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Title: Occurrence of Vibrio parahaemolyticus in and Bacteriological Quality of Oregon Dungeness Crabmeat.

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Margy Woodburn, Ph.D.

Vibrio parahaemolyticus, an organism which causes foodborne infections, is frequently associated with sea water, sea fish and shellfish. There is some indication that this organism may be a part of the flora of many sea fish and shellfish in the United States. This study was conducted to determine the occurrence of V. parahaemolyticus in Dungeness crabmeat from Oregon crab processors and retail markets. Samples of crab were taken from three retail markets and from five points along the processing lines of three Oregon crab processors. The points along the processing lines included: (1) raw, whole crabs; (2) butchered, cooked and cooled crabs; (3) picked crabmeat; (4) brined crabmeat; and (5) crabmeat about to be packed. Plate counts at 20 C and numbers of fecal streptococci were determined to obtain an idea of the bacteriological quality of the crabmeat. To determine the relationship between pH and plate counts, the pH of samples was taken.

Four samples of crab from one of the Oregon crab processors contained halophilic vibrios. These were two samples of raw and two samples of cooked crabmeat. Five of 31 crabmeat samples from Oregon retail markets were positive for halophilic vibrios. The vibrios isolated varied in their degree of correlation with the biochemical characteristics of V. parahaemolyticus. The biochemical characteristics of one sample of raw crabmeat from the processor matched those of V. parahaemolyticus quite well, only varying in the sucrose and citrate utilization. Crabmeat from processors that was ready for market had a median plate count of 8.0×10^3 organisms per gm and a range of 3.7×10^2 to 5.0×10^4 organisms per gm. These counts are within the 100,000 organisms per gm standard of New York City (Food Protection Committee of the Food and Nutrition Board, 1964) and commonly used guideline of other areas. The plate counts of 28 of 31 crabmeat samples from retail markets were over this guideline. The median plate count was 1.2×10^6 organisms per gm and the range was 9.7×10^3 to 3.7×10^8 organisms per gm of crabmeat. Numbers of fecal streptococci on crabmeat from the processors were within the 1000 fecal streptococci per gm limit established by New York City (Food Protection Committee of the Food and Nutrition Board, 1964) as were those from retail markets with a median count of 5.9×10^2 fecal streptococci per gm. No correlation could be found between pH and plate counts on the crabmeat from retail markets.

Occurrence of *Vibrio parahaemolyticus* in and Bacteriological Quality of Oregon Dungeness Crabmeat

by

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INTRODUCTION

There is an indication that market fish and shellfish in the United States may be contaminated with Vibrio parahaemolyticus, an organism which causes foodborne infections. This organism was isolated from samples of market tuna sticks in Japan (Fujiwara, Ohki and Matsushita, 1967). Market fresh frozen Chesapeake Bay blue crab was also found to be contaminated with V. parahaemolyticus, (Fishbein, Mehlman and Pitcher, 1970), as were oyster, clam and mussel samples collected from retail markets on the Atlantic coast of Canada (Thomson and Trenholm, 1971). The "usual" extent of the contamination of market sea fish and shellfish needs to be explored so that guidelines and standards can be established for V. parahaemolyticus on seafood products. In this study, Oregon Dungeness crab and crabmeat were examined for the presence of V. parahaemolyticus.

V. parahaemolyticus has been found to be a part of the natural flora of sea fish, sea water, sea sediment and shellfish in the Puget Sound, state of Washington coastal waters, Gulf of Mexico and

Japanese coastal waters (Ward, 1968; Krantz, Colwell and Lovelace, 1969; Sakazaki, 1969; Baross and Liston, 1970; Vanderzant, Nickelson and Parker, 1970). It has also been isolated from sea fish and sea water in the Philippines, Taiwan, Hong Kong, Singapore, Korea and Germany (Sakazaki, 1969; Fishbein, Twedt and Olson, 1969). It appears that V. parahaemolyticus is ubiquitous, but further studies in other areas of the world are needed to verify this.

V. parahaemolyticus was first incriminated as a food poisoning organism by Fujino et al. in 1951 as a result of a food poisoning outbreak in Japan involving 272 people who had eaten shirasu which is partially cooked, dried sardines. Since then, it has been estimated that 40 to 70 percent of all food poisoning outbreaks in Japan are a result of V. parahaemolyticus contamination (Fishbein et al., 1969). Thus, it is a potential public health problem in the United States. The magnitude of the problem in the United States is unknown since V. parahaemolyticus has been considered in the examination for causative organisms only recently and in most state laboratories, as in Oregon's, it is not routinely looked for on seafood products. In 1969 V. parahaemolyticus contamination of shellfish was presumed to be the cause of two outbreaks of food poisoning at a camp. The outbreaks involved a total of 71 people (Gangarosa and Donadio, 1970). In 1970 two outbreaks of food poisoning presumed to be due to

V. parahaemolyticus occurred in the state of Washington involving about 150 people. The contaminated food in one of these outbreaks was believed to be crabmeat which was eaten at a restaurant. The causative food in the other outbreak was not known (Communicable Disease Center, 1970).

In this study crab samples from Oregon retail markets and from various points along crab processing lines of Oregon processors were analyzed for V. parahaemolyticus to determine the occurrence of V. parahaemolyticus on Oregon crabmeat and whether any spots allowing large increases of V. parahaemolyticus exist along the processing line. Numbers of fecal streptococci and plate counts were obtained to assess the bacteriological quality of the crabmeat.

REVIEW OF LITERATURE

The Dungeness Crab

Natural History and Physiology

The Dungeness crab, Cancer magister, is a large, reddish-brown crab found and fished for in the Pacific Northwest coastal waters from Unalaska to San Francisco, California. The distribution in these coastal waters corresponds to those areas having firm sandy bottoms and relatively shallow waters (Dewberry, 1959).

The Dungeness crab is a carnivore. It prefers fresh food, including oysters, cockles, small fish, shrimp, small clams and other sea animals. At times, the Dungeness crab will become cannibalistic, eating small crab (McKay, 1942). Feeding is one mechanism by which the crab may become contaminated with bacteria from its environment. The food and water which the crab ingests are carriers of bacteria. The bacteria on the food and in the water contaminate the digestive tract of the crab.

The Dungeness crab breathes by means of gills. The openings to the gill chambers are located on the underside of the crab above the base of the claws. The water is drawn through these openings into the gill chambers by means of respiratory currents created by

the beating of appendages near the mouth. Setae, which rim the openings, filter part of the water as it enters the chambers. Once in the gill chambers, the water is drawn through the gills and exits through openings near the mouth. The crab can reverse the flow of water to clean the gills (Lochhead, 1950). The filtering of the water through the gills provides another means of contamination of the crab with microorganisms from its environment. A tremendous amount of water is filtered through the gills. In the lobster, this amounts to 9.6 to 9.9 liters per hr under normal conditions (Kaestner, 1970). Consequently, a large number of microorganisms can be filtered out of the water onto the gills and into the gill chambers.

The habits of the Dungeness crab are believed to be characterized by seasonal migration. From December to February, the crab occupies the off-shore waters and spawns. It then moves inshore where the eggs hatch (Dewberry, 1959). The controlling factor in this migration may be the water temperature (McKay, 1942). The smaller, younger crabs do not take part in this migration until the width across the back of their shell reaches about four inches. The one inch crab is a member of the beach group of sea life. At two inches the young Dungeness crab leaves the beach to occupy littoral waters beyond the low tide mark. At four inches the crab joins the mature group and participates in the migration (Dewberry, 1959).

The crabs which may be legally harvested in Oregon are male, hard-shelled and six and a half inches or greater across the carapace and thus, are in the mature group.

Capture of the Crab

The Dungeness crab is caught by means of wire traps called crab pots and by ring nets. The pots are used most widely. These are wire cages with an entrance and with a special hatch for escape of crabs which are below the minimum legal size. The traps are baited with such things as clams and fish scraps, which lure the crabs into the pots (Dewberry, 1959). The bait is held in a cannister so that the crabs cannot eat it (Hilderbrand, 1970).

After the crab is caught, it is brought aboard the fishing vessel where it is kept either in a dry or a seawater flooded hold until the crabs are taken to the processing plant. In the flooded hold, sea water is pumped through the hold of the boat. The crabs are usually in transit not longer than 24 hours (Hilderbrand, 1970). Once the crabs have reached the plant, which is generally located on the docks, their fate also varies. The crabs which arrive at the processing plant in the morning are processed the same day. However, if they arrive in the afternoon or evening, they are held dry or in live tanks overnight and processed on the following day.

Processing of the Crab

The processing of a food causes changes in its microbial flora. The nature of these changes is determined by the method of processing..

The large crabs with the claws and legs intact are usually processed whole for the fresh market. These crabs are boiled in a brine solution, which is approximately three percent salt by weight, for 15-20 minutes. The length of time depends on the size of the claws. They are cooled in cold water and the shells are brushed to remove any adhering foreign matter. The whole processed crabs are then iced and sent to the market (Hipkins, 1957).

The crabs which are not processed whole are either processed for fresh crabmeat or for canning. The processing procedure for both of these is the same until the crabmeat is packed into cans. The processing procedure described will be directed toward the processing of fresh frozen or iced crabmeat.

The processing begins by butchering the crabs. This is done by hand and involves the removal of the carapace, the viscera and the gills. The crabs are thoroughly bled in fresh municipal water (Nelson, Dassow and Barnett, 1964). The butchered, bled crabs are then broken into two sections and are ready for cooking.

The cooking of the crab may be accomplished by one of two methods. One method utilizes the continuous cooker in which the crabs are carried on a conveyor belt through the cooking chamber containing boiling water qr 212-220 F steam. The crabs are held in the cooking chamber for 10-12 minutes. The other method of processing utilizes the batch cooker. Here, the crabs are lowered in baskets into vats of boiling water and held there for 9-12 minutes (Hipkins, 1957). Either method should reduce the bacterial population of the crab significantly.

The methods of cooling the cooked crab also vary. If the batch cooker was used to cook the crabs, the baskets of crab from the boiling water vats are lowered into a vat of fresh cold water. The crabs on the conveyor belt from the continuous cooker are often sprayed with fresh cold water in order to cool them (Nelson et al., 1964; Hipkins, 1957). The crabs are cooled for about two to four minutes so that they are cool enough to be handled by the "shakers" (Nelson et al., 1964). The crabs may be picked immediately or up to two days after cooking (Damon, 1971).

The term "shakers" is used to refer to the workers who remove the crabmeat from the cooled shell. The crab sections are brought to the shaking tables after the cooling process. Here, the shakers first remove the body meat. This is accomplished by lightly

crushing the body shell against the palm of the hand and table to loosen the meat. The crab section is then tapped against the side of a pan and the meat falls into it. The leg meat is removed by cracking the leg with a small metal mallet. The legs are removed from the body shell, separated at the joints and tapped against the side of a pan. The meat falls out in a whole piece (Hipkins, 1957). The body and leg meat are kept separate.

Pieces of shell and tendons invariably get into the crabmeat during picking. These are removed by brine flotation. The meat is emptied into a 25-26 percent (saturated) brine tank for less than one minute to three minutes (Nelson et al., 1964). Here, the brine is agitated continuously so the meat rises to the top and the shell fragments and tendons sink to the bottom. Since this does not eliminate all of the shell and tendons, the meat is thoroughly inspected as it comes from the tank (Hipkins, 1957), usually under an ultraviolet light under which the shells appear fluorescent. The brine in the tank is changed from every two hours to every day (Nelson et al., 1964).

The crabmeat is debrined by submerging the meat in fresh water for less than one minute or by spraying the meat with fresh municipal water followed by submersion in more fresh water (Hipkins, 1957). After the meat has been debrined, it must be drained

to remove excess moisture. This is accomplished by allowing the crab to set in drain pans for as long as 30 minutes. The body meat is sometimes centrifuged for 10-15 seconds to drain it (Nelson et al., 1964).

The equipment used in the processing of the Dungeness crab is generally stainless steel. It is washed and sterilized each day.

The drained crab is vacuumpacked into number ten, C-enamel cans and sealed with a tight fitting lid. The body meat is placed in the bottom layer and the leg meat is placed in the top layer of the can (Hipkins, 1957). The packed meat is either frozen at such temperatures as 0 or -20 F or packed in ice, and then, transported to the fresh market. At the market cans of fresh, cooked crabmeat are divided into smaller portions. It they are frozen, they are first thawed in a refrigerated room. The crabmeat may be sold in individual meat packages or weighed out individually for each customer.

