ANNUAL REPORT, BUREAU OF COMMERCIAL FISHERIES TECHNOLOGICAL LABORATORY, PASCAGOULA, MISSISSIPPI

Fiscal Year 1965

UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

BUREAU OF COMMERCIAL FISHERIES

Circular 251

UNITED STATES DEPARTMENT OF THE INTERIOR
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By

TRAVIS D. LOVE, Director
MARY H. THOMPSON, Assistant Director

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Travis D. Love, Laboratory Director
Mary H. Thompson, Assistant Laboratory Director
Rachel D. Lightsey, Clerk Typist
Ophelia James, Laboratory Aid

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Mary H. Thompson, Program Leader

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TRAVIS D. LOVE, Director
MARY H. THOMPSON, Assistant Director

Bureau of Commercial Fisheries Technological Laboratory

Pascagoula, Miss. 39567

A HISTORICAL OUTLINE AND GENERAL VIEW OF TECHNOLOGICAL ACTIVITIES IN REGION 2

INTRODUCTION

This part of the circular describes the research activities of the Bureau of Commercial Fisheries Technology Laboratory in Pascagoula since its inception in early 1957 to the end of fiscal year 1965. A resumé of the regional industry and its relation to the Laboratory is given. Because this is the first of a planned series of annual reports, some historical data are included to cover the Laboratory's accomplishments since its beginning.

The Laboratory maintains close liaison with several State and Federal commissions and fisheries associations. The Gulf States Marine Fisheries Commission, a 15-man commission from the five Gulf Coast States, meets semi-annually to consider needed research and legislation. The Commission asks the Laboratory from time to time to report its research results. The Commission may also recommend that State and Federal fishery laboratories perform certain research that may be needed.

The Technology Laboratory works closely with the several State seafood commissions on research problems. Especially successful was a collaborative study with the Florida State Board of Conservation on the utilization of mullet. In addition, the Laboratory has undertaken several cooperative studies with such organizations as the American Shrimp Canners Association, the Texas Shrimp Association, and the National Fisheries Institute to investigate the preservation of quality seafoods.

The Technological Laboratory at Pascagoula is the only laboratory in Region 2 of the Bureau of Commercial Fisheries that is charged with carrying out research on fishery products from point of landing, through processing, and to market. Region 2 encompasses the States and coastlines of North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas and the waters of the South Atlantic, Gulf of Mexico, and Caribbean. The wide diversity and size of the fishing industries served by the Laboratory are well known.

The principal fisheries are described in further detail later in the report, but it is interesting to note that most of the shrimp caught in the United States are landed in Region 2. In 1964 shrimp accounted for 17.9 percent of the total dollars paid for all species of fish and shellfish caught by U.S. fishermen. The value of shrimp was some 20 percent more than for the second most valuable item. In 1964, statistics also show that some 36 percent of the total tonnage of U.S. fishery products was landed within Region 2 borders.

HISTORY

The Technological Laboratory at Pascagoula came into being in late 1957 following the construction of the Pascagoula Fisheries Station building, which it shares with two other Branches of the Bureau of Commercial Fisheries. The Laboratory Director spent considerable time in early 1957 visiting the industry and determining which problems most pressingly needed technological study. Following the installation of the Laboratory furniture and equipment in spring 1958, the Laboratory Director, assisted by a chemist and a laboratory technician, started formal technological studies to aid industry in the region.

As is true in any research organization, one main effort in original form multiplies, finds new directions, and then unifies into a whole. The main object of any technological research laboratory is, of course, to aid the industry and the public. The methods with which this is done vary with the industry it serves and its

Figure 1.--An exterior view of the Bureau of Commercial Fisheries installation in Pascagoula, Miss., where the Bureau's Technological Laboratory is located.

location. From the beginning the Technological Laboratory has held that the interest of the fishing industry of Region 2 in relation to the national welfare is the foremost consideration in planning and conducting research projects. In serving Region 2, the Laboratory has concentrated its efforts both in aiding established fishing industries and in developing the use of underutilized species. At the same time that the research effort and potential at the Laboratory was enlarging, the character of the Region's fishery industry changed from that of a local one to a national one. To keep pace with the growing interests of the industry, the Laboratory began conducting research that was national in scope. As a result the national research effort of the Bureau on composition of fish and shellfish, the control of pesticide residues in fish and shellfish, and the effects and control of microbial flora on fishery products is now concentrated at our Laboratory. Following is a brief resumé of the work accomplished between the start of research in late fiscal year 1958 through fiscal year 1964.

Chemistry

Chemical research has been the major program at the Laboratory in Pascagoula in recent years. A program entitled "The Chemistry of Gulf Marine Products" was started in fiscal year 1958 with the employment of a chemist and a laboratory technician. The initial project, the proximate composition of industrial fish, led throughout the next few years to a characterization of the proximate composition of 17 of the most commonly trawled species in the Gulf. Monthly samples revealed that the composition of these species exhibited a yearly variation--particularly in oil and moisture content. An examination of the oil, moisture, protein, and ash content recorded throughout the year together with other data collected on the size of the fish, the date of catch, and the location of catch revealed

certain characteristics applicable to the fishery as a whole: (1) Geographical and yearly variations have little influence on the ash and protein content of each species and remain fairly constant throughout the year, with the concentration depending upon the species. (2) Geographical and yearly variations appear to have little influence upon the moisture and oil content of the samples, although there is a marked variation throughout the year in almost all species studied. (3) Variations in the individual fish such as size, maturity, sex, and the species reproductive cycle appear to have little influence upon the variation in oil and moisture content. (4) Time-sequence variations in an exact combination of such factors as food, temperature, hours of light, are likely the cause of individual species sample variations. (5) Family relationships, similar eating habits, and a similar mode of life apparently do not influence the seasonal pattern of oil and moisture content variation, although each species' pattern is reasonably reproducible from 1 year to the next. (6) An equation to predict the oil content of a sample of fish of unknown composition was developed (oil content = 65.3 - 0.8 x moisture content) and may be used to predict the oil content with an accuracy of ±0.6 percent. (7) The discovery that another species of fish other than menhaden--i.e., thread herring (Opisthonema oglinun)--has a period of extremely high oil content during the winter raised the possibility of a year-round harvesting of Gulf fish by the fish meal and oil industry.

During fiscal year 1960, a study of the chemical reactions occurring in processed seafoods was added to the chemistry program at the Laboratory. An initial study of the iron sulfide discoloration of canned shrimp, generally known as "blackening", revealed that the presence of citric acid or lemon juice in the liquor of the canned shrimp afforded some protection against the development of the blackening. The removal of tin by the shrimp meats from the tinplate was found to bare the iron base plate at points where the C-enamel had been removed by manufacturing accidents. At this point then the iron was free to combine with sulfur, already present, to form the black iron sulfide deposits. A study in further detail showed that the citric acid or lemon juice facilitated the transfer of tin from the tin plate both at the bare plate and through the C-enamel to the shrimp meats during processing and storage. Thus, sufficient tin was available, reducing the incidence of bared iron. The binding of the tin by the shrimp meats appeared to be related to factors inherent in the raw material; further study of the nature of this reaction has been continuing.

The intense interest of the industry and fishery technologists led us to expand the project on the variation in the composition of fish and shellfish in fiscal year 1962. The project achieved national status and was broadened to include the determination of the quantities of amino acids, trace minerals, fatty acids, sterols, phospholipids, and vitamins present in various species of fish and shellfish. The original species examined were ocean perch, alewife, Chesapeake Bay blue crab, Dungeness crab, and brown shrimp. Samples are once again being collected from a specific geographic area upon a seasonal basis. Interesting variations in total amount of the various constituents, not only from species to species but from season to season, have been found. Such variations and the discussions resulting therefrom are too numerous to mention here but may be found in detail in the publications resulting from this project.

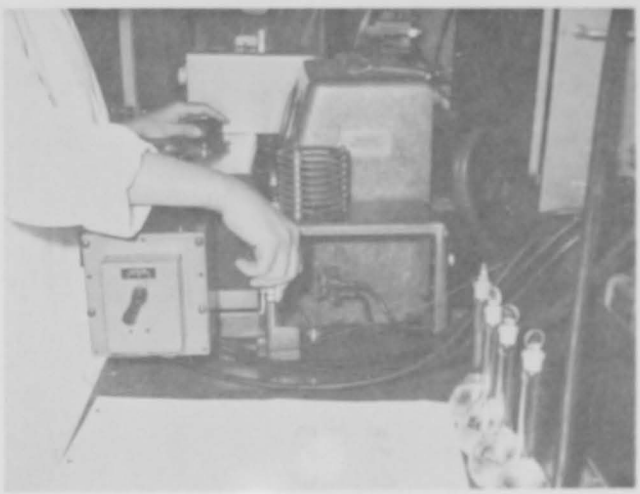

Figure 2.--Analysis of trace minerals in fish by flame photometry.

Studies on the prevention of rancidity in smoked mullet showed that a fillet dip of butylated hydroxytoluene, potassium sorbate, or a combination of both were effective in preventing oxidative rancidity throughout the marketable life of the product. Although butylated hydroxytoluene is an antioxidant and should be expected to help prevent rancidity, potassium sorbate was added as a mold inhibitor, so the retardation of rancidity in the presence of this compound was an unexpected finding. Because potassium sorbate is often used as a dip to decrease the incidence of mold growth on smoked products, the suppression of oxidative rancidity by this compound would be an added economic benefit.

Entering fiscal year 1965, the chemistry program involved more than half of the personnel and research expenditures of the Laboratory. Studies of a more basic nature are being embarked upon and concern such projects as the chemical alterations in proteins and amino acids of various species of shrimp during storage and their relation to change in quality

of the processed product. Cooperative studies with various industry trade organizations are being started. The project on composition and nutritive value of fish and shellfish has increased funding with additional national contributions. A study of the pesticide residue levels in processed fish and fishery products is also being started.

Food Technology

In its early days, the Laboratory embarked upon a fishery technological program for developing methods of using underutilized species. The canning of Gulf marine products was started to encourage a greater use of certain species. Sardinelike fishes obtained from the M/V Oregon were canned. Of the several species canned, round herring and a Sardinella species showed promise as sardines.

Technologists noted that a large amount of oyster juice was lost from the steam box at all oyster canneries. A project was begun at the request of industry to develop a canned oyster soup using concentrated reclaimed juice. The product was successfully manufactured without adding milk. Another recipe for the production of oyster stew using reclaimed juice with milk and an added stabilizer was experimentally completed.

A species of shrimp (Xiphopenaeus kroyeri), known locally as "seabobs", tends to cause a more rapid blackening of the can and its contents than does other species under certain conditions. The appearance of this condition of the shrimp is often indicated by a decrease in acidity of the meats after catch. Pascagoula food technologists collaborated with the chemists in an attempt to control the blackening by chemical additives and lemon juice. This study led to our establishing a formal project to determine the chemical alterations occurring in all commercial species during storage.

