear agreements on acceptable methods must be reached.

VI. SWCB Coordination and Activity Interaction With Agricultural and Shellfish State and Federal Agencies

Close coordination and activity interaction with agricultural and shellfish State and Federal agencies is necessary if Virginia's Animal Waste Management Program is to be effective in protecting the bacteriological quality of shellfish growing waters. The State Department of Health, Bureau of Shellfish Sanitation has worked closely with our agency in this program. Many animal waste raising operations, previously unknown to the Board's staff, are routinely detected by that bureau during their routine sanitary shoreline surveys and reported to us promptly. We, in turn, launch immediate investigations of those operations considered concentrated feed lots and secure corrective action in accordance with the program and strategy established by the Board. Frequent recon-

naissance inspections by the staff are conducted and all resulting pertinent reports are made available to that bureau for their files to document shoreline corrections of concentrated animal waste sources.

The VPI&SU Extension Service and the USDA Soil Conservation Service have established detailed standards and specifications for engineering practices, advisory services, and design services to assist farmers in selecting the proper waste containment facility and animal waste management practice plan to insure no discharge of animal waste into tidal waters.

EPA has undertaken many research projects at its various research laboratories and provided State regulatory personnel with frequent training sessions and research documents. EPA has evaluated 244 different waste containment systems, the majority of which limit water pollution to acceptable levels provided that good management practices are followed. Other agencies that have assisted the Board in its Animal Waste Management Program are the Virginia Department of Agriculture and Commerce, Bureau of Dairy Services, Virginia Institute of Marine Science, Virginia Marine Resources Commission and, occasionally, the U.S. Food and Drug Administration.

VII. Accomplishments and Progress Since January 1, 1972

Since January 1 of 1972, the correction of many concentrated animal feed lot operations and subsequent retention of all waste is partially responsible for the reopening by the Bureau of Shellfish of 156,875 acres of shellfish growing waters as of August 1, 1978. Although many other successful pollution abatement programs were continuing during that period, improved bacteriological quality played a large role in this success.

Over 900 farms have been inspected, and 138 no-discharge certificates have been issued in Tidewater, Virginia.

VIII. Public Health Significance of Animal Waste Problem

Shellfish authorities, public health officials, and others have been repeatedly requested by our staff to demonstrate the public health significance of diffuse animal waste run-off into shellfish watersheds.

No definitive answers have ever been received with the exception of a report by Dr. C. A. Klein in 1972. Dr. Klein reviewed the world's literature on the transmission of diseases through shellfish. He concluded that, in the absence of definitive prospective or retrospective studies, or controlled scientific experiments, it appears unreasonable to implicate shellfish in the transmission of salmonella from non-human sources. In a study published by the Communicable Disease Center, the summary stated that "The experience of the CDC since the inception of records of salmonellosis and shellfish were not considered a part of the epidemiology of salmonellosis from non-human sources." It is possible that further studies will conclude that enteric organisms from animal sources transmitted through oysters to man are not pathogenic.

IX. Future Plans and Goals

A. Continue with Existing and Refined Coordination Plans

There are over 2.3 million farm animals raised in Tidewater, Virginia, according to 1974 census. Using one slaughter and feeder cattle as one animal unit, this amounts to over 176,000 animal units. These are raised on 2,290 animal farms in Tidewater, Virginia.

B. Continue to Seek Precise Guidance Through the NSSP and U.S. FDA

C. Complete Planned Non-Point Source Waste Studies of Selected Shellfish Growing Watersheds

D. Continue to Seek More Refined Animal Waste Control Technology and Program Procedures

X. Summary

Virginia's Animal Waste Management Program has proven in recent years to be adequate for the protection of general water quality and facilitative in Virginia's Shellfish Sanitation Program. Definitive guidelines from shellfish control authorities, both state and federal, for the proper management of unconfined animal feeding operations of diffuse animal waste run-off must be forthcoming if this Virginia management plan is to be considered totally successful for the benefit of the shellfish industry. Public health significance of animal enteric organisms ingested by shellfish needs to be clearly established or

better bacteriological procedures should be established for the differentiation between animal and human enteric organisms. Future nonpoint source watershed studies of specific shellfish growing waters will be conducted and, hopefully, will provide adequate predictive tools.

Until these models are complete, BSS and FDA guidance regarding diffuse animal waste sources becomes more precise, Virginia's management plan will continue with the existing no-discharge certificate program for concentrated feed lots.

The issue of control for diffuse animal waste sources must be resolved before additional expenditures of economic and manpower resources can be justified.

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SHELLFISH SANITATION AND HEALTH EFFECTS STUDY

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The present investigation was initiated in the course of court proceedings in which a few towns in Suffolk County, New York, challenged the validity of the present shellfish sanitation standards as enforced by the State of New York and the Federal government. In view of the tremendous economic impact of the shellfish industry on Long Island, the Suffolk County Executive and the County Legislature requested that the Department of Health Services review and study the Shellfish Sanitation Program, its enforcement, and the health effects of raw clam consumption. The study began in early 1978 as a joint project between the Suffolk County Department of Health Services and the Marine Field Station of the United States Environmental Protection Agency in West Kingston, Rhode Island.