Microbiological Flora of the Crab

Deterioration of the crab sets in soon after death. The high level of free amino acids in crab muscle contributes to this deterioration (Fieger and Novak, 1961). Consequently, the crab should be kept alive until processing. They die quickly if left in a dry environment or in the direct sunlight, so they are kept cool and damp

(Jarvis, 1943). Roach (1956) found that the crab can live for five days in cooled sea water at 35° F.

The meat of freshly caught crab is almost sterile. However, the body surfaces, the intestines and the gills are rich sources of bacteria and can quickly contaminate the meat (Fieger and Novak, 1961). Harris (1932) found the viscera of fresh blue crabs to have an average total aerobic count of 10^6 organisms per gm of crabmeat at 20° C. Any contamination of the crab that has occurred should be greatly reduced by the boiling process. However, not all of the bacteria are destroyed. Ward and Tatro (1970) found counts of less than 3,000 organisms per gm on whole crabs taken directly from the retort and counts of less than 10,000 cells per gm on cooled, boiled whole crabs. Benarde and Austin (1959) found total counts as low as 100 organisms per gm on boiled crabs.

As processing continues, the contamination of the crab increases. Benarde and Austin (1959) found that the bacterial numbers on fresh crabmeat are directly related to total handling of crabs after cooking. Ward and Tatro (1970) found that after picking, 41 percent of the crab samples had total plate counts over the 100,000 organisms per gm of meat which they considered permissible.

Several studies have been conducted to determine the flora of crabmeat after it has been processed and packed into cans.

Shiflett, Lee and Sinnhuber (1966) found the predominating species in the initial flora of packed crabmeat to be Achromobacter. However, Tobin, Alford and McCleskey (1941) found initial counts to be almost exclusively cocci, with some Bacillus, Achromobacter, Flavobacterium, Alcaligenes, Aerobacter, Escherichia and Pseudomonas. Harris (1932) also found these organisms on fresh crabmeat. Total plate counts of the packed crabmeat ranged 10^5 to 3 x 10^6 organisms per gm of crabmeat (Tobin et al. 1941).

With storage, the psychrophilic organisms take over and spoilage occurs. Alford, Tobin and McCleskey (1942) reported that Pseudomonas and Achromobacter predominate in the spoiled crabmeat. Harris (1932) reported that blue crab samples did not show signs of spoilage up to the ninth day of refrigerated storage. The total aerobic count reached a peak on about the 15th or 20 day of storage. The bacterial population of spoiled crabmeat is several million organisms per gm of crabmeat (Tobin et al., 1941).

Fecal streptococci or coliform analysis are often conducted to determine the level of fecal contamination from humans and animals. Raj, Wiebe and Liston (1961) reported that the fecal streptococci occur in higher numbers than do the coliforms on seafoods. They found a usual fecal streptococci count of 10^4 organisms per 100 gm of laboratory heat-treated and frozen fish samples.

Few areas in the United States have standards for numbers of fecal streptococci and plate counts for fresh crabmeat. Oregon follows the federal guidelines which include a standard for Escherichia coli but not for fecal streptococci or for plate counts. The standards of New York City are often used as unofficial guidelines by other areas of the country. These standards include a 1000 count per gm for fecal streptococci and a 100,000 count per gm for plate counts on the crabmeat (Food Protection Committee of the Food and Nutrition Board, 1964).

Vibrio parahaemolyticus

Characteristics of Vibrio parahaemolyticus

An organism which is believed to be a part of the natural flora of the crab is V. parahaemolyticus. V. parahaemolyticus is a pleomorphic, halophilic, facultatively anaerobic, gram negative rod. It grows in ordinary media with a salt concentration from one to seven percent. The optimum pH is from 7.5 to 8.5. The optimum temperature is 37.5 C, but growth occurs between 15 and 40 C (Sakazaki, 1969).

V. alginolyticus was not differentiated from V. parahaemolyticus until 1968 by Sakazaki. Before that time V. alginolyticus was considered to be biotype 2 of V. parahaemolyticus. The literature

before 1968, therefore, does not clearly distinguish between these two microorganisms.

Vibrio parahaemolyticus Foodborne Infection

The ingestion of large numbers of V. parahaemolyticus results in gastroenteritis. The numbers of V. parahaemolyticus required to produce illness in humans is not known. The illness is of the infection type. The symptoms of the gastroenteritis usually occur 15 to 17 hrs. after eating (Fishbein et al., 1969). They include abdominal pain, nausea, vomiting and diarrhea. Recovery from the symptoms occurs in two to five days. The fatality rate is low with the deaths usually occurring in the aged and very young (Sakazaki, 1969).

Habitat of Vibrio parahaemolyticus

The difference in the incidence of V. parahaemolyticus foodborne infection in Japan and the United States is partly due to the difference in the people's consumption of seafood, especially raw seafood, since V. parahaemolyticus is associated with seafood and sea water. The organism has been isolated from sea water, sediment and fish in many parts of the world, including Germany, the United States, Mexico, Korea, Taiwan, Singapore and Hong Kong

(Sakazaki, 1969; Baross and Liston, 1970). In Japan it has been found that counts vary from nondetectable to 10^5 organisms per liter of coastal sea water during the summer season (Horie, Saheki and Okuzumi, 1967).

In the United States V. parahaemolyticus has been isolated from the waters and sediment of the Washington coast and Puget Sound. Water samples taken in a commercial oyster bed in Purdy, Washington were 78 percent positive for V. parahaemolyticus, whereas those from the Puget Sound were 18 percent positive. All of the sediment samples from both areas were positive. The organism was most abundant in inshore sediment and intertidal waters. It was also found that the organism was more abundant in waters with high organic matter, such as some polluted waters, than in waters with a low organic matter content (Baross and Liston, 1970). V. parahaemolyticus has been isolated from other areas of the United States, also. Ward and Tatro (1970) isolated V. parahaemolyticus from sediments taken from the coastal area of the Gulf States. These sediment samples included mud, sand, shell and clay. Bartley and Slanetz (1971) isolated the organism from estuarine waters of New Hampshire. During the month of September, they found counts up to 1000 V. parahaemolyticus per liter of water.

The Puget Sound and state of Washington coastal waters are

the main areas in the United States in which V. parahaemolyticus has been isolated from sea fish and shellfish as they were taken from their normal habitat. Baross and Liston (1970) found that the organism was most abundant in molluscan shellfish. All oyster and clam samples taken from oyster areas in Washington were V. parahaemolyticus positive. The numbers of V. parahaemolyticus in oyster samples were in excess of 1000 cells per gm of shellfish meat. The intestines of crab taken from a polluted area of Bellingham Bay in Washington were all positive for V. parahaemolyticus. Of 15 species of sea fish tested for V. parahaemolyticus, only the Pacific herring, cat shark and sablefish showed no vibrios. Those found to be positive for V. parahaemolyticus were English sole, sand sole, Pacific cod, rockfish, hake, skate, ratfish, staghorn sculpin, starry flounder, pile perch, and Pacific dogfish. V. parahaemolyticus was isolated from the gills, skin and gut of these 12 sea fish species (Baross and Liston, 1970).

Bartley and Slanetz (1971) isolated V. parahaemolyticus from oysters collected from New Hampshire estuarine waters. They found counts of up to 500 per gm of oyster meat.

V. parahaemolyticus seems to thrive in shellfish environments in which stress conditions prevail, such as in laboratory tanks or aquaria for shellfish, and to be pathogenic for some shellfish when

the organism is present in large numbers. This has been found to be true for both the Chesapeake Bay blue crab, Callinectes sapidus, and gulf coast brown shrimp. When blue crabs were being retained in commercial tanks, large numbers of V. parahaemolyticus were isolated from lethargic and moribund crabs. Mortality in the tanks was greater than 50 percent (Krantz et al., 1969). The brown shrimp held in lab aquaria died after being fed white shrimp caught in Oyster Lake on West Galveston Bay. It was concluded that V. parahaemolyticus was the cause of the deaths (Vanderzant et al., 1970).

V. parahaemolyticus has been found to be present on market samples of sea fish and shellfish. In the Netherlands, V. parahaemolyticus was isolated from one of 407 samples of fish and shellfish purchased at three fish shops (Kampelmacher et al., 1970). In Japan, Temmyo (1966) isolated V. parahaemolyticus from five of 55 samples of frozen seafood products. One of the isolates was from sliced cuttlefish, one from cod, one from shellfish and two from sliced tuna. The Most Probable Number of these samples ranged from 29 to 1100 V. parahaemolyticus per gm. Market fresh frozen Chesapeake Bay blue crab was found to be contaminated with V. parahaemolyticus. Twenty-five of 60 samples of the crabmeat were positve for the organism (Fishbein et al., 1970). Fresh oysters, clams and mussels from retail markets on the Atlantic coast of Canada were found to be

positive for V. parahaemolyticus or related halophilic vibrios (Thomson and Trenholm, 1971). All clam samples tested were positive, one of six oyster samples was positive and one of 14 mussels was positive for V. parahaemolyticus.

An interesting development has been the isolation of V. parahaemolyticus from the excrement of birds raised in a zoo in Japan (Ose, 1967). These vibrios were found to be analogous to V. parahaemolyticus except they grew in one percent peptone broth. This isolation could be significant to the public health problem of V. parahaemolyticus because the birds' excrement represents a route of contamination of fishery products.

Seasonal Incidence of Vibrio parahaemolyticus

The incidence of V. parahaemolyticus in sea fish, sediment and water and the incidence of food poisoning outbreaks due to V. parahaemolyticus seems to follow a seasonal pattern. In Japanese waters the incidence of V. parahaemolyticus is highest during spring and summer with the peak in the summer (Miyamoto, Nakamura and Takizawa, 1962). The incidence of the organism on the Purdy oyster from the state of Washington coastal waters was also found to be seasonal with the peak incidence occurring in July. The indicated seasonal incidence of V. parahaemolyticus in sea water and fish

parallels the incidence of food infection due to the organism. In Japan the incidence of V. parahaemolyticus food infection is confined to the months of May to October (Sakazaki, 1969).

Temperature Sensitivity of Vibrio parahaemolyticus

The seasonal incidence of V. parahaemolyticus could be due to the organism's sensitivity to low temperatures. In the case of the Purdy oyster from Washington coastal waters, the temperature of the water paralleled the incidence of V. parahaemolyticus. It appears that the number of V. parahaemolyticus decreases as the temperature of the water decreases. Another indication of the sensitivity of this organism to temperature may be the fact that in the colder waters of the Puget Sound, there are fewer V. parahaemolyticus than in the warmer coastal waters. Also, the incidence of this organism in sediments decreases as the sediments are taken from areas increasingly remote from land (Baross and Liston, 1970). However, these phenomena may be the result of other factors also.

In the laboratory, V. parahaemolyticus has been found to be quite sensitive to low temperatures. Baross and Liston (1970) found that the organism would not grow in pure culture at temperatures below 8 C. In the same laboratory, it was found that freezing at -10 C and -24 C reduced the numbers of V. parahaemolyticus in fish

homogenate. At -10 C a population of 10^7 cells of Oda strain V. parahaemolyticus was reduced to 4 x 10^3 cells in four days and to less than 10^3 cells in eight days of storage. At -24 C a population of 10^7 cells of Oda strain V. parahaemolyticus was reduced to 2 x 10^5 cells in four days (Liston et al., 1967). Temmyo (1966) found that V. parahaemolyticus in peptone solutions with counts of 10^6 cells per gm could be killed within five days at -2 C. At 4 C the bacterial counts were markedly reduced with storage. In pure culture using agar medium, V. parahaemolyticus has been found to be killed at -20 C, -10 C and 0 C in a short period of time. However, the organism inoculated into tuna pieces under similar conditions survived for a considerable time (Asakawa, 1967). This instability of V. parahaemolyticus to low temperatures could create difficulty in the isolation of V. parahaemolyticus from food samples that had been frozen, especially when the organism occurred in low numbers on the food sample.