Food technologists attempted to solve the problem of short shelf life of smoked fish caused by mold growth. A pilot smokehouse was constructed near the Laboratory. Potassium sorbate was found to prevent mold for up to 14 wk. (weeks) when applied to mullet fillets through the use of a 1 percent presmoke dip. Use of the dip, however, appeared to raise a new problem--that of rancidity from oxidation of the polyunsaturated fish oil. Another additive, BHT (butylated hydroxytoluene), an antioxidant approved for cooking oils and oleomargarines and other food products, proved to be an effective antioxidant.

Fishery technologists were asked to study the freezing preservation and storage characteristics of the new calico scallop resource discovered off Cape Kennedy by the M/V Silver Bay. Technologists went aboard the Silver Bay and supervised the shucking of a large sample of the scallops. These scallops were returned to the Laboratory and frozen for later taste-panel tests after the scallops had been held for a prolonged period in frozen storage. During a later period the Silver Bay delivered shell stock to the Laboratory for additional studies. In a collaborative project with the Gulf Exploratory and Gulf Research unit, a device using vacuum and heat for shucking scallops was invented and put into trial operation. These initial experiments formed the basis for a later industrial application.

To obtain an objective test for assessing the storage life of iced scallops, we compared available chemical tests with the more usual organoleptic(sensory) tests. Trimethylamine (a basic substance), volatile acids, pH (measure of acidity and alkalinity), volatile bases, turbidity produced by the addition of picric acid, and taste-panel scores were determined on samples of iced scallops over a 23-day period and on a frozen sample used as the control.[1] Trimethylamine and volatile acids showed an increase in value only after the taste panel had rejected the scallops. Picric acid turbidity closely correlated with the sensory test, particularly after the 16th day, and confirmed advanced decomposition. When the pH had increased to above 6.65 (slightly acid), the panel judged the scallops to be inedible. Volatile base values could not be used to assess quality as they were erratic. All in all, sensory evaluations were the most reliable means for detecting spoilage in the scallops since the chemical analyses did not indicate spoilage until it was well advanced.

On the basis of the foregoing tests, we concluded that the maximum storage life of the scallops stored in iced polyethylene bags was 9 days. We also concluded that a trained taste panel could make organoleptic analysis for quality and freshness more accurately than was possible with the available chemical analyses.

Mullet utilization was the subject of a collaborative project with the Florida State Department of Conservation during fiscal years 1961 and 1964. Our Laboratory planned a pilot canning project designed to determine the best procedure for producing a good light-colored boneless fish suitable for institutional use. Several thousands of pounds of mullet went into both 1-lb. (pound) and 4-lb. cans before a type of pack was formulated that was favorably received by the taste panel. The canning method was widely distributed throughout the Region; and at present there are three firms in Florida packing canned mullet in limited amounts.

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[1] Since we were trying to find a test which might show signs of decreasing quality prior to taste-panel ascertation, chemical tests which showed no discernible changes prior to those ascertained by the taste-panel were judged useless.

Inspection

In November 1958, the functions of the Seafood Inspection Service for Region 2 were transferred from the Bureau's Technological Laboratory at College Park to the Technological Laboratory at Pascagoula. At that time each of the eight plants under continuous USDI (U.S. Department of the Interior) Inspection had an inspector under the immediate direction of our Laboratory. Region 2 Inspection Service increased until, by 1962, 18 plants were under continuous inspection by 20 inspectors, 2 area supervisors, and a regional supervisor. The industry has had some production fluctuations that have caused variations in the number of plants under inspection. By fiscal year 1965 the number of plants in the Region subscribing to USDI Inspection Service was about 16.

The Development of Voluntary Standards of Grade for Fishery Products

This study was funded as a program at our Laboratory in fiscal year 1960. The initial projects were for Frozen Raw Headless Shrimp and Fresh or Frozen Cooked Peeled Shrimp. Later a project was added to develop a standard for Frozen Raw Peeled Deveined Shrimp. In fiscal year 1962 the Bureau asked the Laboratory to provide a revision of the Frozen Raw Breaded Shrimp Standard then in use in the inspected plants. Attempts to revise the standard to reflect market conditions more closely resulted in the industry requesting a large-scale in-plant study. A team was formed with one of our food technologists, a National Fisheries Institute food technologist, and a supervisor from the Region 2 Inspection Service. Their study embodied work at plants in Georgia, Florida, Texas, Arizona, and California. A large amount of data was obtained as to the effect of processing variables on the amount of shrimp meat found by the official debreading method after frozen storage of the material for intervals of 24 hr. (hours), 30 days, 60 days, and 6 mo. (months). The data were statistically analyzed and aided in the promulgation of a revision of the Breaded Shrimp Standard. Peeling the shrimp into an ice-water holding prior to breading, rather than peeling directly onto the line, was shown to affect the control of moisture passage from the shrimp material to the breading. At the beginning of fiscal year 1965, all standards development was transferred to the Bureau's Laboratory at Gloucester; the Pascagoula staff was assigned other technological duties.

Figure 3.--Picking shrimp prior to breading at a USDI inspected plant.

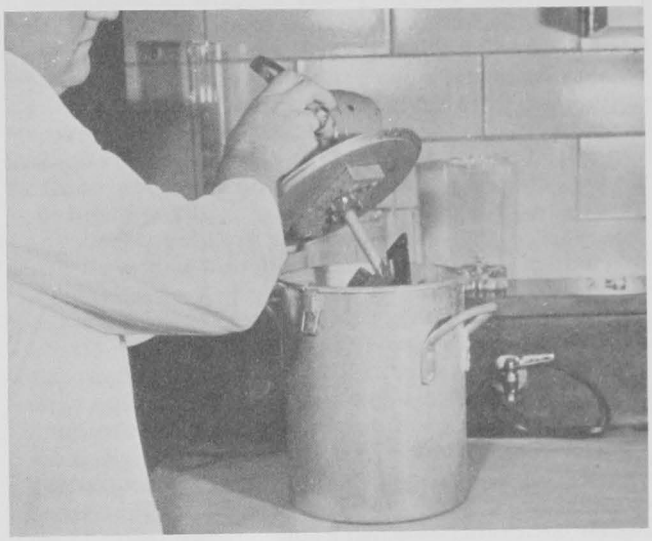


Figure 4.--Debreading equipment is used to determine the amount of shrimp meat in frozen raw breaded shrimp.

Microbiology

Microbiological studies have formed a portion of the Laboratory's research program since 1957. With the start of the Seafood Inspection Service in October 1958, our Laboratory analyzed larger samples of frozen seafoods. Arrangements were made with the plant inspectors to ship large samples of production-line material in dry ice via air express to Pascagoula for microbiological analyses. A series of daily samples was obtained from each plant under continuous inspection.

Each sample submitted was analyzed for Escherichia coli, the coliform group, the Salmonellae, Streptococcus faecalis, and coagulase-positive Staphylococcus aureus. Results showed a tremendous variation in bacterial count between plants and even between days in the same plant. An attempt was made to correlate the plant's sanitary condition with the counts obtained. We soon found,

however, that apparently clean plants might have high counts, whereas sanitarily poorer plants might have lower counts. Closer scrutiny revealed that handling and processing methods affected the microbiological flora more than did poor housekeeping. We showed each operator the results of the bacteriological survey of his plant and product and suggested ways of improving the conditions. The industry reacted most favorably.

In addition, the Seafood Inspection Service required that all new products offered to be packed under continuous USDI inspection must be examined bacteriologically first before approval for the use of the USDI shield on the new label was given. Samples from a pilot run were collected by the inspector and shipped in dry ice via air express to the Pascagoula Laboratory for a thorough examination. If the bacterial flora indicated satisfactory conditions, permission for the product to be packed under the shield was immediately wired to the inspector. In the few instances of a bacteriological report showing unsatisfactory conditions, handling and processing methods had to be altered before permission could be granted. The use of bacteriology as a tool for indicating the need to improve the product was well received by plant managers.

In a comparative study, a series of samples of breaded shrimp from noninspected plants was obtained from local supermarkets for bacteriological analysis. These samples also exhibited tremendous variability in numbers and kinds of bacteria commonly used as indicators of improper sanitation and handling methods. In general, raw frozen seafoods had more bacteria than did cooked frozen seafoods.

THE REGION'S FISHING INDUSTRIES

The industries in Region 2 are among the largest in the United States in terms of both production and dollar value. The Laboratory has tried to serve these industries throughout its existence as well as help find ways to create new industries through the use of underutilized species. A brief description of the larger fishery industries in Region 2 will serve to develop the connection between the Laboratory's main research efforts and the industry.

The domestic shrimp industry, located almost wholly in Region 2, is the nation's number one fishing industry in terms of dollar value. During calendar year 1964, the shrimp fleet caught 208 million lb. of shrimp valued at $70 million. Most of the shrimp catch is sold as fresh or frozen headless shrimp. A significant amount of shrimp, however, is now passing into the consumer chain as raw frozen breaded shrimp (about 33 percent of the total volume). The shrimp canning industry accounts for a considerable portion of the processed shrimp sold each year (about 7 percent).

Oysters in Region 2 brought over $7.5 million to the fishermen, who harvested the shell stock that yielded 22.7 million lb. of meats in 1962. The oyster industry is completely based upon the American oyster, Crassostrea virginica, which supplies the oyster industry throughout the eastern United States.

The same year a catch of 65.7 million lb. of crab meat brought the fishermen $3.4 million. The industry, consisting in a large measure of small scattered plants, presents many possibilities for improvement through technological study. The crab meat industry is also supported by a single species in Region 2. The blue crab, Callinectes sapidus, is the sole source of supply for the crab meat industry along the Atlantic and Gulf of Mexico coasts.

The menhaden fishery led all other fisheries in the Region in tonnage with over 1,214 million lb., worth $13.2 million, being landed in 1962. Brevoortia patronus and B. smithii are the two species of menhaden commonly caught in the South Atlantic and Gulf of Mexico. Menhaden are used for reduction into fish meal, fish solubles, and fish oil. Other industrial fish in the Region are used as raw material for such products as petfood, mink feed, and fish meal. In 1962 some 96.9 million lb. were landed for these purposes. Total value of this fishery was $1.2 million in 1962.

Fresh-water fish, crayfish, mussels, saltwater finfish, clams, and scallops are but a few of the fishery resources needing technological attention.