This interim report describes the objectives of the investigation and the study design, and touches briefly on the initial findings to date. In no way was the study designed to challenge the current standards or to validate present enforcement procedures. Rather, it was intended to explore several microbiological, epidemiological and public health components of the Shellfish Sanitation Program.

Objectives of the Investigation:

1. To study the bacterial contaminants in the water of selected areas in the Great South Bay.
2. To study the bacterial and viral contaminants of samples of shellfish in the same areas.
3. To study the correlation between the bacterial indicators in the water samples and the shellfish samples collected in the same areas. This aspect of the investigation will examine the relationship, if any, of clean, conditional, and contaminated waters.
4. To study the effect of several variables on the bacterial indicators.
5. To study the health effects of consuming raw clams among selected groups and to correlate them with the various bacterial and/or viral contaminants in water and shellfish. The clams used in this aspect of the investigation will be obtained from approved areas only.

Study Design

Phase I: During this period, 275 surface and bottom water samples were collected from five areas in the Great South Bay of Long Island. Sampling was performed at different time periods in order to study the role of the tidal cycles and other variables on the bacterial counts.

The following variables were recorded and tested:

1. Temperature
2. Dissolved oxygen
3. Salinity
4. Rainfall
5. Wind direction and speed
6. Total coliforms
7. Fecal coliforms
8. Fecal streptococci

Results obtained during this 7-week phase indicate that the tidal cycle does not significantly affect the bacterial counts. The effect of the other variables on bacterial counts is still being studied.

Phase II: During this 10-week period, approximately 300 water and shellfish samples were collected from seven areas and examined for bacterial contaminants. A subsample of shellfish was examined for viral contaminants at the University of New Hampshire. Microbiological testing was performed for the identification of the following organisms (qualitatively and, whenever possible, quantitatively):

1. Total coliforms - MF, MPN
2. Fecal coliforms - MF, MPN, Al
3. *Escherichia coli*
4. Aeromonas
5. *Clostridium perfringens*
6. *Vibrio parahaemolyticus*
7. Vibrio species
8. Enterococci

Streptococcus faecalis *Streptococcus bovis*
Streptococcus faecium *Streptococcus avium*

9. Bifido bacteria
10. *Candida albicans*
11. Salmonella

Comparisons were made between the various sampling areas and during different time periods. Bacterial counts were presented according to the intensity of rainfall during the period. Bottom samples were compared with surface samples in the approved and contaminated areas. In view of the fact that the study is still in progress, any definite conclusions at this time would be premature.

Phase III: During this phase, water and shellfish are being examined in a few areas for the feeding experiment.

Many of the microbiological standards which have been adopted or proposed for recreational waters, perishable foods, or shellfish have not been fully supported by epidemiologic studies of the health effects on human populations. Some were based on risk factors or criteria which were applicable several decades ago but are not as important at present. The lack of the health effects studies precipitated the frequent challenges of the validity of the current standards or guidelines. It is for this reason that the current investigation is geared toward determining the health effects of raw clam consumption.

The ideal study design in a situation such as this would be to take several thousand volunteers, divide them at random into several groups or strata, and feed them clams collected from waters with different coliform counts or other bacterial indicators. Volunteers would be followed for a pre-determined period, and any health effects would be recorded. A decision would then be made of the count or counts which provide a reasonable degree of protection. Needless to say, the various ethical and legal problems involved made this approach practically impossible. The only alternative is to collect clams from certified areas, examine them for various indicators, and determine the health effects in volunteers following the consumption of such clams.

Criteria for Selection of Volunteers: The main criterion for the selection of volunteers was to recruit a group who could be reached in large numbers in a few sites and who would be amenable to follow-up.

An ideal group was, of course, the student population. Several colleges were contacted and informed of the purpose of the study, and administrative approvals were requested. Other groups considered to be suitable for this type of investigation were employees of the various municipal agencies and industrial plants. The acceptability of volunteers in the study was contingent on their willingness to eat raw clams, on the absence of history of allergic reactions to clam consumption, on their willingness to report any sickness during a follow-up period of two months and, if need be, to provide biological samples.

Stratification: Volunteers will be divided at random into three blocks.

Block I	To be fed raw clams.
Block II	To be fed depurated clams.
Block III	Will not be fed any clams, but will be asked to fill out a questionnaire and be available for follow-up.

The reason for feeding Block II depurated clams is to exclude the gastroenteric symptoms which are occasionally caused by consumption of the clam meat irrespective of any microbiological contamination. Block III will serve to provide information relating to the background sickness in this group of volunteers.

Size of the Sample: Considering that we do not have a rough estimate of the expected incidence of gastroenteric disease among the study population, it was very difficult to estimate with precision, the optimum sample size. To be on the safe side, a decision was made to choose about 8,000 volunteers.

The study group will continue the examination of water and shellfish in the Great South Bay as long as weather conditions permit, and will conduct the feeding experiment during 1979. It is hoped that the current investigation will help clarify many of the pertinent and ambiguous issues in the Shellfish Sanitation Program.