Very little work has been conducted on the heat sensitivity of V. parahaemolyticus. However, Liston et al., (1967) concluded that the organism is more readily inactivated by heat than other food poisoning organisms such as Staphylococcus. They found that a population of 4 x 10^6 Oda and Nake strains of V. parahaemolyticus was reduced to less than 10^3 cells in five minutes at 60 C both in a

phosphate buffer and fish homogenate. Freitas Leitão (1970) also found that V. parahaemolyticus strain ATCC17802 is quite sensitive to heating. At 50 C a population of 10^5 cells could be reduced to 10^1 within two minutes.

MATERIALS AND METHODS

Introduction

To determine the occurrence of V. parahaemolyticus on Oregon Dungeness crabmeat, samples of crab were collected from Oregon crab processors and Oregon retail markets. The samples from the crab processors gave an idea of initial contamination and of any trouble spots along the processing line. The samples from the retail markets revealed the extent of contamination on the crabmeat that the consumer would purchase. The samples from the two sources would give an index of the potential health hazard of fresh Dungeness crabmeat in Oregon.

Description of Processors

Crab samples were taken from the processing lines of three Oregon crab processors. The choice of processors was based on the recommendations of Ken Hilderbrand, Oregon State University Seafood Extension Agent. It was desirable that the crab be sold fresh or frozen in the retail markets. Processors X and Y processed crab caught along the northern Oregon coast, and Processor Z used crab caught along the central Oregon coast. Processor Z processed crab which was sold in retail markets used in this study. It is labelled as

brand b.

These processors followed the same general processing procedure for the crab as described in the Review of Literature. Figures 1, 2 and 3 outline the processing procedure of processor X, Y and Z, respectively. The method by which these processing steps was carried out varied among the processors. Processor Z used a rotating brush to remove the viscera and gills from the butchered crab before it was cooked. Processors X and Y did not. Processors X and Y utilized a continuous cooker to boil the crabs whereas processor Z used a batch cooker. Only processor Z weighed out the cooked, drained crab into wire baskets before picking. The other two processors allowed the crab to fall from a conveyor into the wire racks as they were carried from the cooking tank to the cooling tank. After picking and weighing, only processor X rinsed the crabmeat by means of a hose sprayer.

Another difference in the procedures occurred in the brining-inspecting step. Processor Z emptied the crab from picking pans into a brine tank, scooped the crab out with a wire ladle into the same picking pan, drained it, emptied it onto a packing table, inspected it under an ultraviolet light and packed it. Processors X and Y carried the crab from the brine tank via a metal-mesh conveyor belt under an ultraviolet light to be inspected, rinsed the crab in a flow-through

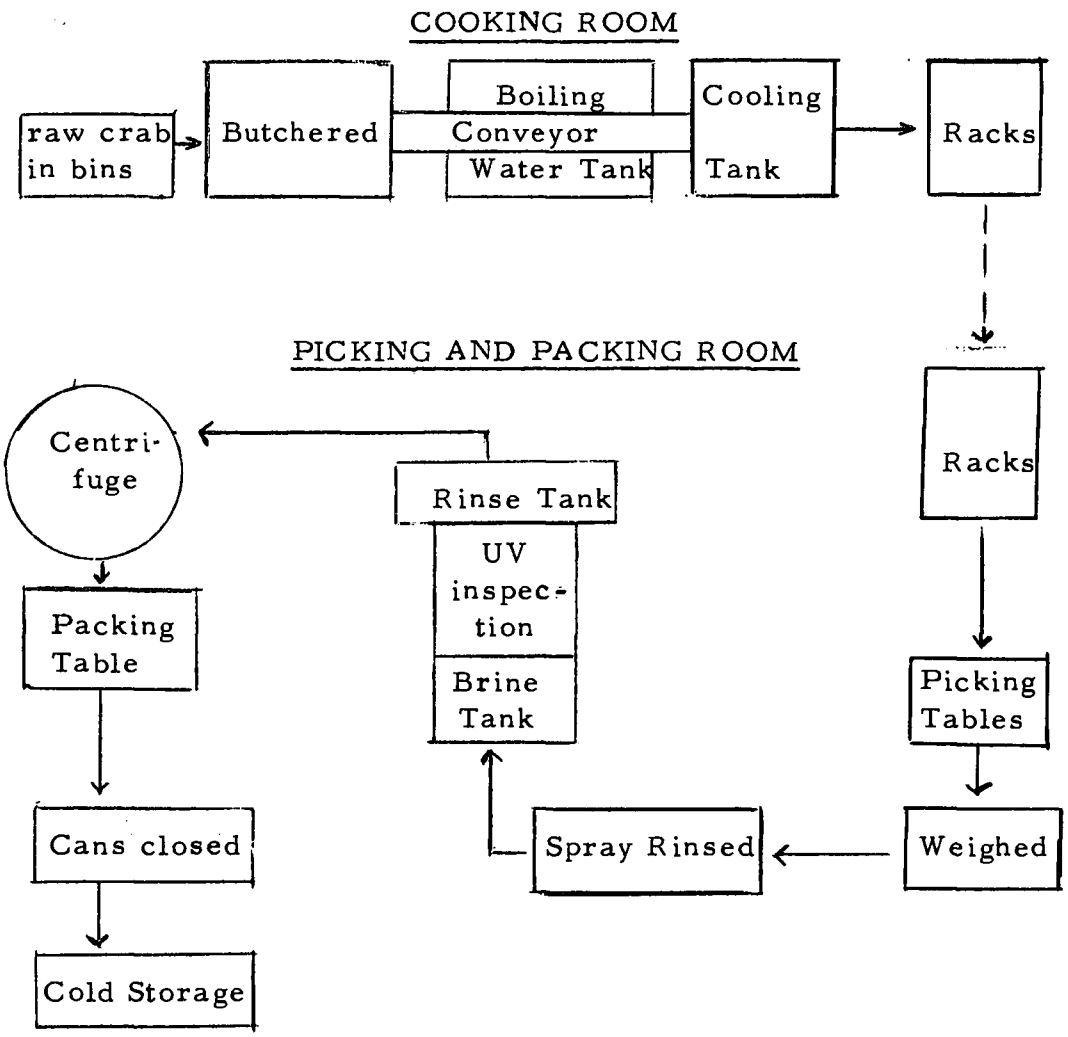

Figure 1. Flowsheet for the crab processing procedure in the plant of processor X.

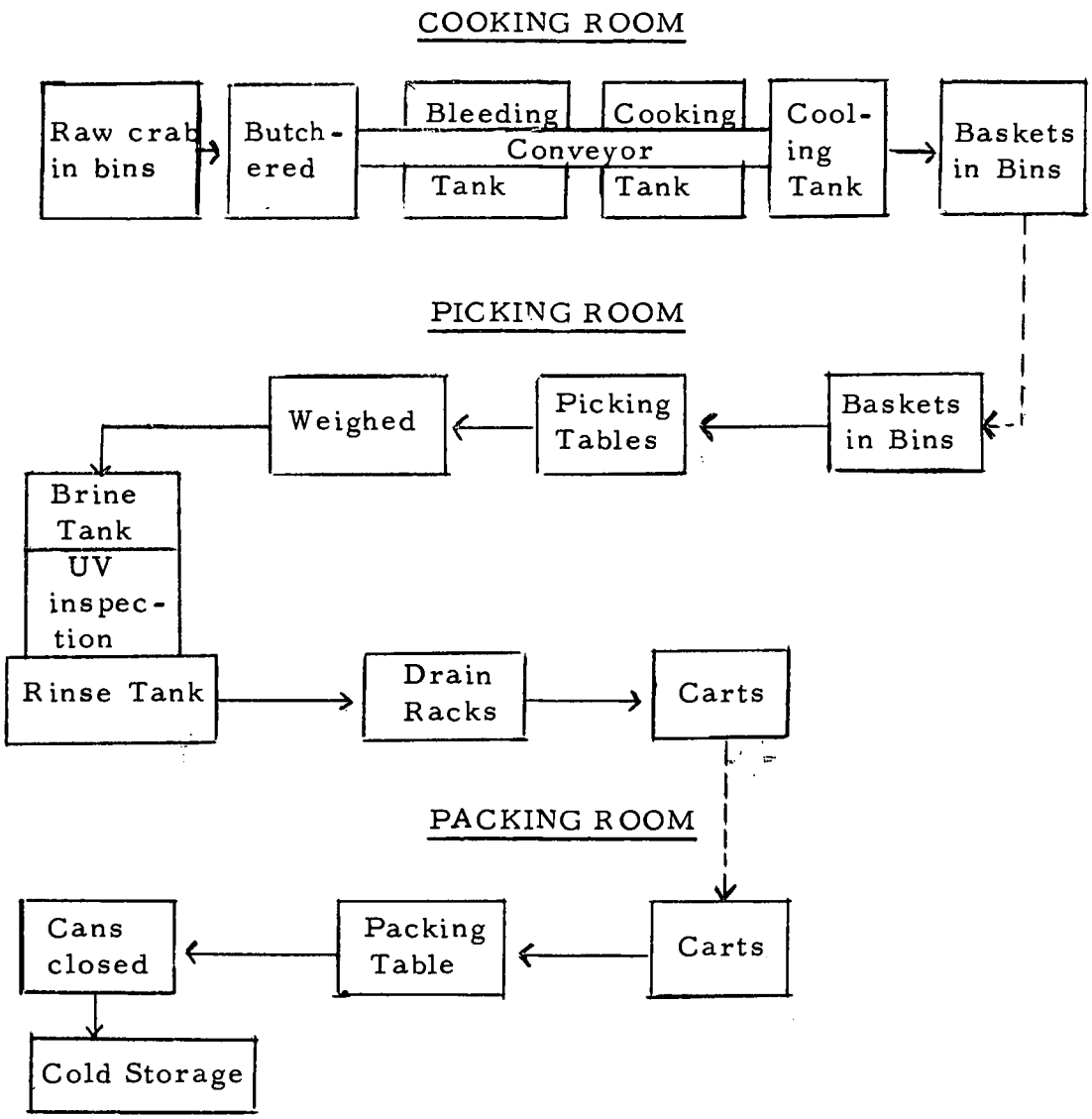

Figure 2. Flowsheet for the crab processing procedure in the plant of processor Y.

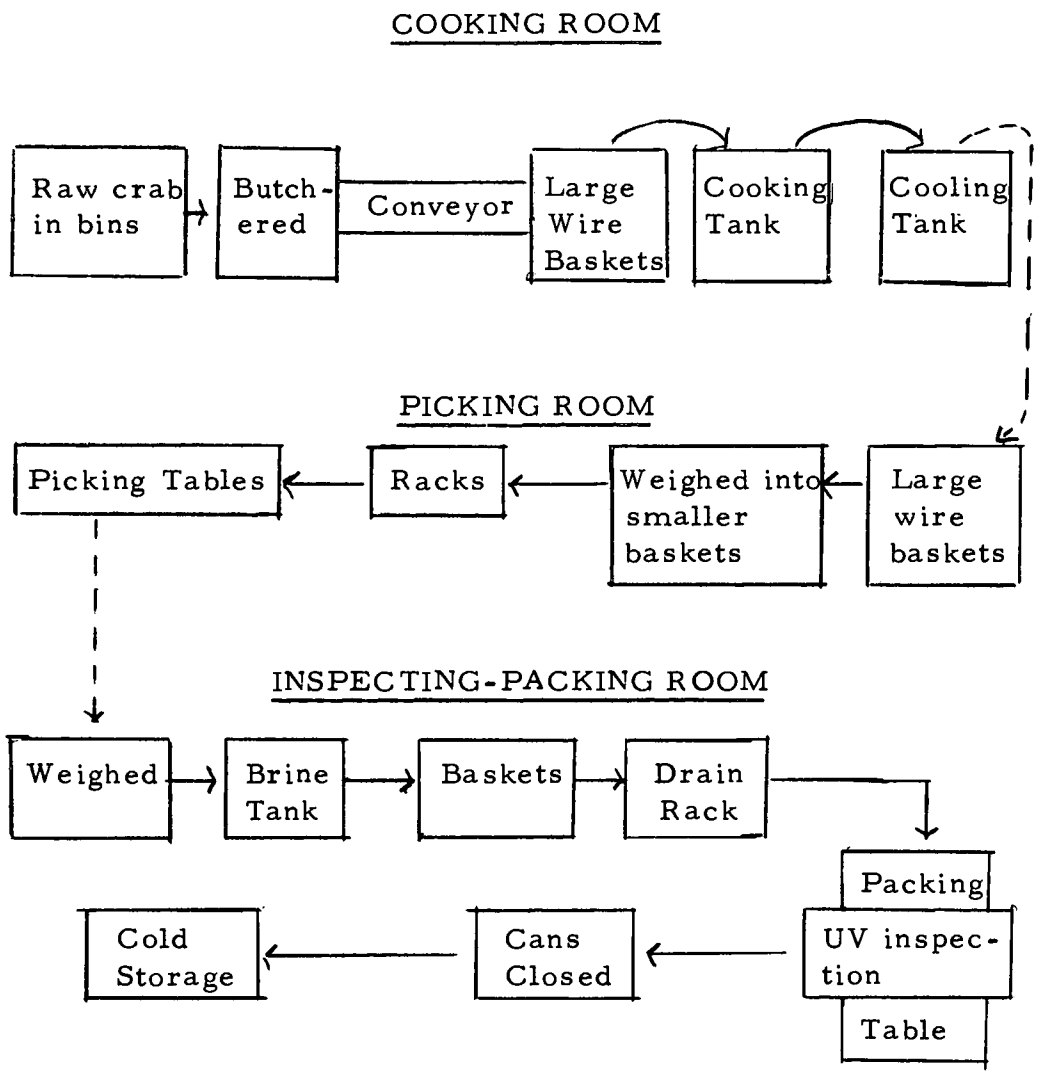

Figure 3. Flowsheet for the crab processing procedure in the plant of processor Z.

tank, drained it and packed it. Processor X drained the crab by means of a centrifuge; processors Y and Z drained it in pans on a metal rack.