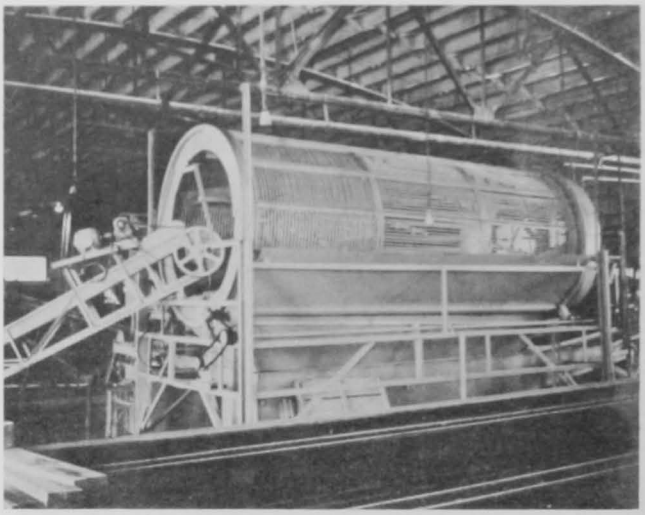


Figure 5.--An oyster shucking operation.

OTHER FISHERY RESEARCH FACILITIES IN THE AREA

The Technological Laboratory at Pascagoula shares information and research publications

with several other fishery research laboratories nearby. Through an exchange of information, all laboratories are cognizant of the others' projects so as to prevent duplication of effort.

The Gulf Coast Research Laboratory at Ocean Springs, Miss., is a biological teaching and research facility under the direction of the State of Mississippi College Board. Graduate and undergraduate courses are offered for accreditation at several southern universities. A program of marine biological research is carried out by a permanent staff in a new modern laboratory.

The U. S. Public Health Service's Dauphin Island Shellfish Research Center located nearby in Alabama is devoted to a wide variety of projects related to pollution in the shellfish growing areas. In the field of pesticide research this USPHS Center maintains especially close contact with the Laboratory in Pascagoula because both are studying pesticides in shellfish but at different levels in the processing chains. Microbiologists at the Pascagoula Laboratory are interested in the Dauphin Island Center's studies on pollution by the enteric organisms which might be carried over in the final consumer package.

The Bureau of Commercial Fisheries Biological Research Laboratory at Pensacola, Fla., is studying pollution by pesticides and their effect on the marine animals in their native habitat. Since the Laboratory at Pascagoula begins measuring pesticide residues at the point of catch, there is close rapport between the two laboratories.

The U.S. Department of Agriculture maintains an insecticide research laboratory at Gulfport, Miss. There has been an exchange of information on methods of analysis and on those insecticides that might pollute the estuarine areas, be picked up by fish or shellfish, and thus be found in some processed seafoods. With a more complete knowledge of the usage of these dangerous chemicals, the Bureau of Commercial Fisheries will be able to discuss limitations for their use on or near the fishing grounds.

THE CHEMICAL RESEARCH PROGRAM

In all our chemical research projects, we emphasized the development of methods of analysis of fishery products. Several significant contributions to the knowledge of seafoods and their processing were made. The past year's research, including a few related facts from previous research, will be discussed by individual project.

THE COMPOSITION AND NUTRITIVE VALUE OF FISH AND SHELLFISH

The composition of fish and shellfish varies not only from species to species but from one sample to another. Research workers in the field are not in agreement about the underlying reasons for this variation, and a number of factors have been set forth as being causes. Among these are (1) season, (2) geographic area of catch, (3) size, (4) sex, (5) maturity of gonads, (6) physical activity prior to capture, (7) feed, and (8) type of flesh. Detailed studies at Pascagoula have shown that, in all probability, the variations cannot be explained by a simple assumption that one of these factors causes the variation in all species. Indeed, it is quite probable that either there is a multiplicity of causes or that there is a specifically different cause in each case. Samples being analyzed currently are (1) Chesapeake Bay blue crab body meat, claw meat, and offal, (2) Dungeness crab body meat and claw meat, (3) brown shrimp tail meat and offal, (4) fillets from high-weight male and female as well as fillets from low-weight male and female ocean perch, and (5) whole alewife.

Variation in Composition

Seasonal variations.--Moisture and oil are generally accepted to be the most variable of the constituents on a seasonal basis and in general bear an inverse relation to each other. Although moisture and oil did vary seasonally in all samples examined, the usual inverse relation did not hold true in alewife ground whole, nor in brown shrimp tail meats. Nitrogen content varied seasonally in all but alewife, Dungeness crab body meat, brown shrimp tail meat, and brown shrimp offal. Although the nitrogen content either remained stable or varied according to the sample of the species examined, certain individual amino acids have been found to vary seasonally in all samples tested so far (Dungeness crab has not been analyzed to date). Further, the total concentration of the protein amino acids generally reach a low point as the nitrogen content reaches a high point. Table 1 lists the amino acids that varied significantly at the 5-percent level or better in concentration throughout the season for each of the samples assayed. In general, the variation in individual amino acids appeared to depend more on a state of activity of the animal than it did on any other one particular factor. The trace mineral content, as far as chloride, phosphate, sodium, and potassium are concerned, also varied seasonally in all samples studied.

Differences in type of sample.--There were significant differences in oil and moisture content between samples in any 1 month of (1) blue crab body meat, claw meat, and offal material; (2) brown shrimp tail meat and offal;

Table 1.--Amino acids and related compounds that vary significantly (at the 5-percent level or greater) throughout the year in various fish and shellfish

Blue crab (*Callinectes sapidus*):	
Body meat:	Claw meat:
Alanine	Alanine
Glycine	Arginine
Histidine	Aspartic acid
Hydroxylysine	Glycine
Hydroxyproline	Histidine
Ornithine	Leucine
Proline	Lysine
Tryptophan	Ornithine
Urea	Phenylalanine
	Proline
Offal:	Tryptophan
	Tyrosine
Lysine	
Proline	
Taurine	
Brown shrimp (*Penaeus aztecus*):	
Tail meat:	Offal:
Alanine	Alanine
Arginine	Arginine
Aspartic acid	Aspartic acid
Cystine/2	Cystine/2
Glutamic acid	Glycine
Glycine	Ornithine
Histidine	Serine
Isoleucine	Taurine
Leucine	Threonine
Lysine	Urea
Methionine	
Phenylalanine	
Proline	
Serine	
Threonine	
Valine	
Alewife (*Alosa pseudoharengus*):	
Ground whole:	
Aspartic acid	
Ethanolamine	
Hydroxylysine	
Hydroxyproline	
Ornithine	
Taurine	
Urea	
Valine	

and (3) low-weight male or female ocean perch fillets and high-weight male or female ocean perch fillets. The body meat, claw meat, and offal of the blue crab do not differ significantly throughout the year in aspartic acid, cystine, hydroxyproline, phenylalanine, proline, serine, threonine, tyrosine, and ethanolamine. The other amino acids do vary in concentration in

Figure 6.--Chemist preparing a run on the automatic amino acid analyzer.

the three types of samples at one time of the year or another. Lysine and arginine, however, were significantly higher in the claw meat and the body meat of the blue crab during all of the sampling period. A number of the amino acids differ in brown shrimp tail meat and waste material during the same month. Throughout the year the tail meat, however, was consistently higher in arginine, whereas the offal was consistently higher in alanine, histidine, ornithine, taurine, urea, and valine. The chloride and phosphorus content of the three types of blue crab samples and the two types of brown shrimp samples differed from each other throughout the year. There was no difference, however, in the content of these two components in the ocean perch fillets.

Species differences.--Suggestions by several taxonomists have been considered concerning the probability of significant variance among the amino acid concentrations of various species and the use of this variation as a taxonomic measure. The consensus is that the individual amino acids of the whole animal will vary so widely in concentration owing to the previously mentioned influences that identification would become improbable if not impossible. Recently, however, attention has been paid to the profile concept wherein the pattern of certain of the amino acid concentrations has been used to distinguish between species. During February 1964 the R/V Oregon

had an opportunity to collect samples of brown shrimp, white shrimp, and pink speckled (Penaeopsis megalops) shrimp from nearly the same geographic area and within 1 day of each other. In fact, the browns and whites were caught in the same trawl and could be considered as living in a similar environment. The pink speckled shrimp, however, are a deepwater shrimp found in the Gulf near the 200 fath. (fathom) curve. Table 2 shows the amino acid concentration of the tail portion of each of these shrimp together with the significant differences. As is readily apparent, the brown shrimp were significantly different from either the white or the pink speckleds in a number of the amino acids; the difference in the browns and the pink speckleds were the most pronounced. Little difference existed between the whites and the pink speckleds. Only hydroxyproline had a significant difference in all three species. Although the browns and the whites are more closely related in a taxonomic sense, the quantitative amino acid levels do not clearly bear out this similarity. If this type of analysis were to be useful, the browns and whites, which belong to the same genus, should be more closely related to each other in amino acid level than either is to the pink speckleds, another genus. It may be that amino acid patterns may be of use in a taxonomic sense, but the analysis of only three species does not allow a logical interpretation of pattern. As a further corollary, it appears that depth or environment has little to do with changes in amino acid concentration. If this were true, the browns and whites should be more closely related than either of them are to the pink speckleds.

Yearly and geographic variations.--Three samples of brown shrimp obtained at different times--two from the same area and in the same year, one from a different area and in a different year--showed little difference in amino acid concentrations (table 3). Where

Table 2.--Average amino acid concentrations in three species of Gulf of Mexico shrimp tails caught February 1964

Amino acid	Average amino acid concentration			Significant species differences in amino acid content		
	Brown	White	Pink speckled			
	μ moles/mg. N					
Alanine	3.64	4.36	4.88		B≠PS	
Arginine	3.81	3.81	4.06			
Aspartic acid	4.14	5.57	5.17	B≠W	B≠PS	
Cystine/2	.36	.38	.48			
Glutamic acid	4.97	6.22	6.23	B≠W	B≠PS	
Glycine	6.19	6.96	5.07		B≠PS	W≠PS
Histidine	.79	.86	.94		B≠PS	W≠PS
Hydroxylysine	.03	.04	.01			
Hydroxyproline	.11	.01	.21	B≠W	B≠PS	W≠PS
Isoleucine	2.02	2.59	2.61	B≠W	B≠PS	
Leucine	3.59	4.32	4.36	B≠W	B≠PS	
Lysine	3.52	3.46	3.82		B≠PS	W≠PS
Methionine	1.04	1.25	1.44		B≠PS	
Ornithine	.10	.11	.13			
Phenylalanine	1.20	1.42	1.51		B≠PS	
Proline	2.29	2.47	2.81			
Serine	1.96	2.40	2.52	B≠W	B≠PS	
Taurine	.31	.65	1.05	B≠W	B≠PS	
Threonine	1.77	2.16	2.31	B≠W	B≠PS	
Tyrosine	.85	.89	.87			
Urea	.08	--	.12			
Valine	2.31	2.80	2.98	B≠W	B≠PS	
Latitude N.	29°01'	29°01'	28°04'			
Longitude W.	90°12'	90°12'	90°11.5'			
Depth (fath.)	7	7	190			
Date	Feb. 20, 1964	Feb. 20, 1964	Feb. 21, 1964			

significant differences did occur, it is apparent that it is the progression from the amino acid concentration prevalent in the winter to the amino acid concentration prevalent in the summer that results in the development of the significant difference. For example, the January and February samples encompassing the different years and different geographic locations might not be significantly different, but the March sample would be significantly different from the others in a particular amino acid concentration; or the February and March samples might not be different, but the January one would. Thus, except for lysine, shrimp caught in different years at different locations had essentially the same concentration of amino acids on a nitrogen content basis. Of the amino acids that varied in concentration, the only amino acid not in this category of single variation is lysine, for which all three samples were significantly different. From an examination of the seasonal variation, however, it is apparent that the February sample represented the high point of the characteristic seasonal curve for lysine.

Table 3.--Amino acid concentration of brown shrimp tail meats collected in two years and in two geographic areas in the Gulf of Mexico, 1963-64

Amino acid	Amino acid concentration in samples taken in:		
	1/14/63[1]	2/20/64[2]	3/25/63[1]
	μmoles/mg.N		
Alanine.....	3.70	3.64	4.26
Arginine.....	3.16	3.81	3.60
Aspartic acid..	3.98	4.14	4.64
Cystine/2.....	.33	.36	.09
Glutamic acid..	4.90	4.97	5.64
Glycine.....	6.35	6.19	6.45
Histidine.....	.67	.79	.76
Hydroxylysine..	.05	.03	.01
Hydroxyproline.	.09	.11	.03
Isoleucine.....	1.86	2.02	2.13
Leucine.....	3.35	3.59	3.87
Lysine.....	2.82	3.52	3.11
Methionine.....	.87	1.04	1.06
Ornithine.....	.13	.10	.12
Phenylalanine..	1.11	1.20	1.30
Proline.....	2.11	2.29	2.46
Serine.....	1.92	1.96	2.33
Taurine.....	.47	.31	.57
Threonine.....	1.70	1.77	1.93
Tryptophan.....	.39	--	.45
Tyrosine.....	.77	.85	.74
Urea.....	.07	.08	.16
Valine.....	2.06	2.31	2.57

[1] Mouth of Pascagoula River, Miss.
[2] Off Timbolier Bay, La.

Sex.--In one sample of the Chesapeake Bay blue crab there were sufficient males and females to divide the body meat sample according to sex. There were no significant differences discernible in proximate composition, amino acid (taurine is an exception), chloride, phosphate, sodium, or potassium concentrations.

Nutritive Value

The amino acid pattern of all species tested so far indicates that the nutritive value of these fish and shellfish is as good as or superior to other animal meats. Thus, portion for portion, equal protein value is obtained when meat of these tested species are eaten.

Results previously obtained indicate that the Chesapeake Bay blue crab and the Dungeness crab contain very low amounts of cholesterol: Blue crab body meat averages 98 mg. (milligrams) per 100 g. (grams) of meat; Dungeness crab body meat, 63 mg. per 100 g.; and Dungeness crab claw meat, 52 mg. per 100 g. The cholesterol content of brown shrimp is higher--156 mg. per 100 g.

The sodium and potassium concentration in a variety of fresh and processed seafoods were analyzed in connection with the development of a method for the Association of Official Agricultural Chemists (table 4). Although the sodium and potassium content of the five intensively studied (Chesapeake Bay blue crab, ocean perch, Dungeness crab, alewife, and brown shrimp) samples varied throughout the year, a yearly average value for these species is also included in the table.

Thiaminase Studies

At the request of industry, a cooperative project with the Bureau's Technological Laboratory at Ann Arbor was undertaken to determine the presence of thiaminase in six species of industrial fish. Thiaminase is a biologically active enzyme, sometimes present in fish, that destroys thiamine in the diet. Thiaminase is inactivated by heat. The species selected for testing are those that form the major part of the industrial fish catch--Atlantic croaker (Micropogon undulatus), Atlantic cutlassfish or silver eels (Trichiurus lepturus), Southern kingfish or king whiting (Menticirrhus americanus), searobin (Prionotus sp.), spot (Leiostomus xanthurus), and silver seatrout or white trout (Cynoscion nothus). Samples were collected by commercial vessels in 6 to 11 fathoms of water near Grand Isle, La., July 19, 1964; November 4, 1964; February 19, 1965; and June 27, 1965. No thiaminase activity appeared in any of the samples tested.

Table 4.--Market survey of the sodium and potassium concentration in various fishery products

Product[1]	Average concentration in:	
	Sodium	Potassium
	mg./100 g.	
Cod, frozen fillet.....	74	332
Flounder, fresh fillet...	76	436
Haddock, frozen fillet...	168	396
Mullet, fresh fillet.....	83	307
Salmon, canned.....	482	336
Tuna, canned.....	482	382
Clams, canned, salt added	434	205
Crab, Alaskan King, cooked and frozen.....	446	230
Lobster, African Rock, frozen.....	450	334
Oysters, raw washed.....	149	198
Shrimp, raw washed.....	220	320
Crab, Chesapeake Bay blue, frozen body meat.	299	316
Crab, Chesapeake Bay blue, frozen claw meat.	375	282
Crab, Dungeness, frozen body meat.....	239	371
Crab, Dungeness, frozen claw meat.....	354	337
Crab, Dungeness, canned, cooked in brine.....	791	163
Crab, Dungeness, canned, cooked without brine...	169	286
Shrimp, brown, frozen tails.....	197	357
Alewife, frozen whole....	110	311
Ocean perch, frozen fillet.....	111	345

[1] Samples were obtained in local supermarkets at the end of the distribution chain.

CHEMICAL REACTIONS IN PROCESSED SEA FOODS

A year was devoted to a cooperative study between the Laboratory and the American Shrimp Canner's Association. The purpose of the study was to process and store a large test pack of canned shrimp to determine means of increasing the shelf life and quality of this product. Twenty-four variations of processing conditions were used, and withdrawals from storage were set at 0, 3, 6, 12, 18, and 24 mo. (months). To date, samples up to and including the 12-mo. storage period have been analyzed; however, it will not be possible to predict the best method of processing among the variables tried until after the 24-mo. withdrawal. Particular attention is being paid to texture, taste, iron sulfide discoloration, opaqueness of the liquor, and nitrogen content of the liquor. Several pertinent facts are evident, however, without waiting until the tests are complete. The shrimp meats have a small loss in net weight over the first 3 mo. of storage. The pH of the liquor remains fairly constant until after the third month of storage when it begins to decline. The opaqueness of the liquor (a measure of the amount of protein material being solubilized) slowly increases during storage except for those packs that are extremely milky at the beginning of the storage period. The increase in opacity correlates well with the increase in nitrogen content of the liquor and the development of undesirable texture. At the completion of the storage test we expect that it will be possible to predict the most desirable process for producing canned shrimp with a long shelf life and desirable quality.

A study, also at the request of industry, of the possibilities of increasing the Grade A iced storage life of brown, white, and pink shrimp resulted in a series of papers presented at a Symposium, sponsored by our Laboratory and attended by industry members from Florida to Texas, that was held in Pascagoula April 14, 1965. Since the results of this study are most clearly presented in terms of microbiology, chemistry, histology, and food technology, they will be presented in a special section at the end of this circular.

PESTICIDE RESIDUES IN FISH AND SHELLFISH

At the beginning of fiscal year 1965, the Laboratory undertook a new national project concerned with assaying the pesticide residues present in processed fishery products and developing methods of control. The changes in agricultural procedures in the past 20 yr., (years), necessitated by feeding a growing population, have also brought changes in all of the foodstuffs we eat. Pesticide residues of varying proportions are found in almost all our food supply. To keep abreast of the situation a number of Government agencies--among them the U.S. Department of Agriculture, the U.S. Department of Health, Education and Welfare, and the U.S. Department of the Interior--have agreed to monitor certain portions of the nation's food supply falling under their various jurisdictions. As the methods of detection of the various pesticide residues have increased in sensitivity, agricultural scientists have shown that most foods contain minute traces of the more common residues--amounts well under tolerances allowed. A survey of fishery

products as marketed was started. One of the most significant facts to emerge from this initial survey was that--at least in the limited number of samples analyzed--there is little evidence of the presence of chlorinated pesticides other than DDT, DDD, and DDE. These last three, however, are present to some degree in almost every fishery product tested. There is some evidence that heat processing, particularly of oily products, reduces the pesticide residue level somewhat (table 5). A study of the effect of heat processing upon residue levels will be started next year. The effect of dressing fishery products in reducing residue levels is shown in table 6. As would be expected, a large amount of the residue present in the fish is removed along with the vital organs, leaving somewhat less than half in the edible portion. Both the cod and haddock samples shown in table 5 were obtained in-the-round at the same time from vessels fishing the Grand Bank of Newfoundland. The cod samples contained considerably more residues than did the haddock samples. The pesticide residue content of six species of industrial fish is shown in table 7. At least 16 fish of each species were ground whole for each analysis. The fish were obtained at three widely spaced times throughout 1 year's period from nearly the same geographic area. The variability of residue levels shown indicates that investigation into seasonal variations in pesticide residue levels should be undertaken.

Difficulties with the Florisil[2] separation of chlorinated pesticide residues necessitated our study of the properties of this absorbent. We found that successful separation and recovery could be achieved by adding 0.7 percent by weight of water after the absorbent was activated by heating it at 130° C.

[2] Trade names referred to in this publication do not imply endorsement of commercial products.

Figure 7.--The analysis of pesticide residues in fishery products conducted by electron capture gas chromatography.

Table 5.--Pesticide residue levels in various fishery products

Product[1]	Concentration of:		
	DDT	DDD	DDE
	Parts per million		
Oysters, fresh	0.010	0.010	T[2]
	.010	.010	T
	.010	.010	T
	.010	.010	T
	.010	.010	T
Oysters, canned	None	T	T
	..do...	T	T
	..do...	T	T
	..do...	T	T
	..do...	T	None
	..do...	T	Do.
	..do...	0.014	Do.
	..do...	.014	T
	..do...	T	T
	..do...	T	T
	..do...	.015	None
	..do...	T	T
	0.02	.030	T
	None	.040	0.03
Shrimp tails, fresh	None	None	None
	T	T	0.063
	None	None	None
	..do...	..do...	Do.
	T	T	T
	T	T	T
Shrimp, canned	None	T	0.043
	..do...	None	T
	0.020	0.020	T
	.040	.030	T
	.010	.020	T
	None	.020	T
Petfood	0.014	.035	0.078
	.015	.036	.058
	.011	.024	.059
Tuna loins, frozen	0.343	0.181	0.157
Tuna, canned (6 samples)	None	None	None
Salmon, canned (6 samples)	None	None	None
Crab meat, canned (3 samples)	None	None	None

[1] Samples were obtained in local supermarkets at the end of the distribution chain.