Sanitation measures used were similar for the processors. All three processors cleaned and sanitized the equipment daily. At each coffee break the floor was hosed down with water. All workers, except those at the inspection table, wore rubber gloves. Each time the picker brought a pan of picked crabmeat up to be weighed, she rinsed her gloves and apron front in chlorinated water. Each time the shaker from processor X finished picking a batch of crab, her area was hosed down with chlorinated water. All equipment used was stainless steel or metal, except some of the bins in which the raw crabs were transported which were wooden.

Descriptions of Markets

Three large retail markets were used as sources of fresh crabmeat. A survey of local markets was conducted; and the choice of the three markets to be used was made so as to include a variety of crab wholesalers and brands of crabmeat. Each of these markets purchased crabmeat from a different wholesaler. The wholesalers provided the markets with a variety of brands which utilized crab caught along the Oregon and Washington coasts.

According to the market butchers, the crabmeat usually arrived at the store the day after it had been processed. It arrived in vacuum-packed no. 10 cans. Markets A and B packaged the crabmeat into individually Saran-wrapped, plastic foam trays. Since market B received seafood deliveries once a week, part of the packages were refrozen until needed and part were put into the meat case. Market A received seafood deliveries two times per week, so all of the individually wrapped crabmeat was put into the meat case until it was sold. Market C emptied the crabmeat into a 12" x 24" stainless steel pan which was displayed on ice in an enclosed glass case. The crabmeat was then portioned, using a metal scoop, as requested by the customer, and wrapped in waxed paper and butcher's paper. Market C received crabmeat two or three times per week.

Sampling Procedure

From the Processors

Samples were collected from processors X and Z on two different occasions.. Processor Y was sampled once. Samples were taken at the processing plants during the morning hours. The first sampling from processor X was from 10:30 to 11:30 a.m. All of the other sampling periods at processors X, Y, and Z were from 8:30 to 9:30 a.m.

Samples were taken from the following points along the processing lines: (1) raw, whole crab in bins ready to be butchered; (2) butchered, cooked and cooled crab, either whole or halved, ready to be picked; (3) picked crabmeat, including both leg and body meat; (4) crabmeat after brining or after brining and inspection under a UV light; and (5) crabmeat, leg and body, that was about to be packed into cans.

Six samples were taken at each sampling spot. Six whole crabs were taken of both raw and cooked crabs. With the picked, inspected and packed samples, six approximately one-fourth pound samples were taken from each spot.

Disposable plastic gloves were used to pick up the samples. The raw and butchered cooked crabs were placed in unused heavy polyethylene bags which were closed with wire twists. The crabmeat samples were placed into fold-up cardboard freezer boxes and sealed with masking tape.

Since it has been reported that V. parahaemolyticus is sensitive to low temperatures (Baross and Liston, 1970), three of the six samples from each sampling location were put into insulated containers with two packs of frozen plastic gel refrigerant so the temperature remained about 10 C. These samples were used for V. parahaemolyticus determinations. The other three samples from each

location were put into similar insulated containers with abundant ice or packs of frozen gel refrigerant to maintain the temperature near 0 C. These samples were used for total plate counts, fecal streptococci determinations and pH.

Three and a half to four hours elapsed between samplings from the processor and the analysis of the crab for processor X and Y. One and a half to two hours elapsed for processor Z.

From the Markets

The markets were sampled on any day of the week except Sunday. The samples were taken from 11:00 a.m. to 1:00 p.m. The samples were purchased at the markets and transported to the laboratory in an unrefrigerated state, as the customer would do. Not more than 45 minutes elapsed between the time of purchase and that of analysis.

Laboratory Sampling

For crabmeat samples from the market, the same package of crabmeat was used for all analyses performed. All anayses were begun the day of purchase.

For the processors, the six samples of crabmeat from each point along the processing line were divided into two groups of three

samples each. One of these groups was used for V. parahaemolyticus analysis and the other was used for fecal streptococci, pH and total plate counts. The V. parahaemolyticus analysis was begun the day of collection of samples. The others were begun early the following day.

For all crab samples 50 gm of crabmeat were weighed into 450 ml of three percent sodium chloride solution in a quart jar to make a 1:10 dilution of the crabmeat.

For the raw, whole crabs, the meat was scooped out of the body section and legs with an alcohol-flame sterilized spoon after the crab had been washed in cold running tap water, and had had its carapace, viscera and gills removed. The meat from the body section of the cooked crab halves was scooped out of the body section and legs. For both the raw, whole crabs and the cooked crab halves, sterile plastic, disposable gloves were worn at all times when handling the crabs, and an alcohol-flame sterilized metal hammer was used to crack the shells of the legs.

The crabmeat samples in boxes from the processors and in packages from the retail markets were weighed directly from the box or package into the quart jar.

The blade and gasket of an electric blender were sterilized in 70 percent ethanol, rinsed three times in sterile distilled water

and transferred to the quart jar. The crabmeat was then blended on low speed for three minutes.

The 1:10 dilution of crab in the quart jar was used to make further dilutions. Dilutions of 1:100, 1:1000 and 1:100,000 were made of the market crabmeat. Crab from processors had dilutions of 1:100 and 1:1000 for all samples. These dilutions served for V. parahaemolyticus analysis, fecal streptococci determinations, total plate counts and pH. A summary of the procedure is presented in Figure 4.

Vibrio parahaemolyticus Analysis

Two methods were utilized to isolate V. parahaemolyticus: the method developed by Liston and Baross, University of Washington, for use on water, sediment and fish samples and that developed by Japanese workers for use on stool samples. By using these methods, both quantitative and qualitative methods were utilized. The procedure for these methods was adapted from that presented in Bacteriological Analytical Manual (1969). Changes that were made included alterations in dilutions used, the incorporation of the Most Probable Number procedure (MPN) and the substitution of Kovac's oxidase test (Steel, 1961) for the cytochrome oxidase test presented.

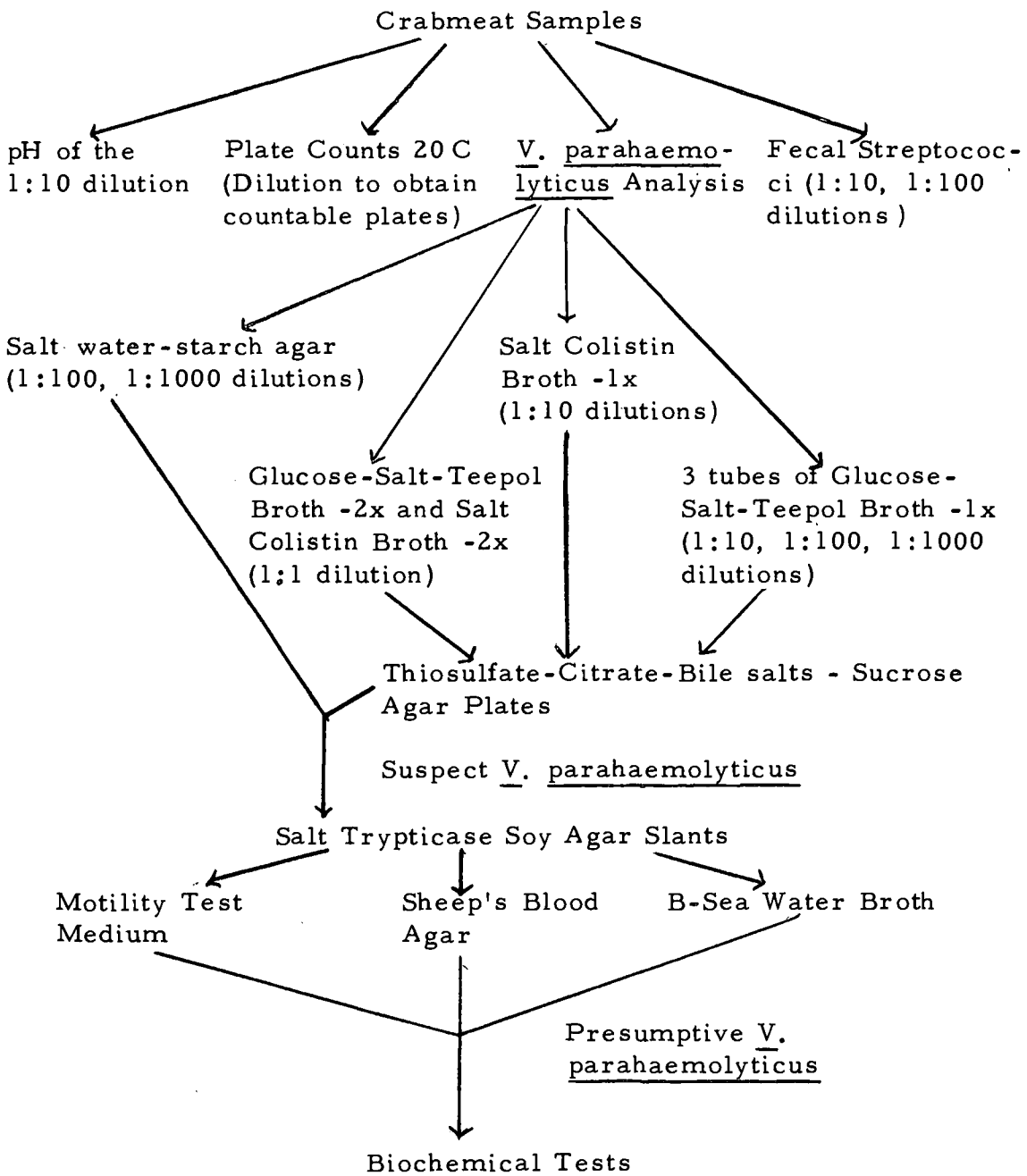

Figure 4. Outline of analytical procedure on crabmeat samples.

Liston-Baross Method

This method involved the direct plating of the crabmeat dilutions onto salt water-starch agar (SWSA) (Food and Drug Administration, 1969). This medium was composed of 0.3 percent peptone (Difco Laboratories, Detroit), 0.1 percent yeast extract, 0.5 percent soluble potato starch, 1.5 percent agar and 4.0 percent sodium chloride. Suspect V. parahaemolyticus colonies were those that were white, non-spreading and amylase positive, indicated by a clearing or halo around the colony.

From the 1:10 and 1:100 dilutions of crabmeat, one-tenth ml was pipetted onto the surface of SWSA plates and spread by means of the spread plate technique. These plates were incubated anaerobically (GasPak Anaerobic System, Baltimore Biological Laboratories, Baltimore) at 37 C for 48 hours.

Suspect V. parahaemolyticus colonies, as described above, were transferred to slants of Trypticase soy agar (BBL) with three percent sodium chloride and incubated 24 hours at 37 C.

Japanese Method

The Japanese method involves the use of an enrichment in broth and consequent plating of the broth onto a selective medium. Two enrichment broths were used, glucose-salt-Teepol broth (GSTB)

(Food and Drug Administration, 1969) and salt-Colistin broth (SCB) (Tanabe Seiyaku Co., Ltd., Osaka, Japan). These were used in single (1x) and double (2x) concentrations. GSTB-1x was composed of 0.3 percent meat extract, 1.0 percent peptone, 3.0 percent sodium chloride, 0.5 percent glucose, 0.0002 percent methyl violet and 0.4 percent Teepol. SCB-1x contained 0.3 percent yeast extract, 1.0 percent peptone, 2.0 percent sodium chloride and 500 ug/ml Colistin methanosulfate. The GSTB-2x and SCB-2x had double these concentrations, respectively. The selective agar medium used was thiosulfate-citrate-bile salts-sucrose agar (TCBS) (BBL).

Presumptive positive V. parahaemolyticus colonies on TCBS are round, two to three millimeters in diameter with a green or blue center. V. alginolyticus colonies, which appear larger and are yellow on this medium, were also subcultured.

One tube containing 20 ml of SCB-2x and one tube containing 20 ml of GSTB-2x were inoculated with 10 ml of the 1:10 dilution of the crabmeat. One ml of the 1:10 dilution was inoculated into 10 ml of SCB-1x. Three tubes of GSTB-1x were inoculated for each of the 1:10, 1:100 and 1:1000 dilutions so as to utilize the MPN procedure.

All of the SCB and GSTB tubes were incubated for 18 hr at 37 C. After the incubation, a three mm loopful of the broth was streaked onto TCBS agar plates. These plates were incubated at 37C for 24 hr.

Typical V. parahaemolyticus colonies were transferred to STSA and incubated 24 hr at 37 C. Large yellow colonies indicating V. alginolyticus were also transferred to STSA slants. It was felt by the researcher that V. alginolyticus is important also as it may be a cause of illness (Baross, 1971).

In preliminary work using pure cultures of V. parahaemolyticus strain YMK33 from Dr. R. M. Twedt of the Food and Drug Administration in canned crabmeat samples, it was found that the Japanese method using GSTB and TCBS could detect an inoculum of eight V. parahaemolyticus per gm of crabmeat.

Identification of Vibrio parahaemolyticus

The cultures isolated by means of the Liston-Baross and Japanese methods were tested for V. parahaemolyticus through the use of biochemical tests.

The isolates on the STSA slants were inoculated onto blood agar base (Difco) containing five percent sterile, defibrinated sheep's blood and into motility test medium (BBL) and B-sea water broth (BSWB) (Food and Drug Administration, 1969), (0.5 percent Bacto-peptone, 0.4 percent BBL Phytone, 0.1 percent Bacto-yeast extract and 4.0 percent sodium chloride). Presumptive V. parahaemolyticus were gram negative, pleomorphic, motile rods which were hemolytic on sheep's blood agar. These

presumptive V. parahaemolyticus were carried through further biochemical testing.

The following biochemical tests were conducted on suspect V. parahaemolyticus cultures according to the procedure outlined in Bacteriological Analytical Manual (1969): growth in Trypticase broth with 0, 3, 7, and 10 percent sodium chloride; nitrate reduction; indole production; cholera red reaction; methyl red and Voges-Proskauer reaction; gelatinase, phenylalanine deaminase, urease and catalase production; malonate and citrate utilization; glucose fermentation or oxidation and the fermentation of carbohydrates including adonitol, arabinose, cellobiose, inositol, lactose, maltose, mannitol, salicin, sucrose, trehalose and xylose. Oxidase production was tested for by Kovac's oxidase test as presented by Steel (1961). Cultures isolated on TCBS were tested for starch hydrolysis on SWSA. Sensitivity to penicillin was tested for by growth at 37 C on SWSA with 2-2.5 units of penicillin per ml.

The above biochemical tests were also conducted on known V. parahaemolyticus cultures, including strains ATCC17802; YMK33 from Dr. R. M. Twedt, Food and Drug Administration, and SB04-422 from Dr. John Liston, University of Washington; on a gram positive cocci which was a common contaminant from SCB on the TCBS agar plates and on a gram positive rod isolated on SWSA.

Analysis for Fecal Streptococci

Analysis for the detection of fecal streptococci was conducted to determine the level of fecal contamination. It was thought that the fecal contamination might correlate with V. parahaemolyticus numbers since it is believed that V. parahaemolyticus occurs in higher numbers in water with a higher organic matter content, i.e., is more polluted (Baross and Liston, 1970). The fecal streptococci were used rather than the more commonly used coliforms because of the evidence that the coliforms are more sensitive to low temperatures than the fecal streptococci (Raj et al., 1961; Digirolamo, Liston and Matches, 1970) and that the fecal streptococci occur in higher numbers in seafood samples than the coliforms (Raj et al. 1961).

KF-streptococcal agar (Difco) was used in the analysis for fecal streptococci. This medium is selective for Streptococcus faecalis, S. bovis, S. equinus, S. mitis and S. durans. These appear as pink or red colonies in the agar.

In preliminary work both KF-streptococcal broth and KF-streptococcal agar were tested. It was found that the agar and broth gave approximately equal counts. Consequently, it was decided to use only the agar plate method.

One ml of each of the 1:10 and 1:100 dilutions of the crabmeat samples was pour plated with KF-streptococcal agar. These plates were incubated 48 hr at 37 C. The pink and red

colonies were counted as fecal streptococci.

Total Plate Counts

Total plate counts at 20 C were conducted to get an idea of the total number of mesophiles. These counts give an index of contamination and growth during processing and handling and of the spoilage of the crabmeat. It was thought that perhaps the plate counts would correlate with the V. parahaemolyticus counts of the crabmeat samples.

Dilutions of $1:10^3$, $1:10^4$, $1:10^5$, and $1:10^6$ for the market crabmeat were pour-plated with plate count agar (Difco). For the crabmeat from processors, $1:10^1$, $1:10^2$, $1:10^3$, and $1:10^4$ dilutions were pour-plated with plate count agar. These plates were incubated at 20 C for 48 hr. All colonies were counted.

pH

Some workers have indicated that the pH of crabmeat rises as spoilage proceeds (Harris, 1932) and thus, it has been commonly believed that the pH of crabmeat can be used as an index of spoilage. Other workers believe that pH is not a good index of spoilage since it cannot be correlated with numbers of bacteria and it varies with the kind of crab (Fieger and Novak, 1961). To study the relationship

between bacteriological counts of Dungeness crab and pH, the pH of the crab samples was taken using the 1:10 dilution of the crabmeat samples from Oregon processors and retail markets. A Corning pH meter was used.

Statistical Analysis

To determine the relationship between numbers of fecal streptococci and plate counts and between pH and plate counts of crabmeat, the correlation coefficient, r, was calculated for fecal streptococci counts and plate counts of crabmeat from processors, for fecal streptococci counts and plate counts of crabmeat from retail markets and for plate counts and pH of crabmeat from retail markets. A table of correlation coefficients at the 5 percent and 1 percent levels was used to determine the significance of r.

RESULTS AND DISCUSSION

Description of Sample Collection From the Processors

The crab samples that were collected from the processors were slightly different depending on which processor they came from and on the sampling period.

With processor X, the raw crabs were taken from both metal and wooden bins. All bins were inside the building. The butchered, cooked crabs were taken from racks in the butchering area during the first sampling period but from racks in the picking room during the second sampling period. During both periods, each of the crab samples was taken from a different shelf. The picked crabmeat was taken from pans after weighing and before rinsing. During the first sampling period, the leg meat samples were taken from two pans and the body meat was from one pan. During the second sampling period, only leg meat was taken. It came from two different pans, each sample had meat from each pan. In both sampling periods, the to-be-packed crabmeat came from two pans for leg meat and one pan for body meat.

Processor Y's samples were taken in a similar fashion. The raw crabs were taken from three different metal bins; two crabs were taken from three different metal bins, two crabs from each bin,

that were located outside of the building. The butchered, cooked crab samples were taken from bins in the picking room, with a different rack being used for each sample. The picked crabmeat samples were taken from two pans of leg meat and two pans of body meat. The inspected crabmeat was taken from the conveyor rack as it came from the UV inspection. The to-be-packed crabmeat came from leg meat that was on the canning table and from different pans of body meat which were ready to be emptied onto the table.

The samples from processor Z are described as follows: the raw crabs for both sampling periods were taken from one bin in the butchering room. The butchered, cooked crab samples for both periods were taken from one bin in the picking room as they were being weighed into wire baskets. On both sampling periods the crabmeat taken was all body meat. The picked crabmeat samples from the first sampling were taken from five different pans, and on the second sampling period, from three different pans, with half of each box from a different pan. For both samplings the brined crabmeat samples were taken after the meat had been brined and to-be-packed samples were from the inspection tables.

Occurrence of *Vibrio parahaemolyticus*

Five of 31 samples of crabmeat from Oregon retail markets

were positive for halophilic vibrios (Table I). Three of these samples were purchased from store B and two from store A. Samples 5 and 9 had been collected in April, sample 13 in May, sample 24 in June and sample 27 in July. All of these samples, except sample 5, were brand e which is from a Washington processor. Sample 5 was brand c which utilized crab caught along the central Oregon coast. The crabmeat from which the isolates were obtained had been held at the store before sampling for varying periods of time from one to six days. Nine isolates of halophilic vibrios were obtained from four samples of crab from processor Z in June (Table II). Two of these samples were meat of raw, whole crabs and the other two were picked crabmeat.

The isolates were classified in the genus Vibrio on basis of cultural, morphological and biochemical characteristics. They were gram negative, pleomorphic, motile rods which were not sensitive to penicillin. The biochemical characteristics of these isolates are presented in Table III. Also presented in Table III are the biochemical characteristics of V. parahaemolyticus strains YMK33, ATCC17802 and SB04-422 as tested in this laboratory. Table IV summarizes the biochemical characteristics of V. parahaemolyticus isolates as reported in the literature. A great deal of variation can be noted. Baross and Liston (1970) found that a large percentage of their V. parahaemolyticus isolates produced acid from

TABLE I. Occurrence of halophilic vibrios, numbers of fecal streptococci, plate counts, (20 C) and pH of fresh Dungeness crabmeat from Oregon retail markets.

Sample no.	Date of samp. 1971	Market	Brand	Days held at store	Halophilic vibrios	Fecal streptococci (count/g)	Plate count[1] 20 C (count/g)	pH
1	3-31	A	a	5	-	2.6×10^2	4.9×10^5	---[2]
2[1]	3-31	D	b	6	-	9.2×10^3	5.7×10^7	---[2]
3	3-31	C	b	2	-	4.9×10^2	1.5×10^6	---[2]
4	4-7	C	b	1/2	-	3.1×10^2	1.7×10^5	7.80
5	4-7	B	c	6	2.3[4]	2.5×10^3	3.7×10^8	7.20
6	4-7	A	a	2	-	2.5×10^3	3.3×10^6	7.23
7	4-21	C	d	1/2	-	2.8×10^2	9.7×10^3	7.25
8	4-21	A	a	1	-	1.1×10^2	2.1×10^5	7.15
9	4-28	A	e	2	3.6[4]	3.2×10^2	3.9×10^6	7.45
10	4-28	B	-[3]	6	-	1.8×10^2	5.7×10^6	7.25
11[1]	4-28	C	b	2	-	9.2×10^3	3.2×10^7	7.56
12	5-4	A	f	1/2	-	5.0×10^1	5.0×10^4	7.55
13	5-4	B	e	5	+	1.7×10^2	9.3×10^4	7.61
14	5-4	C	b	1/2	-	8.2×10^2	2.2×10^5	7.10
15	5-12	A	a	1	-	4.5×10^2	1.5×10^5	7.32
16	5-12	C	b	1	-	2.2×10^2	7.8×10^4	8.13
17	5-18	A	e	1/2	-	1.3×10^3	3.8×10^6	7.15
18	5-18	C	b	1	-	5.9×10^2	1.6×10^6	7.86
19	5-21	B	e	1	-	1.4×10^3	1.2×10^6	7.45
20	5-28	A	e	1	-	6.3×10^2	1.8×10^6	7.20
21	5-28	C	b	1	-	1.1×10^3	1.8×10^7	7.41
22	5-28	B	e	1	-	1.0×10^3	1.4×10^7	7.42
23	6-8	C	b	3	-	1.1×10^4	1.4×10^8	7.71
24	6-25	A	e	1	+	6.5×10^2	3.0×10^5	7.82
25	6-25	B	e	1	-	9.9×10^2	1.3×10^6	8.32
26	6-25	C	b	1	-	7.7×10^2	6.5×10^5	7.85
27	7-1	B	e	2	+	4.2×10^2	5.6×10^5	7.62
28	7-1	C	b	1	-	1.3×10^2	2.4×10^5	7.53
29	7-1	A	e	2	-	4.1×10^2	2.0×10^7	7.84
30	7-8	B	e	1/2	-	1.0×10^3	3.4×10^5	7.83
31	7-8	D	g	1/2	-	7.9×10^2	5.4×10^6	7.02

[1] + = positive, - = negative

[2] Not determined

[3] Not known

[4] Most probable number

TABLE II. Occurrence of halophilic vibrios, numbers of fecal streptococci, plate counts (20 C) and pH of Dungeness crabmeat from Oregon processors.