[2] T = Trace, less than 0.01 parts per million.

Table 6.--Effect of dressing fishery products upon the reduction of residue levels in the edible portion of three fishery products obtained in the North Atlantic during the fall of 1964

Product	Concentration of:					
	DDT in:		DDD in:		DDE in:	
	Offal	Edible portion	Offal	Edible portion	Offal	Edible portion
	Parts per million		Parts per million		Parts per million	
American lobster (average of 6).....	0.162	0.035	0.224	0.122	0.327	0.132
Cod: No. 1.....	.024	.011	T[1]	.010	.048	.020
No. 2.....	.097	.051	.035	.021	.076	.035
No. 3.....	.291	.064	.084	T	.150	.045
No. 4.....	.703	.104	.496	.064	.451	.064
No. 5.....	.474	.057	.198	T	.450	.073
Haddock: No. 1.....	.151	None	0.065	None	.050	None
No. 2.....	T	None	None	None	T	Do.
No. 3.....	T	None	T	None	T	Do.
No. 4.....	.138	None	0.054	None	.038	Do.
No. 5.....	.187	T	0.107	T	.063	T
No. 6.....	T	None	T	None	T	None

[1] T = Trace, less than 0.01 parts per million.

Table 7.--Pesticide residue levels in six species of fish obtained at different times at the same location west of the Mississippi River Delta, 1964-65

Common name	Scientific name	Concentration of:								
		DDT in:			DDD in:			DDE in:		
		11/4/64	2/19/65	6/28/65	11/4/64	2/19/65	6/28/65	11/4/64	2/19/65	6/28/65
		Parts per million			Parts per million			Parts per million		
Silver seatrout or white trout.....	Cynoscion nothus.....	0.020	T[1]	T	0.020	T	T	0.058	T	T
Searobin.....	Prionotus sp.	None	T	None	None	T	None	.020	T	None
Spot.....	Leiostomus xanthurus...	.015	T	None	.015	T	T	.015	T	T
Atlantic croaker.....	Micropogon undulatus...	None	T	T	None	T	T	.020	T	T
Atlantic cutlassfish or silver eel.....	Trichiurus lepturus....	None	T	T	None	T	None	.020	T	T
Southern kingfish or king whiting.....	Menticirrhus americanus	None	T	T	None	T	T	.022	T	T

[1] T = Trace, less than 0.01 parts per million.

THE MICROBIOLOGICAL RESEARCH PROGRAM

The present microbiological program embodies separate projects for marine and terrigenous (land-derived) organisms; however, only the terrigenous project remains active following the Iced Shrimp Symposium.

BACTERIA OF TERRIGENOUS ORIGIN

Bacteriology of Frozen Foods

The analyses formerly made as a part of the U.S. Department of the Interior Inspection Service were incorporated into a project in early fiscal year 1964. Many samples of frozen raw breaded shrimp were assayed for bacteria of public health significance--e.g., total plate counts, the coliform group, Escherichia coli, Salmonella, Streptococcus faecalis, and coagulase-positive staphylococci. This project on organisms of terrigenous origin was pointed toward developing data to establish the necessity for baselines for bacterial standards on breaded shrimp. The Pascagoula staff was in close contact with a committee from the Association of Food and Drug Officials of the United States whose purpose was to suggest standards for all frozen foods. We hoped that the Pascagoula data would assist the industry to obtain realistic standards if State and Federal regulatory agencies promulgate such standards. The data showed considerable unexplained variability in counts and isolations even though we had a large number of samples. Higher counts from plants having good sanitary practice indicated that any standard counts promulgated must be high enough to allow for the variability of number of organisms found

in a frozen raw product produced under good commercial practice.

With the addition of a fourth microbiologist in 1965, the project on frozen seafoods was broadened to include precooked items. Samples were taken by the inspectors of plants under continuous USDI inspection and shipped in dry ice to the Laboratory. Full recognition must be given to the fact that freezing may prevent growth and reproduction of from 60 to 80 percent of the terrigenous microorganisms found on the frozen product. The initial results on precooked products in inspected plants showed that the incidence of bacteria of public health significance was encouragingly low. A complete survey will require more months of work.

Thus far, microbial analyses have been completed on 36 samples of various precooked fishery products. Samples examined included breaded shrimp, shrimp creole, breaded cod portions, haddock, and pollock fish sticks. The study was designed to determine total plate counts, E. coli, coliforms, Streptococcus faecalis, Salmonella, and coagulase-positive staphylococci. All of the samples analyzed were collected by resident USDI inspectors from their respective plants.

Preliminary results indicate a total absence of E. coli, Streptococcus faecalis, Salmonella, and coagulase-positive staphylococci in the products thus far examined. The overall total plate counts for samples examined range from 20 to 2,200 cells per gram of precooked breaded shrimp; 10,450 to 20,700 cells per gram for shrimp creole; 5,200 to 61,000 cells per gram for precooked breaded fish. These counts reflect a rather good product picture. The coliform MPN[3] values ranged from 0 to 230 per 100 g. for precooked breaded shrimp; 2,300 to 46,000 per 100 g. in shrimp creole; 91 to 230 per 100 g. in precooked fish.

Studies on Clostridium botulinum

In midyear of fiscal year 1964, we again increased the staff to allow fuller attention to the problem of botulism in fishery products. We obtained additional laboratory space by remodeling underutilized freezer rooms into isolated botulism research areas. A project on the nutritional requirements of type E Cl. botulinum was started with the D-8 strain.

Amino acids, vitamins, fatty acids, classical preservative acids, and the constituents of nucleic acid were evaluated in synthetic media for their ability to support Cl. botulinum spore germination, vegetative growth, sporulation, and toxin formation. We found that the D-8 strain of Cl. botulinum had no absolute nutritional requirements. Toxin was never produced in synthetic media until citric or lactic acid was added. Once begun, however, toxin formation would continue when the culture was transferred to media not containing these acids. We tested some strains other than D-8 type E and found them not to have this requirement. We concluded that attempts to control the organism through its nutritional requirements would be less likely to produce immediate success than other possible control methods.

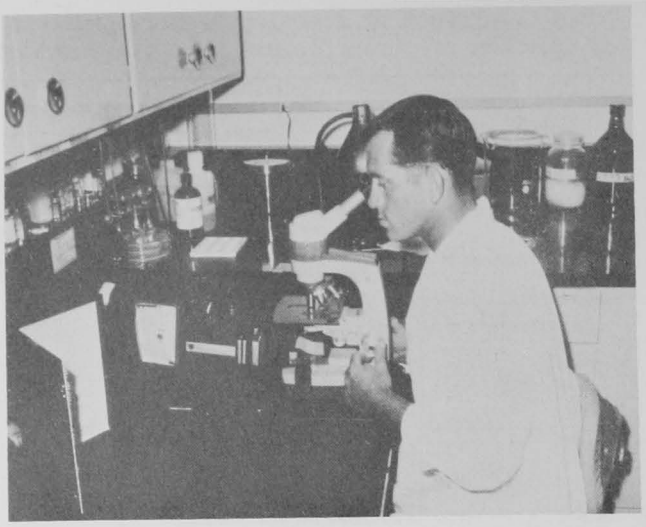


Figure 8.--A microscope is an essential part of the fluorescent antibody technique used to detect Cl. botulinum.

A verbal report from a foreign investigator was the only previous record of type E Cl. botulinum in the Gulf of Mexico. As an adjunct to the phase of work concerned with type E, a very limited survey was undertaken to confirm this verbal report which dealt with the presence of this type E from Freeport, Tex. In an effort to confirm this report, the Pascagoula microbiologists tested 36 mud samples taken from various points in Galveston Bay. On the 36 mud samples, two were positive for type E Cl. botulinum. In view of these isolations and the verbal report, the concept that endemic Cl. botulinum is peculiar to northern land masses above 40° N. should be re-examined.

During the latter part of fiscal year 1965, AEC (U.S. Atomic Energy Commission) became interested in our botulism studies. A contract was signed between the AEC and the BCF, adding a microbiologist to the Pascagoula staff. The planned survey will require the collection of samples from Gulf inshore areas to determine the incidence of Cl. botulinum type E. The microbiologists will gather samples from the bays, rivers, and estuarine areas for analysis in the laboratory. Samples have already been obtained from Key West to Panama City, Fla., and from the Biloxi Bay-Pascagoula area in Mississippi. In preparation for this additional project, part of the unused space of the pilot canning plant was converted into a third bacteriology laboratory.

[3] MPN = most probable numbers of bacteria.

The new fluorescent antibody staining method developed by British workers to microscopically identify Cl. botulinum types will be compared with the toxin production-mouse injection method already accepted. Pascagoula samples now receive analysis under both methods.

THE SYMPOSIA

On several occasions, our Laboratory has started special "crash" programs to solve a particularly pressing industry problem. Often the results of the studies are published and distributed to interested parties. On two occasions the staff held conferences, or symposia, with members of the industry to present more fully the data and to answer questions. These symposia have proved to be good methods for gaining industry acceptance of the results and for quickly disseminating a large volume of technical information. Members of the industry have shown an eagerness to cooperate by traveling great distances to attend the meetings.

THE CANNED SHRIMP SYMPOSIUM

A canned shrimp technological symposium was held in the Pascagoula Technological Library in 1964. Almost every major shrimp canning firm between Mobile Bay and Galveston Bay was represented. The group was welcomed by Hermes Gautier, then Chairman of the Gulf States Marine Fisheries Commission. In addition to the industry members, the meeting had representatives from the National Fisheries Institute, the American Can Company, the Continental Can Company, Sunkist Growers Citrus Products, and private technological consultants.

Pascagoula staff members presented talks on "The Chemistry of Iron Sulphide Discoloration," "Quality Control by Bacteriological Analysis," "Shelf Life and Quality Extension by Use of Additives," and "Compliance with State and Federal Regulatory Acts." Following the talks, a series of open-panel discussions were held with full industry participation. In general the industry accepted the suggestions for improvements; however, for economic reasons, not all suggestions were adopted in the processing plants.

THE FRESH ICED SHRIMP SYMPOSIUM

A fresh iced shrimp symposium was held on April 14, 1965, following an intensive 60-day study by the Pascagoula technological staff, whose members took short trips on shrimp vessels and collected samples of three commercial shrimp species--pinks, whites, and browns. The results of this study are described below.

ICED SHRIMP STORAGE STUDY

EXPERIMENTAL

Sampling Methods

Brown shrimp and white shrimp were caught on trashy, muddy bottoms near the mouths of rivers, and pink shrimp were taken from clean sand bottoms off Tortugas. Three types of samples were taken directly from the trawl as it emptied on deck. An aseptically collected sample was picked up with sterile forceps and placed in sterile jars of ice. Another sample was washed exceedingly well, after the shrimp were headed by hand, and mixed with ice in a 2:1 ratio. The third sample was washed following normal commercial practice and iced in a 2:1 ratio. All shrimp were brought to the Laboratory in less than 3 days.