Processor	Date of sampling (1971)	Place on processing line	Halophilic Vibrios[1]	Fecal streptococci[1] (count/g)	Plate count 20C[1] (count/g)	pH
X	3-18	Raw	-	4.0×10^1	8.0×10^2 [3]	---[2]
		Cooked, cool	-	9.3×10^1	$<10^2$ [3]	---[2]
		Picked	-	1.5×10^3	2.0×10^2 [3]	---[2]
		Brined, insp.	-	2.5×10^2	5.0×10^2 [3]	---[2]
		To-be-packed	-	---[2]	2.3×10^3 [3]	---[2]
X	3-25	Raw	-	$<10^1$	4.0×10^1	---[2]
		Cooked, cool	-	$<10^1$	2.0×10^1	---[2]
		Picked	-	1.1×10^3	1.5×10^3	---[2]
		Brined, insp.	-	4.2×10^2	7.4×10^2	---[2]
		To-be-packed	-	1.8×10^2	3.7×10^2	---[2]
Y	4-26	Raw	-	$<10^1$	1.4×10^2	6.62
		Cooked, cool	-	$<10^1$	4.4×10^3	7.56
		Picked	-	2.0×10^2	7.2×10^4	7.09
		Brined, insp.	-	$<10^1$	1.8×10^3	7.48
		To-be-packed	-	7.0×10^1	8.0×10^3	7.84
Z	5-25	Raw	-	$<10^1$	8.2×10^2	6.78
		Cooked, cool	-	1.0×10^2	9.7×10^4	7.52
		Picked	-	4.8×10^2	9.1×10^5	7.95
		Brined	-	3.0×10^2	1.4×10^8	7.72
		To-be-packed	-	3.0×10^1	5.0×10^4	7.75
Z	6-8	Raw	+	$<10^1$	2.5×10^3	6.78
		Cooked, cool	-	$<10^1$	4.3×10^4	7.22
		Picked	+	5.3×10^2	1.4×10^5	7.30
		Brined	-	1.9×10^2	2.0×10^4	7.00
		To-be-packed	-	1.2×10^2	3.1×10^4	7.01

[1] Average of three samples [2] Not determined [3] Lowest dilution plated was 1:100

TABLE III. Biochemical characteristics of halophilic Vibrio isolates and of Vibrio parahaemolyticus strains ATCC17802, YMK33 and SBO4-422.[1]

SAMPLE NUMBER	TB[4] 0% NaCl	TB[4] 3% NaCl	TB[4] 7% NaCl	TB[4] 10% NaCl	Hemolysis	Kovac's Oxidase	Catalase	Gelatinase	Phenylalanine deaminase	Urease	Nitrate reduction	Indole production	Cholera red reaction	Voges-Proskauer reaction	Methyl red reaction	TSI reaction	Glucose fermentation[5]	Adonitol[5]	Arabinose[5]	Cellobiose[5]	Inositol[5]	Lactose[5]	Maltose[5]	Mannitol[5]	Salicin[5]	Sucrose[5]	Trehalose[5]	Xylose[5]	Malonate utilization	Citrate utilization	Starch hydrolysis
S-3[1]	-	+	+	-	+	+	+	+	-	-	+	-	-	-	+	a	+	-	-	+[7]	-	-	+	+	-	+	+	-	-	-	+
S-3[2]	-	+	+	-	+	+	+	+	-	-	+	-	-	-	+	a	+	-	-	+[7]	-	-	+	+	-	+	+	-	-	-	+
S-3[2]	-	+	+	-	+	+	+	+	-	-	+	+	-	-	+	a	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+
S-3[2]	-	+	+	-	+	+	+	+	-	-	+	+	-	-	+	a	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+
S-3[2]	-	+	+	-	+	+	+	+	-	-	+	+	-	-	+	a	+	-	+	-	-	-	+	+	-	+	+	-	-	-	+
S-3[1]	-	+	+	-	+	+	+	+	-	-	+	+	-	-	+	a	+	-	±	-	-	-	+	+	-	+	+	-	-	-	+
S-1[1]	-	+	+	-	+	+	+	+	-	-	+	-	-	+	+	a	+	-	-	+[7]	-	-	+	+	-	+	+	-	-	-	+
U-2[1]	-	+	+	-	+	+	+	+	-	-	+	+	-	+	+	b	+	-	+	+[7]	-	-	+	+	-	+	±	-	+	±	+
U-3[1]	-	+	+	-	+	+	+	+	-	-	+	+	-	+	+	b	+	-	+	+[7]	-	-	+	+	-	+	-	-	+	±	+
27	-	+	+	-	+	+	+	+	-	-	+	+	-	+	+	b	+	-	+	+[7]	-	-	+	+	-	+	+	-	±	+	+
5[1]	-	+	+	+	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+
5[1]	-	+	-	+	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+
5[1]	-	+	+	-	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	+	+	+	+	-	+	+	-	-	+	+
5[1]	-	+	+	+	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+
5[1]	-	+	+	+	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+
5[1]	-	+	+	+	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	+	+	+	+	-	+	+	-	-	-	+
24[2]	-	+	+	+	+	+[6]	+	+	-	-	-	-	-	-	+	a	+	-	-	+[7]	-	+	+	+	-	+	+	-	-	-	+
9[1]	-	+	+	-	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	-	-	+	+	-	+	+	-	-	+	+
9[1]	-	+	+	-	+	+[6]	+	+	-	-	-	+	-	-	+	a	+	-	-	-	-	-	+	+	-	+	+	-	-	+	+
9[1]	-	+	+	-	+	+[6]	+	+	-	-	-	-	-	-	+	a	+	-	-	-	-	-	+	+	-	+	+	-	-	+	+
9[1]	-	+	+	-	+	+[6]	+	+	-	-	-	-	-	-	+	a	+	-	-	-	-	-	+	+	-	+	+	-	-	+	+
13[1]	-	+	+	-	+	+[6]	+	+	-	-	-	-	-	-	+	a	+	-	-	-	-	-	+	+	-	+	+	-	-	+	+
YMK33	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	b	+	-	+	-	-	-	+	+	-	-	+	-	-	-	+
ATCC17802	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	b	+	-	+	+[7]	-	-	+	+	-	-	+	-	-	+	+
SBO4-422	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	b	+	-	+	+[7]	-	-	+	+	-	-	+	-	-	+	+

[1] + = positive; - = negative; a = all acid; b = alkaline slant, acid butt [2]Isolated on thiosulfate-citrate-bile salts-sucrose agar
[3]Isolated on salt water-starch agar. [4]Trypticase broth [5]Acid only [6] Delayed positive [7]After 24 hrs.

TABLE IV. Biochemical characteristics of Vibrio parahaemolyticus isolates as reported in the literature.[1]

Biochemical tests	Colwell[2]	Sakazaki[3]	Baross and Liston[4]	Fishbein et al.[5]	BAM[6]	Thomson & Trenholm[7] Japanese culture	Thomson & Trenholm[7] Canadian isolate	Twedt et al.[8]
Growth in broth with:								
0% NaCl	-	-	-	-[9]	-	-	-[9]	-
3% NaCl	+	+	+	+	+	+	+	+
7% NaCl	+	+	+	+	+	+	+[9]	+
10% NaCl	+	-	-	-	-	-	+, -	-
Hemolysis	+	+	+	+	+	+	-	+
Oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	NR	+	+	NR	NR	+
Phenylalanine deaminase	NR	-	NR	-	-	NR	NR	-
Urease	+[9]	-	NR	-	-	NR	NR	-
Gelatinase	+	+	+	+[9]	+	-	-	+[9]
Nitrate reduction	+	+	+	+	+	+	+	+[9]
Indole production	+[9]	+	NR	+	+	+	-	+[9]
Cholera red reaction	NR	+, -	NR	-	+, -	NR	NR	+[9]
Voges-Proskauer reaction	-	-	-	-	-	-	-	-, +
Methyl red reaction	+[9]	+	+	+[9]	+	NR	NR	NR
TSI reaction	NR	NR	NR	a	a	NR	NR	NR
Glucose fermentation	+	+	NR	+	+	+	+	+
Adonitol - acid only	-	-	NR	-[9]	-	-	-	-
Arabinose - acid only	+[9]	+, -	NR	NR	+, -	+	+, -	+, -
Cellobiose - acid only	+[9]	-, (+)	NR	-	(+), -	-	+, -	+, -
Inositol - acid only	-	-	NR	-[9]	-	-	-	NR
Lactose - acid only	-	-	NR	-	-	-	-	-
Maltose - acid only	+[9]	+	NR	+[9]	+	+	+[9]	+[9]
Mannitol - acid only	+	+	NR	+	+	+	+	+
Salicin - acid only	-	-	NR	-	-	-	-[9]	-
Sorbitol - acid only	-	-	NR	-	-	NR	NR	-
Sucrose - acid only	-	-	+, -	-	+, -	-	-	+, -
Trehalsoe - acid only	+[9]	+	NR	-[9]	+	NR	NR	+
Xylose - acid only	-	-	NR	-	-	NR	NR	-
Malonate utilization	+[9]	-	NR	-	-	NR	NR	+, -
Citrate utilization	+	+, -	NR	+	+	NR	NR	+, -
Starch hydrolysis	+	+	+	+	+	+	-[9]	+[9]

[1] NR = not reported; a = alkaline slant, acid butt, no H_2S or gas; + = positive; - = negative; (+) = late positive

[2] Colwell, R. R. 1970. Polyphasic taxonomy of the genus Vibrio. Numerical taxonomy of Vibrio cholerae, Vibrio parahaemolyticus, and related Vibrio species. Journal of Bacteriology 104:410-433.

[3] Sakazaki, R. 1969. Halophilic vibrio infections. In: Food-borne infections and intoxications, ed. by H. Riemann, New York, Academic Press. 698 p.

[4] Baross, J. and J. Liston. 1970. Occurrence of Vibrio parahaemolyticus and related hemolytic vibrios in marine environments of Washington state. Applied Microbiology 20:179-186.

[5] Fishbein, M., I. J. Mehlman and J. Pitcher. 1970. Isolation of Vibrio parahaemolyticus from the processed meat of Chesapeake Bay blue crabs. Applied Microbiology 20:176-178.

[6] Food and Drug Administration. 1969. Bacteriological analytical manual. 2d ed. Washington, D. C., U. S. Department of Health, Education and Welfare. Public Health Service. various paging.

[7] Thomson, W. K. and D. A. Trenholm. 1971. The isolation of Vibrio parahaemolyticus and related halophilic bacteria from Canadian Atlantic shellfish. Canadian Journal of Microbiology 17:545-549.

[8] Twedt, R. M., P. L. Spaulding and H. E. Hall. 1969. Morphological, cultural, biochemical and serological comparison of Japanese strains of Vibrio parahaemolyticus with related cultures isolated in the United States. Journal of Bacteriology 98:511-518.

[9] Small number of strains studied varied from this reaction.

sucrose. Twedt, Spaulding and Hall (1969) noted this variation also in isolates from wound infections.. Another variation in the sugar fermentations was reported by Fishbein et al. (1970) who gave a negative trehalose fermentation. All other workers reported a positive trehalose fermentation. It has been reported that the ability to ferment cellobiose and arabinose varies with the strain of V. parahaemolyticus (Table IV). Thomson and Trenholm (1970) reported that strains of V. parahaemolyticus they isolated did not produce indole nor did they hydrolyze starch. Colwell (1970) reported that the strains she tested utilized malonate and were urease positive. Sakazaki (1969) and Twedt et al. (1969) reported that the V. parahaemolyticus strains they tested varied in citrate utilization reaction. Twedt et al. (1969) also reported a variable malonate utilization. There also seems to be some disagreement concerning the growth of V. parahaemolyticus in ten percent sodium chloride broth. It was originally presented (Sakazaki, 1968) that V. parahaemolyticus will not grow in broth with ten percent sodium chloride but V. alginolyticus will. This was used as a differentiating characteristic between these two organisms (Table V). More recently, Colwell (1970) and Thomson and Trenholm (1970) reported that strains of V. parahaemolyticus they tested did grow in broth with ten percent sodium chloride.

Obviously, there is a wide variation of biochemical reactions

TABLE V. Differentiating characteristics of Vibrio parahaemolyticus, Vibrio alginolyticus, and Vibrio anguillarum.[1]

Characteristics	V. parahaemolyticus	V. alginolyticus	V. anguillarum
Growth in peptone water:			
with 0% NaCl	-	-	-
with 3% NaCl	+	+	+
with 7% NaCl	+	+	-
with 10% NaCl	-	+	-
Voges-Proskauer reaction	-	+	-
Sucrose fermentation	-	+	±
Cellobiose fermentation (within 24 hours)	-	-	+

[1] Differentiation scheme based on data of Sakazaki, R. 1969. Halophilic vibrio infectious. In: Food-borne infections and intoxications, ed. by H. Riemann, New York, Academic Press. p. 115-129.

including variable reactions in indole production; growth in broth with ten percent sodium chloride; urease and amylase production; cholera red reaction; malonate and citrate utilization and arabinose, cellobiose, trehalose and sucrose fermentations (Table IV). This makes it very difficult to classify organisms into V. parahaemolyticus using biochemical tests.

Another complicating factor is that, according to Baross

(1971), an isolate can match all of the biochemical reactions of a known V. parahaemolyticus culture and still not be V. parahaemolyticus as based on other techniques of classification. This creates a problem in using biochemical tests alone in the classification of halophilic Vibrio isolates.

As can be seen from Table III, some of the halophilic Vibrio isolates vary considerably from the biochemical characteristics of known V. parahaemolyticus cultures. It is difficult to come to any conclusions about the classification of these organisms as to species in the genus Vibrio. However, the six isolates from the raw crab sample S-3 closely resembled V. parahaemolyticus. They only varied in that they produced acid from sucrose, they did not utilize citrate as a sole carbon source and two of the six isolates did not produce indole. These seem to be within the variability of V. parahaemolyticus. Numbers of these halophilic vibrios on sample S-3 were 3.6 organisms per gm MPN and 1600 organisms per gm from counts on SWSA plates.

Isolates from the raw crab sample S-1, the picked crab samples U-2 and U-3 from processors and the crabmeat sample 27 from a retail market were probably V. parahaemolyticus or V. alginolyticus. These isolates did not grow in Trypticase broth with ten percent sodium chloride, they produced acetylmethylcarbinol, they did not produce acid from cellobiose within 24 hr of incubation and

they produced acid from sucrose. Isolates from samples U-2 and U-3 utilized malonate and isolates from sample S-3 did not utilize citrate, but these reactions are within the variability of V. parahaemolyticus. Table V lists the differentiating characteristics of V. parahaemolyticus and V. alginolyticus as presented by Sakazaki (1968). Note that isolates from these samples in this study have some characteristics of both organisms. Isolates with these characteristics were classified as V. parahaemolyticus by Twedt et al. (1969).

The remainder of the isolates from crabmeat samples 5, 9, 13 and 24 vary considerably from the biochemical characteristics of V. parahaemolyticus. Isolates from sample 5 and 24 varied in that they had a delayed positive oxidase reaction, did not reduce nitrate, did not utilize citrate, had variable growth in Trypticase broth with ten percent sodium chloride and produced acid from lactose and sucrose. In addition isolates from sample 5 produced acid from inositol and isolates from sample 24 did not produce indole. The isolates from samples 9 and 13 varied in that they had a delayed oxidase reaction, did not reduce nitrate and produced acid from sucrose. Also, the isolate from sample 13 and two of the four isolates from sample 9 did not produce indole. These isolates from samples 5, 9, 13 and 24 are probably halophilic vibrios which are related to V. parahaemolyticus. Isolates from sample 5 occurred

in counts of 23 organisms per gm MPN and isolates from sample 9 occurred in counts of 3.6 organisms per gm MPN. Numbers in the crabmeat of the other isolates could not be determined.

Even if all of the vibrios recovered are V. parahaemolyticus, they occurred in a small proportion of samples (9 of 106 samples would be positive) and in small numbers. There are several factors which may be related to the low level of V. parahaemolyticus contamination. First is the water temperature. The average monthly water temperatures during the spring and summer of 1965-1969 and 1971 for points along the northern and central Oregon coast are presented in Table VI. Temperatures of the water which crab inhabit were not available, but they would be close to the temperatures given. Crab do not inhabit waters as far from shore as the Columbia River Lightship is located.

Note that for both periods of time these temperatures were on the lower limit of the V. parahaemolyticus growth temperature range, which is 15 to 40 C (Sakazaki, 1969) and some are in the range where V. parahaemolyticus will not grow (<10 C). The temperature of the water during 1971 was slightly lower than during 1965-1969. but not more than 2 C in each comparison. Japanese waters in which V. parahaemolyticus has been isolated are about 24 C (Horie et al., 1967). Consequently, in regards to temperature, these waters are

TABLE VI. Average monthly temperatures of ocean waters along the northern and central Oregon coast.

Month	Northern Oregon Coast				Central Oregon coast at Yaquina Bay	
	Shore temperature at Seaside[1] (°C)		Mouth of Columbia[2] (°C)		(°C)	
	1971	1965-69	1971	1965-69	1971	1965-69
March	8.5	9.8	7.8	9.2	----[4]	9.8
April	9.9	11.0	9.6	10.0	10.0	10.2
May	10.9	12.3	11.2	11.5	10.4	12.4
June	14.4	15.0	12.6	13.6	----[4]	13.4
July	15.1	15.0	----[4]	14.6	----[4]	12.6
August	----[4]	14.6	----[4]	14.6	----[4]	12.3
September	----[4]	15.3	----[4]	14.9	----[4]	13.4

[1] At pump outlet into Seaside Aquarium settling tank from surf inlet pipe on beach.

[2] Columbia River Lightship, five miles southwest of Columbia River South jetty.

[3] OSU Marine Science Center at pump outlet into the center from bottom of Yaquina Bay.

[4] Temperatures not available.

not conducive to the growth of V. parahaemolyticus. The water is warmest during the months of July, August and September and during these months, the temperature would be best for the growth of V. parahaemoltyicus. However, crab are not commercially caught during these months. The crab fishing ends during or before June. The colder water temperature in the early spring could have also contributed to the fact that no halophilic vibrios were isolated from

processors X and Y which were sampled in March and April; whereas some were isolated from processor Z which was sampled in June.

Another contributing factor is the degree of pollution of the water. Baross and Liston (1970) reported an apparent relationship between degree of pollution and V. parahaemolyticus counts. Panshin (1971) stated that pollution along the Oregon coast (excluding estuaries) is negligible, with more pollution at the opening of bays and the mouth of a river. It is interesting to note that all but one of the market samples from which halophilic vibrios were isolated were processed by a Washington processor. Perhaps the greater level of pollution in waters around and in the Puget Sound could account for this.

Since most of the samples were processed crabmeat samples, the numbers on them should be low if the crab was properly handled. Most or all of the V. parahaemolyticus should be killed during cooking and then, if the crab was further processed under good sanitary conditions, the crab should have minimal recontamination. Also significant is the finding that V. parahaemolyticus is killed rapidly in fresh water (Temmyo, 1966). Thus, the boiling in fresh water would be especially lethal for V. parahaemolyticus, and the many fresh water rinses during the processing of the crab would serve to reduce V. parahaemolyticus counts considerably.

If the samples collected in this study can be considered typical, it would appear that V. parahaemolyticus contamination of crabmeat from Oregon retail markets and processors may not constitute a public health problem. However, V. parahaemolyticus does contaminate some crabmeat. If this crab were mishandled before or after it reached the consumer, the V. parahaemolyticus on the crab could multiply and reach high numbers; and, since the crabmeat is usually not reheated after purchase, it would cause illness.

The sports fishing of crab may constitute more of a public health problem than the commercial. Sports fishermen often fish for crabs in bays and throughout the summer. These two factors would provide opportunities for the crab to be in contact with warmer and more polluted water and, thus, more likely to be contaminated with higher numbers of V. parahaemolyticus.

Limitations of the Media

A large variety of media and methods have been used to isolate V. parahaemolyticus from sea fish and shellfish. At the time this study was initiated, it appeared that a combination of the Liston-Baross method and the Japanese method, as described in Bacteriological Analytical Manual (1969), was the best available procedure. However, it has been found that these methods do have their problems.

Liston and Baross' SWSA is selective for V. parahaemolyticus which is identified on the agar as being a nonspreading, white colony with a clearing around it, indicating starch hydrolysis. However, another organism with these same growth characteristics repeatedly grew on this agar. This organism was easily differentiated from V. parahaemolyticus through the use of a gram stain since it was a gram positive, short, fat straight rod which occurred singly, in pairs or short chains. The biochemical characteristics of this organism are given in Table VII. Perhaps, the incorporation of pencillin into the media would help to reduce the numbers of false positives caused by these gram positive rods.

Another problem with the SWSA is that it often missed the presence of halophilic vibrios when these occurred in small numbers. On samples 5, 9, 13, S-1, U-2, U-3 and 27, the SWSA did not pick up the halophilic vibrios on the samples. It appears that when working with samples, such as crabmeat, in which low numbers are expected, an enrichment step is needed.

The Japanese method of isolation provides this enrichment step in using GSTB and SCB. However, this method was also found to have shortcomings. The SCB did not pick up any vibrios. It did allow a gram positive cocci that grew in tetrads and packets to grow in the SCB and consequently, appear on the TCBS. The GSTB did not do this. The biochemical characteristics of this gram positive cocci

TABLE VII. Biochemical characteristics of gram prositive rods isolated on salt water-starch agar and gram positive cocci isolated on thiosulfate-citrate-bile salts-sucrose agar from crabmeat.[1]

Biochemical Tests	Gram Positive rod	Gram Positive cocci
Growth in Trypticase Broth with:		
0% NaCl	+	+
3% NaCl	+	+
7% NaCl	+	+
10% NaCl	-	-
Hemolysis	NT	NT
Oxidase	-	-
Catalase	+	+
Phenylalanine deaminase	-	-
Urease	-	-
Gelatinase	-	-
Sensitivity to penicillin (2-2.5 U/ml)	+	+
Nitrate reduction	+	-
Indole production	-	-
Cholera red reaction	-	-
Voges-Proskauer reaction	-	+
Methyl red reaction	NT	+
TSI reaction	NT	NT
Glucose fermentation	-	+
Adonitol - acid only	-	-
Arabinose - acid only	-	-
Cellobiose - acid only	+	+
Inositol - acid only	-	-
Lactose - acid only	-	+, -
Maltose - acid only	+[2]	+
Mannitol - acid only	-	-
Salicin - acid only	-	-
Sorbitol - acid only	-	+
Sucrose - acid only	-	+
Trehalose - acid only	-	-
Xylose - acid only	-	-
Malonate utilization	NT	-
Citrate utilization	-	NT
Starch hydrolysis	+	-

[1] NT = not tested

[2] Gas produced

are given in Table VII. V. parahaemolyticus colonies are differentiated from other bacteria on TCBS on the basis of their negative sucrose fermentation. Baross and Liston (1970) reported that some of their isolates were sucrose fermenters. Thus, these would not appear as "typical" V. parahaemolyticus colonies on the TCBS. Consequently, discretion must be used in picking suspect V. parahaemolyticus colonies on the TCBS.