At the Laboratory, a large amount of ice was placed in the bottom of commercial shrimp boxes before the shrimp were added; the ice was well packed into the corners and around the sides. This was done to keep the shrimp from coming in contact with the box as the ice melted. The shrimp and ice were mixed in a ratio of two parts ice to one part of shrimp, and a large cap of crushed ice was placed over the shrimp. The boxes were all placed so that they would drain adequately. We added a fresh layer of ice to the top of each box daily and turned and re-iced the shrimp on an average of every 2 days.

Daily withdrawals were made for study at the Laboratory. Because the aseptically collected sample had only a few shrimp, we could not perform a large number of analyses on it; consequently, although we made routine daily withdrawals for microbiological purposes, we withdrew only a few for chemical and organoleptic analyses.

Organoleptic Analysis

One of the tests on the shrimp was daily grading by organoleptic analysis. The major criteria for these analyses were appearance and odor of the raw shrimp, and taste of the cooked shrimp. The grades were designated as A, B, substandard, and inedible. Figure 9

shows the time in days that the shrimp remained in each grade. Usually, the shrimp that had a good wash reached Grade B much later than did the same species that were washed in the usual manner. The good-washed shrimp often remained in Grade B longer than did the average-washed shrimp.

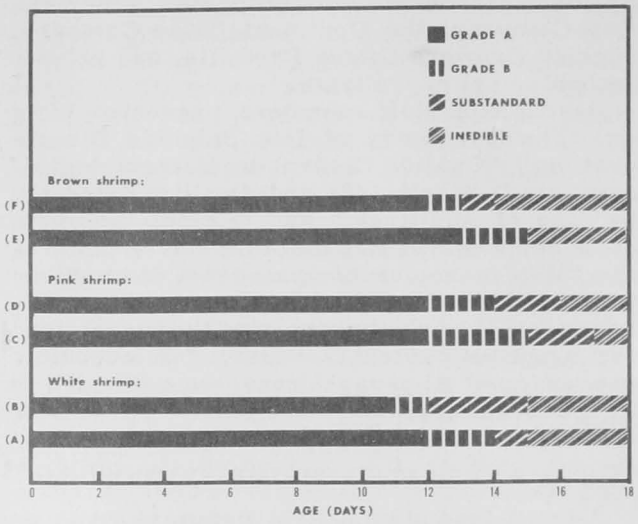


Figure 9.--Organoleptic grade patterns obtained during the iced storage of (A) white shrimp, good wash; (B) white shrimp, average wash; (C) pink shrimp, good wash; (D) pink shrimp, average wash; (E) brown shrimp, good wash; and (F) brown shrimp, average wash.

Bacteriological Methods

We used the standard total plate count method to determine daily the number of bacteria per gram of shrimp. The procedure for making the total plate count was as follows: 50 g. of headless shrimp with shell intact were placed in a sterile Waring blendor jar containing 450 cc. of sterile phosphate buffer and blended for 2 min. (minutes). From this blended portion further dilutions were made as needed. Each dilution was plated by pipetting 1.0 cc. of the dilution into an empty petri dish and pouring sterile melted Tryptone Glucose Extract Agar into the plate, mixing, and allowing to solidify. The medium was prepared with both distilled water and with filtered sea water. Once inoculated, the plates were divided into two groups--one being incubated at 25° C. (77° F.) and the other group at 37° C. (98.6° F.). The plates were incubated at these temperatures for 48 hr. at which time they were removed and the colonies counted. The total plate count for each sample was determined by averaging duplicate plates of the same dilution.

Chemical Methods

Biochemical analyses were planned so as to follow protein and connective tissue degradation by enzymes and bacteria. Fractions of the protein followed were: (1) the nonprotein nitrogen (NPN) fraction composed of amino acids and small peptides; (2) the salt-soluble fraction composed of enzymes, muscle proteins, sarcoplasmic proteins, and a portion of the connective tissue; and (3) the acid-soluble fraction composed of most of the remainder of the connective tissue.

The fractionation was accomplished in the following manner. Six shrimp were blended at 4° C. (39.2° F.) in a Waring blendor (equipped with baffle) with a volume of phosphate buffer, pH 7.0, ionic strength 1.0, about 10 times their weight for 90 sec. (seconds). The homogenate obtained was centrifuged at 3,000 x gravity for 20 min. at 4° C. The supernate was carefully removed from the insoluble protein material. The NPN fraction was prepared by taking a portion of the supernate and precipitating the protein material with trichloracetic acid. We considered the salt-soluble fraction to be the difference in value between the supernate and the NPN fractions. The insoluble material collected above was reblended with a pH 3.6 Sorenson citrate buffer in a Waring blendor at 4° C. The blend was allowed to stand for 24 hr. in the cold and was then centrifuged at 16,000 x gravity to remove insoluble materials. We considered the supernate from this fraction to be the acid-soluble fraction.

Duplicate nitrogen values were obtained on each fraction every day to follow the degradation of the shrimp tissues. Nitrogen values were obtained with the standard micro-Kjeldahl technique and are reported as the average of the duplicate values. An additional analysis, that of the amino acid hydroxyproline, was used to reflect changes in the connective tissue. The colorimetric method of Bergman and Loxley was adapted to our needs. Duplicate determinations of hydroxyproline were made for each fraction; we report the results as averages of the duplicate value.

Histological Methods

In order to follow the changes in the muscle and structural parts of the shrimp during the iced-storage period, we used classical histological methods of microscopic analysis. The sixth abdominal somite of the shrimp was selected as the point from which to obtain the tissue sections. This somite was chosen because it was the smallest and thereby the easiest to embed and section.

Following the removal of the shell, a section of tissue about one-quarter inch long was removed from the somite and placed in Zenker's fixative, dehydrated, imbedded in paraffin, sectioned, and stained.

RESULTS AND DISCUSSION

Bacteriological Results

From a bacteriological standpoint, the first important fact noted about the bacteriology of shrimp is that the numbers of bacteria have a very high degree of variability. This variability may be due to one or more of the following factors: size of shrimp, type of bottom inhabited, intestinal flora, and chance contact with extraneous contaminants. We noted that the bacterial counts on the white shrimp were initially higher than on the browns, which were higher than the pinks (figs. 10, 11, and 13). This order was followed throughout the study: the white shrimp reached much higher counts than did the brown shrimp; and they, in turn, reached much higher counts than did the pink shrimp. The simplest explanation, although not the only, for these differences is the type of bottom the shrimp came from--since a muddy bottom tends to have a much higher bacterial count per gram of sediment than does a sandy bottom.

Following the removal of the shrimp from its natural habitat, a series of standard practices begins, each of which influences subsequent bacterial developments. First the shrimp are headed. Heading reduces the bacterial load from 50 to 80 percent, because the major portion of the shrimp's bacterial load is found in the cephalothorax, or head. At the same time this native load is being drastically reduced, handling is introducing extraneous bacteria. Actually, this introduction of bacteria begins prior to heading and continues throughout the storage period. Once the shrimp are entrapped in the trawl and brought aboard a vessel, they are immediately exposed to contamination by foreign microorganisms. Most of these contaminants are introduced from either the vessel, equipment, or crew and are of terrigenous origin. Examples of these types of contaminants would be coliforms, E. coli, streptococci, staphylococci, Salmonella, Proteus, and many others.

The second major event in this series is the process of washing the shrimp. Efficient washing may reduce the initial bacterial count as much as 75 percent. Results from this study indicated that washing had no great effect in maintaining lower bacterial counts during the first week of storage; however, during the second week of storage it became clear that this initial washing played a very important role in its ultimate effect on bacterial counts (figs. 10, 11, and 13). The type of bottom from which the shrimp are taken should influence the type and extent of washing the product is given. A shrimp caught on a muddy bottom requires a much better wash than a shrimp caught on a clear sandy bottom.

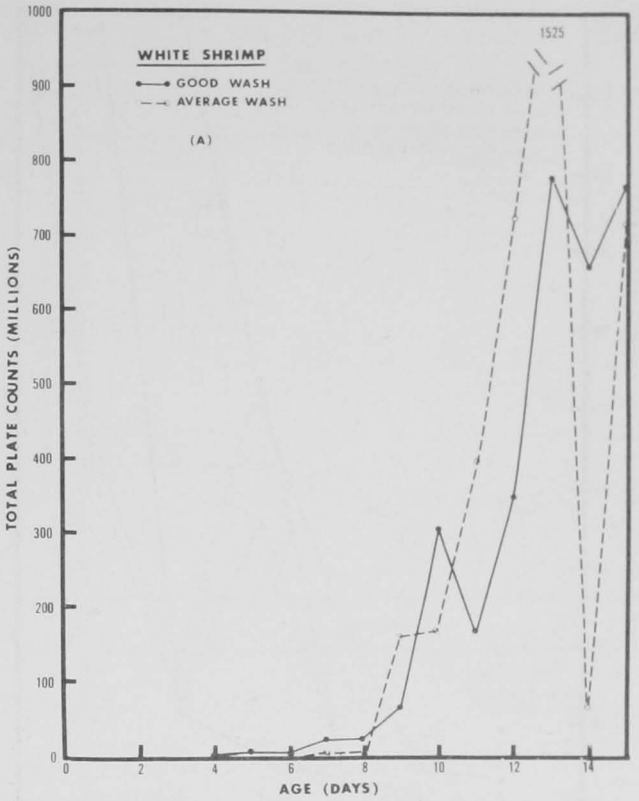


Figure 10.--Total plate counts of homogenates of white shrimp on salt-water media at 25° C.

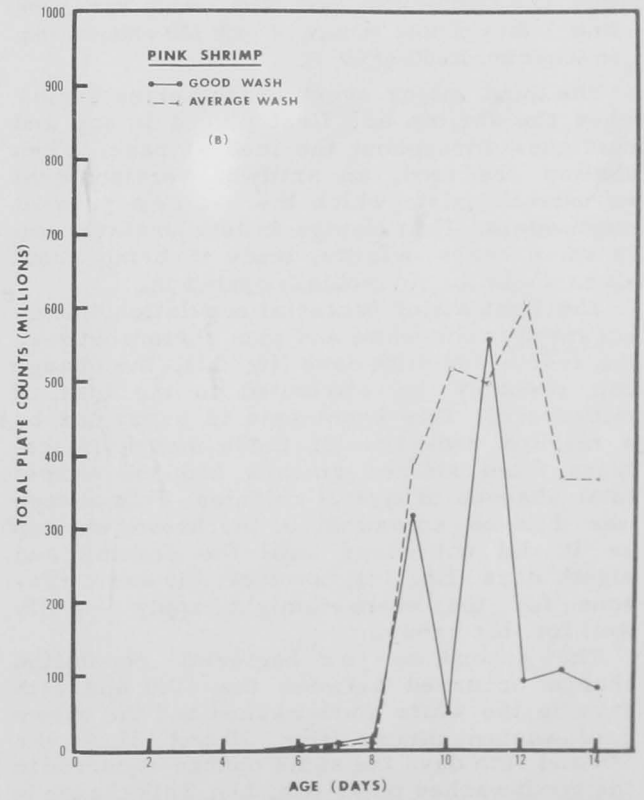


Figure 11.--Total plate counts of homogenates of pink shrimp on salt-water media at 25° C.