In further studies, when examining food samples with expected low numbers of V. parahaemolyticus, it would be recommended that an enrichment step be used. The use of GSTB along with a less inhibitory broth, such as a meat broth with five percent sodium chloride as used by Kampelmacher et al. (1970) as enrichment broths would be desirable. For plating the broth, TCBS appears to be quite adequate. Thomson and Trenholm (1970), Bartley and Slanetz (1971) and Kampelmacher et al. (1970) used bromothymol blue-Teepol-salt agar with good results. This agar could be used in conjunction with TCBS or by itself. To date a good quantitative method for the detection of small numbers of V. parahaemolyticus in a sample has not been developed. However, the MPN procedure could be used in conjunction with the above enrichment technique.

Plate Counts and Fecal Streptococci

Comparison of Processors

As expected, the counts on the raw crabmeat were low (Table II). The plate counts ranged from 4.0×10^1 to 2.5×10^3 organisms per gm with a median count of 8.0×10^2; and the fecal streptococci counts ranged from $<10^1$ to 4×10^1 with a median count of $<10^1$. There had been some contamination of the meat due to death and invasion of the tissue and to sampling. The counts of the butchered, cooked and cooled crabmeat varied. The expected trend after cooking would be that of reduction of numbers of bacteria. With processor X, the plate counts were lower on the cooked crab than on the raw, but with processors Y and Z, the plate counts went up. The crab from processor X had been cooked the same morning it was sampled, whereas the cooked crab samples from processor Z had been cooked two or three days before sampling and iced until they could be picked. Damon (1971) found the holding of cooked crabs for up to two days before picking to be a problem in the blue crab industry. The difference of processor Y was probably due to basic differences in its handling technique as compared to processor X or Z.

After picking, plate counts and fecal streptococci had increased. Plate counts ranged from 2.0×10^2 to 9.1×10^5 organisms per

gm with a median count of 7.2×10^4 organisms per gm. Fecal streptococci numbers ranged from 2.0×10^2 to 1.5×10^3 organisms per gm with a median count of 4.8×10^2 organisms per gm. This is as expected because of the handling of the crab during picking. The counts were reduced or remained static after brining, except in the case of the first sampling from processor Z. The brined crabmeat from processor Z was not rinsed after brining before it was sampled and was placed into the same pan into which it had been picked. The brine in the brine tank was changed once a day so that it could serve as a source of contamination of the crabmeat. The brined crabmeat samples from processors X and Y had been rinsed in a tank through which fresh water flowed, which would tend to reduce numbers that had increased during picking and brining. The brined crabmeat samples had a plate count range of 5.0×10^2 to 1.4×10^8 organisms per gm with a median count of 1.8×10^3 organisms per gm and a fecal streptococci range of 10^1 to 4.2×10^2 organisms per gm with a median of 2.5×10^2 organisms per gm. The counts of the to-be-packed crab also varied. Plate counts varied from 3.2×10^2 to 5.0×10^4 organisms per gm and fecal streptococci counts varied from 3.0×10^1 to 1.8×10^2 organisms per gm. In most cases, there was no real change in bacterial numbers. The expected change would be a slight increase in numbers or no significant change, depending on the length of time between

inspection and packing and on the amount of handling.

There seems to be a rather large difference among the processors with respect to plate counts, but no appreciable difference with respect to fecal streptococcal numbers. The trends in the plate counts among processors paralleled observations made during preliminary examination of the plants. Processor X, with very low plate counts in all cases, had several extra measures to keep the growth of bacteria down. Most of these were aimed toward the picking process. A flume ran along the picking tables and carried the shells to the ends of the tables to be emptied in waste cans. This kept most of the shells off the floor and away from the picking area. Each time the picker had cleared her area of a batch of crabs, that area of the table was hosed down with chlorinated water. Also, after weighing the picked crabmeat, the pans of crab were rinsed with fresh water.

Processor Y had slightly higher counts which could be accounted for by the fact that it did not have these extra procedures. The counts on samples from processor Y were still quite low and well within the 100,000 organisms per gm standard of New York City (Food Protection Committee of the Food and Nutrition Board, 1964).

Processor Z's higher counts can partly be accounted for by the fact that the cooked, picked, inspected and to-be-packed samples

had been cooked three days before. However, higher counts would be expected by the extra steps in the procedure this processor used. After butchering, the crabs were loaded into large wire baskets before cooking. It takes about 15 to 20 minutes to fill these baskets, thus allowing time for growth of bacteria. Before picking, the crabs are taken from the wire baskets and weighed into smaller wire baskets. This is done by hand. Also, after brining, the step to inspection is interrupted by scooping the crab out of the brine tank into pans and draining, thus the crab is not rinsed after the handling for inspection. However, note again that final counts on the to-be-packed crab with a range of 3.7×10^2 to 5.0×10^4 organisms per gm. were below the 100,000 organisms per gm commonly used guideline.

The variability among the fecal streptococci counts and the plate counts of the three samples for each point along the processing line was quite small. Very seldom did it even amount to a difference of 10^1.

Comparison of Retail Markets

A wide variation in plate counts among and within retail markets occurred. Each market had about the same range of counts from about 10^4 to 10^7 or 10^8 cells per gm of crabmeat. No realistic comparison of markets can be made since the crab was sampled from them after it had been in the store a varying number of days. All of

the markets did seem to have fairly good sanitary conditions. However, occasionally when a sample was collected from markets A and C, the butcher picked up the crab with his bare hands. The temperature of the cases of store A and B was maintained at approximately -1 C at the coldest part; that of the case of market C was maintained at about 5-6 C. Store C had the quickest turnover; store B the slowest.

The overall view of plate counts on the crabmeat from these markets was not ideal. The median count was 1.2×10^6 organisms per gm of crabmeat and the mean count was 2.0×10^7 organisms per gm. These are well above the 100,000 organisms per gm commonly used guideline. The larger number of high counts, i.e., those several million and over, is of concern. Crabmeat with counts in this range have been considered spoiled by Tobin and McCleskey (1941). The experimenter noticed that some of the samples in this range did have an odor of ammonia, indicative of spoilage. This would mean that the consumer would sometimes purchase crabmeat that has spoiled or is near the spoilage point. It appears that greater care or supervision of the marketing of crabmeat is needed.

The fecal streptococci counts were similar among and within markets. The range was from 5.0×10^1 to 1.1×10^4 fecal streptococci per gm of crabmeat, with a median of 5.9×10^2 fecal streptococci per gm and a mean of 1.3×10^3 fecal streptococci per gm.

The majority of counts were within the commonly used guideline of 1,000 fecal streptococci per gm of crabmeat.

Relationship Between Fecal Streptococci and Plate Counts

A correlation coefficient of 0.73 for the relationship between fecal streptococci and plate counts of crabmeat samples from retail markets was found. This value of r is significant at the one percent level. This would indicate that high plate counts are generally paralleled by high fecal streptococci counts on crabmeat from the retail markets studied. However, for the comparison of numbers of fecal streptococci and plate counts of crabmeat from processors, the correlation coefficient was 0.32, which is not significant.

These results would appear to contradict each other. However, this could be due to the fact that the comparison of crabmeat from the processors utilized samples from all five points along the processing line, but significant fecal streptococci contamination would occur during and after picking. The greatest amount of contamination would probably occur in the market.

Relationship to Vibrio parahaemolyticus

Plate counts at 20 C and fecal streptococci determinations were conducted to determine whether there was any relationship

between these and the incidence of V. parahaemolyticus. No correlations between V. parahaemolyticus counts and plate counts or fecal streptococci counts, because of the low incidence of recovery of V. parahaemolyticus, were made.

pH

The correlation coefficient, r, for the comparison of plate counts and pH of crabmeat samples from retail markets was -0.14, which is not significant. This indicates that pH is not a good indicator of bacterial numbers and thus, should not be used as an index of them. These results confirm those reported by Fieger and Novak (1961).

SUMMARY

The occurrence of V. parahaemolyticus on crabmeat samples from Oregon retail markets and from Oregon crab processors was studied. Crab samples from the following five points along the processing lines of three Oregon processors were taken: (1) raw, whole crabs, (2) butchered, cooked and cooled crabs, (3) picked crabmeat, (4) brined or brined and inspected crabmeat and (5) to-be-packed crabmeat. Two processing plants were sampled twice and the other was sampled once. A total of 31 crabmeat samples from three Oregon retail markets was also taken. The study was conducted from March to July 1971.

V. parahaemolyticus was recovered by the Japanese method and that of Liston-Baross as outlined in the Bacteriological Analytical Manual (1969). Salt-water-starch agar was used for primary plating of crabmeat samples. Glucose-salt-Teepol broth and salt Colistin broth were used as enrichments before plating on the sulfate-citrate-bile salts-sucrose agar. The presumptive positive V. parahaemolyticus were tested biochemically.

Numbers of fecal streptococci and plate counts at 20 C were also determined. The pH of the crabmeat sample was taken to study the relationship between plate count and pH.

Five of 31 samples of crabmeat from Oregon retail markets

were positive for halophilic vibrios. They were isolated between April and July 1. Four of 75 samples of crabmeat from Oregon crab processors were positive for halophilic vibrios. Two of these were raw crab samples and two were picked crab samples. They were all from the same processor and had been collected in June.

The halophilic Vibrio isolates varied in their biochemical characteristics. The isolates from only one sample matched the accepted characteristics of V. parahaemolyticus closely. This was a sample of raw crabmeat from a processor. The isolates fermented sucrose and did not utilize citrate, but these variations have been reported. One isolate from raw crabmeat, two isolates from picked crabmeat and one from crabmeat from retail markets appeared to be either V. parahaemolyticus of V. alginolyticus. These isolates did not grow in Trypticase broth with ten percent sodium chloride, they produced acetylmethylcarbinol and acid from sucrose and did not produce acid from cellobiose within 24 hours.

The plate counts varied among the processors. The processor which took the most precautions to minimize contamination had the lowest plate counts. In general, plate counts were low for raw crab, with a median of 1.4×10^2 organisms per gm; remained the same or increased slightly on cooked, cooled and ready-to-be-picked crabmeat, with a median count of 4.4×10^3 organisms per gm; increased on picked crabmeat, with a median count 7.2×10^4 organisms per gm;

decreased in three of four sampling periods on brined crabmeat, with a median count of 1.8×10^3 organisms per gm; and remained static on to-be-packed crabmeat, with a median count 8.0×10^3 organisms per gm. Plate counts of to-be-packed crabmeat from all of the processors were within the 100,000 organisms per gm New York City standard(Food Protection Committee of the Food and Nutrition Board, 1964) and commonly used guideline for crabmeat.

Fecal streptococci counts of crabmeat from processors were within the 1000 organisms per gm standard of New York City (Food Protection Committee of the Food and Nutrition Board, 1964).

Plate counts at 20 C varied considerably on crabmeat from Oregon retail markets. They ranged from 9.7×10^3 to 3.7×10^8 with a median of 1.2×10^6 organisms per gm. The plate counts of 25 of 31 of these samples were over 100,000 organisms per gm. Fecal streptococci numbers were similar for all samples. The median of 5.9×10^2 fecal streptococci per gm was below the 1000 fecal streptococcus per gm commonly used guideline.

A correlation coefficient of 0.73 was found for the comparison of fecal streptococci counts and plate counts from retail markets. This is significant at the one percent level indicating that there is a relationship between fecal streptococci numbers and plate counts. However, the correlation coefficient for the same relationship for crabmeat from processors was not significant. The apparent contradiction could be due to the fact that the comparison of crabmeat

from the processors utilized samples from all five points on the processing line, but significant fecal streptococci contamination would occur during and after picking.

The correlation coefficient for the relationship between plate counts and pH of market crab was not significant. This indicates that plate counts of crabmeat samples cannot be predicted from pH.

Dungeness crabmeat from Oregon processors and retail markets in the season sampled appeared to harbor only small humbers of V. parahaemolyticus, if any. It also appeared to be of good quality on the basis of fecal streptococci counts and on plate counts in the case of processors. However, the plate counts of the crabmeat from retail markets would indicate that excessive multiplication of bacteria had been allowed to occur. Consequently, greater care in the marketing of crabmeat is recommended.

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