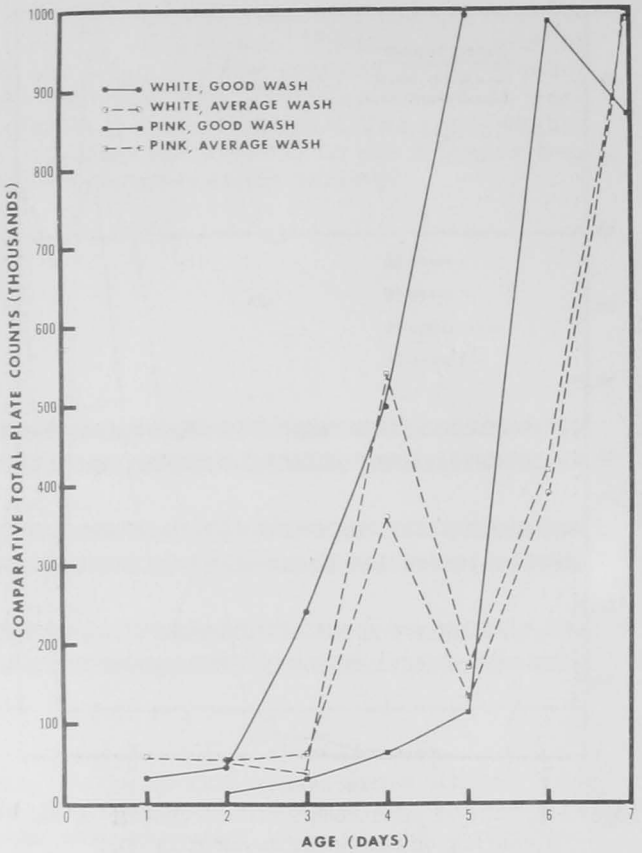


Figure 12.--Comparative total plate counts during the first 7 days of iced storage of pink and white shrimp on salt-water media at 25° C.

The third major event in the series begins when the shrimp are first placed in ice and continues throughout the iced storage. When shrimp are iced, an artificial environment is established to which the bacteria present must adapt. This change in temperature, and in some cases salinity, tends to bring about a change in the microbial population.

The first major bacterial population change occurred in the white and pink shrimp between the fourth and fifth days (fig. 12). The change can probably be attributed to the loss of micrococci. This hypothesis is supported by a marked reduction of these morphological types from stained smears and the almost total absence of typical colonies. This change was delayed somewhat in the brown shrimp as it did not occur until the seventh and eighth days (fig. 13); however, the same reasons for the change might apply equally well for this group.

The second major bacterial population change occurred between the 10th and 11th days in the white good-washed and the brown good-washed shrimp (figs. 10 and 13). On the 9th and 10th days the same change occurred in the good-washed pinks (fig. 11). This change in population corresponds to the sharp drop in the number of Flavobacterium. The change might be attributed to the reduced salinity of the shrimp because the Flavobacter are more adapted to live in higher salinities than are the other species present.

The third major change in population took place between the 12th and 15th days in all three species (figs. 10, 11, and 13). This change seems to represent the points where a reduction occurs in the number of pseudomonads remaining and an increase occurs in the number of Achromobacter. In this period, Achromobacter becomes the dominant type of microorganism in the spoilage pattern.

When shrimp are iced, they are usually placed in alternate layers of shrimp and ice. This layering may bring about another change in the shrimp bacteriologically. When shrimp are layered in a bin or box, the thickness of the layers should be held to a minimum and ice should be scattered in with the shrimp. The added ice within the shrimp layers facilitates the washing effect that normally takes place. It also reduces the density of packing, which could easily result in anaerobic conditions. This dense packing may result in "bilgy shrimp" due to anaerobic microorganisms. Studies have been made of the differences in bacterial counts in the different layers of shrimp (Green, 1949[4]). Green found that the bacterial counts of shrimp in the uppermost

[4] Green, Margaret, 1949. Bacteriology of shrimp. II. Quantitative studies on freshly caught and iced shrimp. Food Res. 14: 372-383.

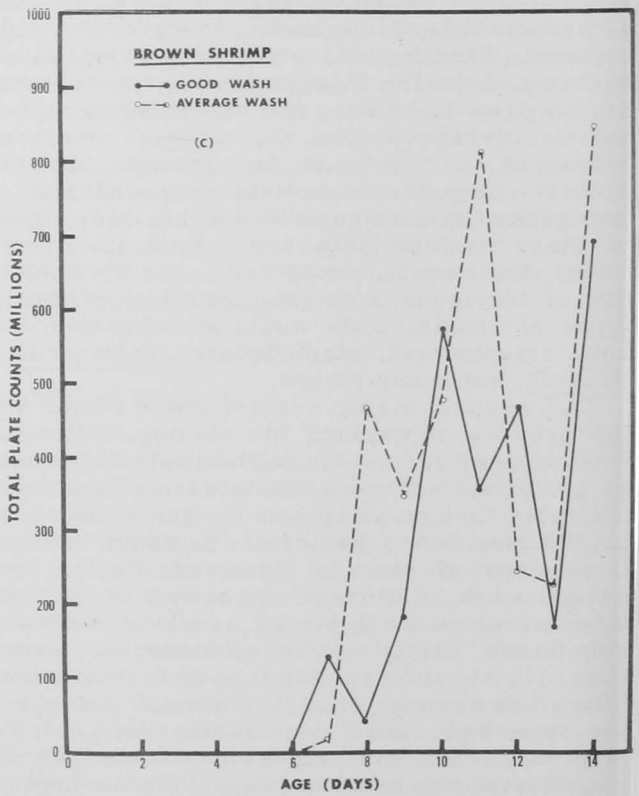


Figure 13.--Total plate counts of homogenates of brown shrimp on salt-water media at 25° C.

layer of a bin or box had about a twofold increase in bacterial counts, whereas the shrimp in the bottom layer had about a thousandfold increase. The difference in numbers is produced by a steady washing that takes place as a result of melting ice. Consequently, the shrimp in the lower layer are immersed, or at least subjected to the washings from the upper layers, which produces an enormous increase in bacterial counts. Thus, "melt water" should be prevented from percolating through more than two or three layers of shrimp.

The microbial populations of the white and pink shrimp that were aseptically collected (fig. 14) compare well with those of the good- and average-washed shrimp. As we expected, counts on the white shrimp were consistently higher. Both whites and pinks showed two major population changes occurring between the 10th and 14th days. Although the bacteria counts were never as high as for the commercially collected samples, the spoilage pattern was the same except for the time required. The commercially collected species spoiled long before the aseptically collected species.

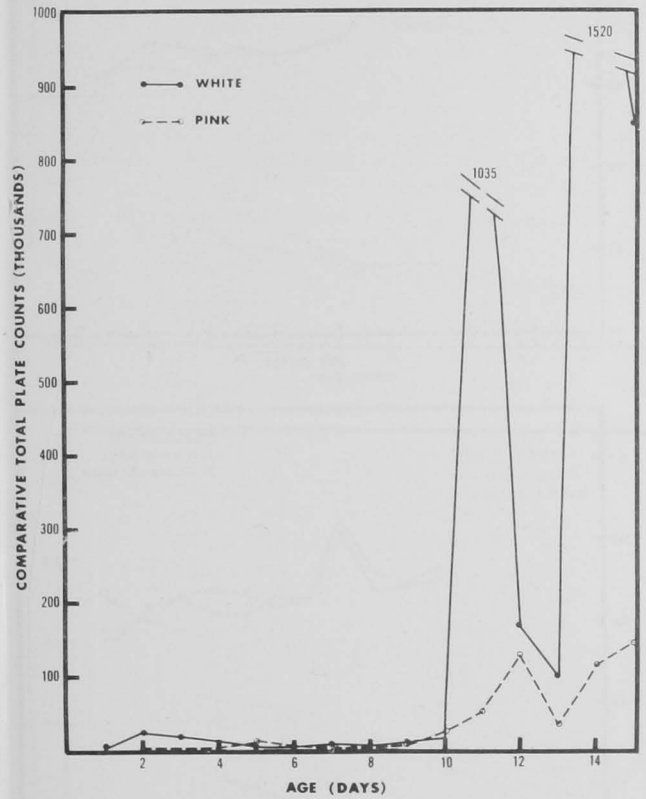


Figure 14.--Total plate counts of aseptically collected white and pink shrimp homogenates on sea-water media at 25° C.

Chemical Results

The nitrogen content of the acid-soluble fraction of both the average- and good-washed white shrimp follow each other closely throughout the period of storage--both begin at 0.5 mg./g. of shrimp and proceed to a value almost half of that through the period of Grade A life of the shrimp (fig. 15(A)). The

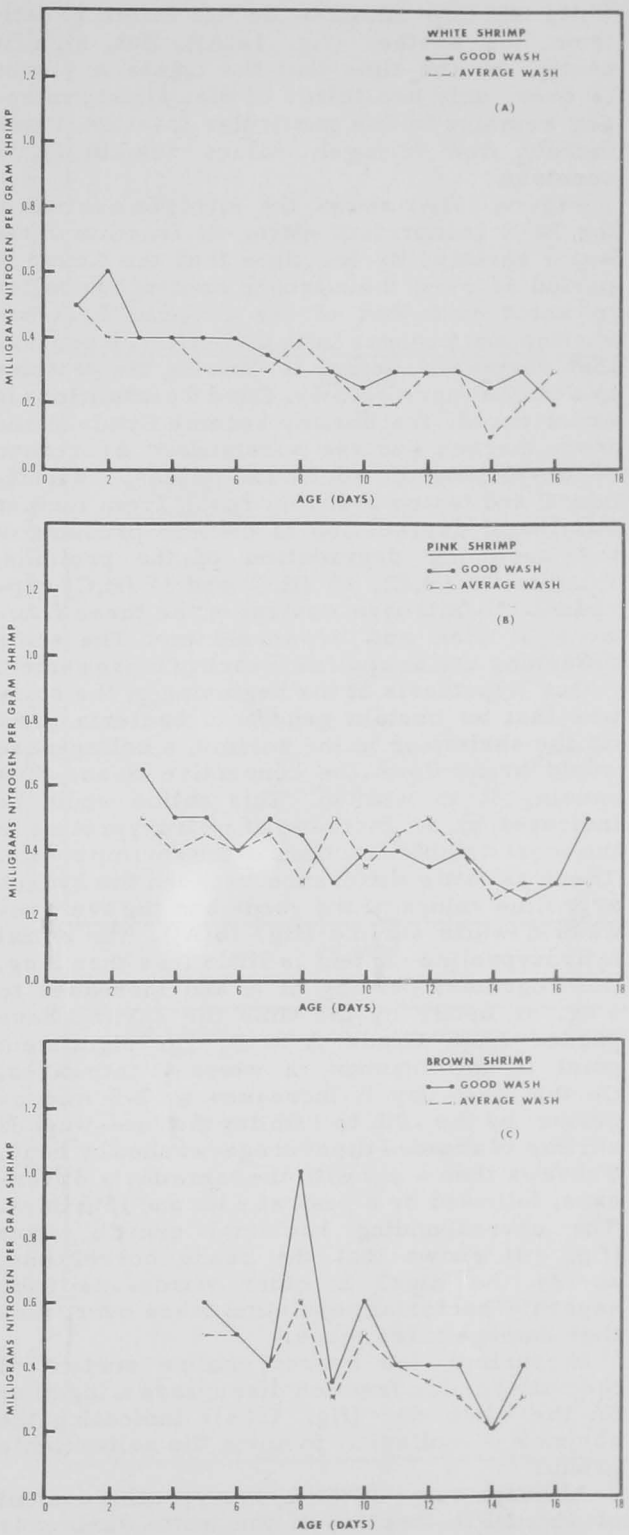


Figure 15.--Change in nitrogen content of the citric acid-soluble fractions of (A) white shrimp, (B) pink shrimp, and (C) brown shrimp during iced storage.

nitrogen then remains much the same in this fraction through the periods of Grade B, Grade Substandard, and Inedible.

The nitrogen content of the salt-soluble fraction of the average- and good-washed white shrimp samples do not differ greatly from one another (fig. 16(A)). But, significantly, by the time that the Grade A period is over, only two-thirds of the original nitrogen remains in this particular fraction. From thereon the nitrogen values remain fairly constant.

Figure 17(A) shows the nitrogen content of the NPN (nonprotein nitrogen) fraction of the white shrimp. By the time that the Grade A period is over the nitrogen content has fallen to about half that of the original first-day shrimp and changes little thereafter. It appears that enzymatic action is causing the proteins to become more soluble. Once a certain loss is experienced, the shrimp become Grade B and from thereon become substandard by reason of developing off-odors and tastes. The off-odors and tastes probably result from further enzymatic degradation of certain products of the beginning degradation of the proteins. Figures 15 (B,C), 16 (B,C) and 17 (B,C) represent the nitrogen content of the three fractions of pink and brown shrimp. The same reasoning can be applied to each of these series.

Our hypothesis at the beginning of the study was that as certain genera of bacteria grew on the shrimp or in the shrimp, a collagenase would break down the connective tissue thus causing it to weaken. This action would be indicated by an increase of hydroxyproline in the most soluble fractions of the shrimp meats. There is little difference between the hydroxyproline values of the good- and the average-washed white shrimp (fig. 18(A)). The initial hydroxyproline content is little less than 2 μg. (micrograms) per mg of N and increases to 5 μg. or better by the time the shrimp have passed from Grade A to B. The significant point is the manner in which it increases. On the sixth day it increases to 7-8 micrograms; by the 10th to 11th day the good-washed shrimp is ahead of the average-washed by 1 day. There is then a dip with the same day's difference, followed by a peak at 13th and 15th days. The corresponding bacterial growth curve (fig. 10) shows that the peaks correspond, as do the dips. In other words--as each separate bacterial population takes over, further damage takes place.

In shrimp, the hydroxyproline content of the salt-soluble fraction disappears altogether on the ninth day (fig. 19(A)) indicating the absence of collagens forming the salt-soluble group.

Although some of the hydroxyproline content of the NPN fraction of the white shrimp is almost certainly washed out with the ice

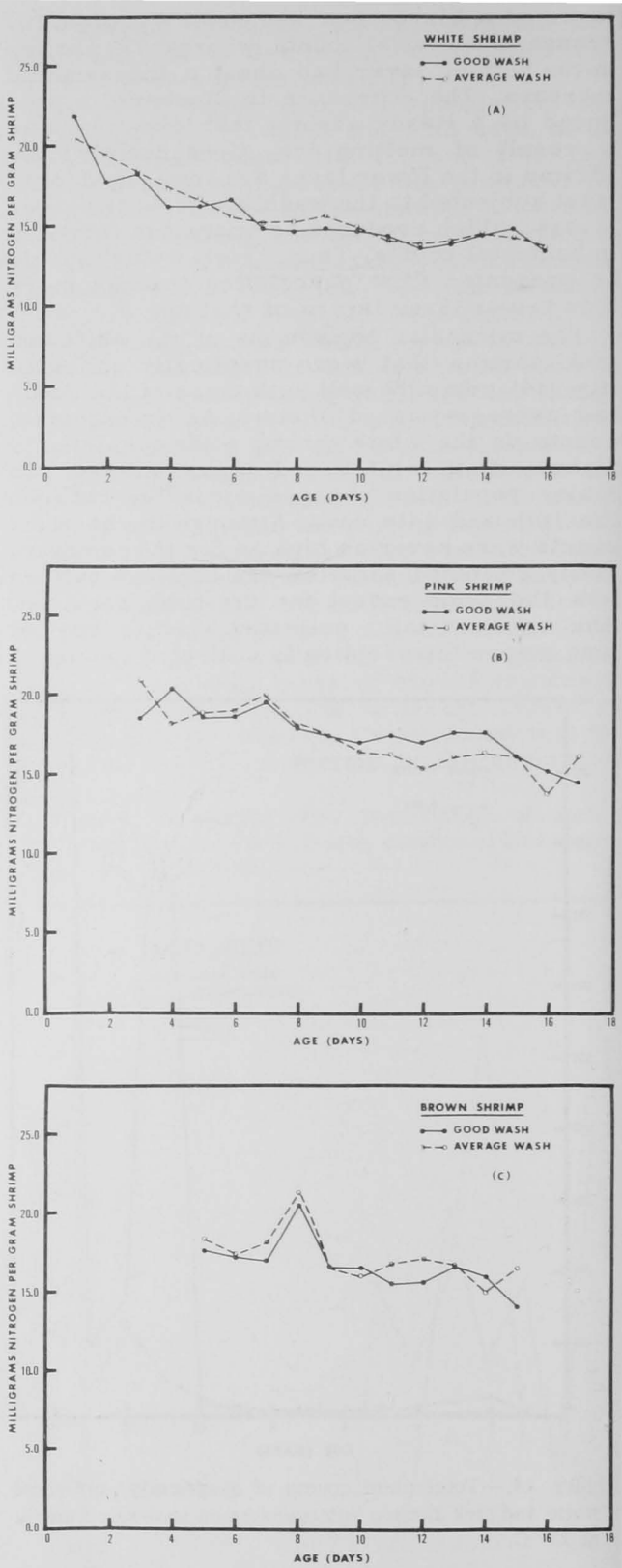
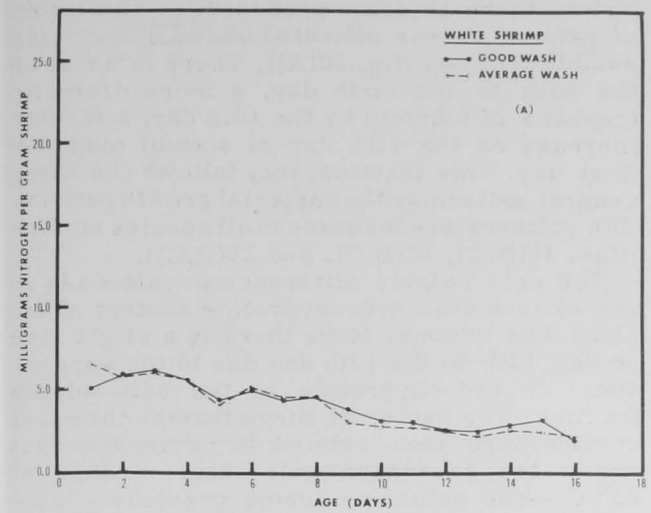
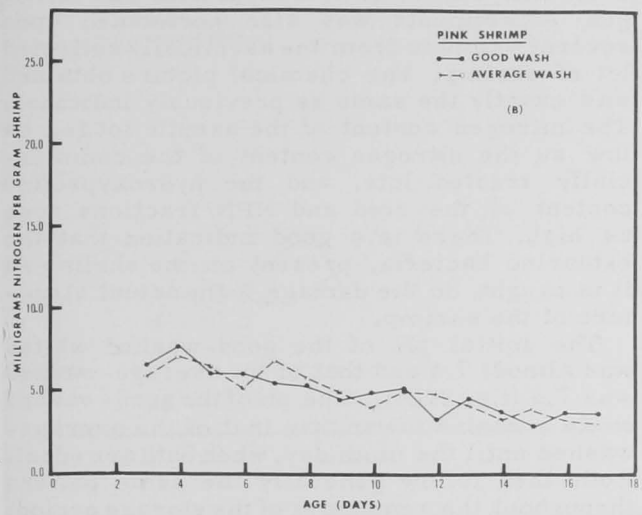


Figure 16.--Change in nitrogen content of the salt-soluble fractions of (A) white shrimp, (B) pink shrimp, and (C) brown shrimp during iced storage.





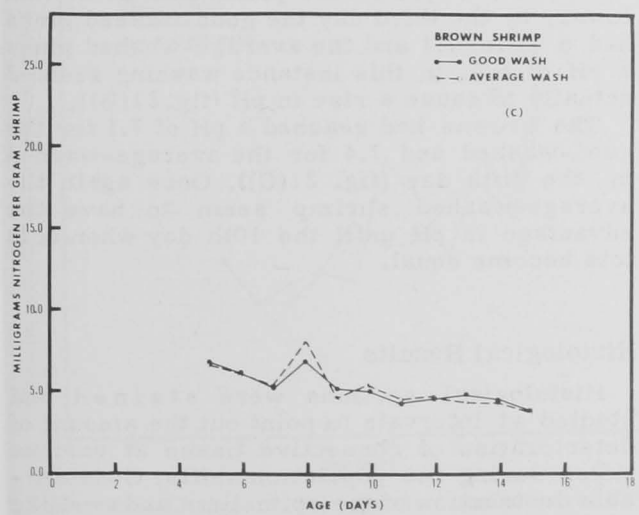
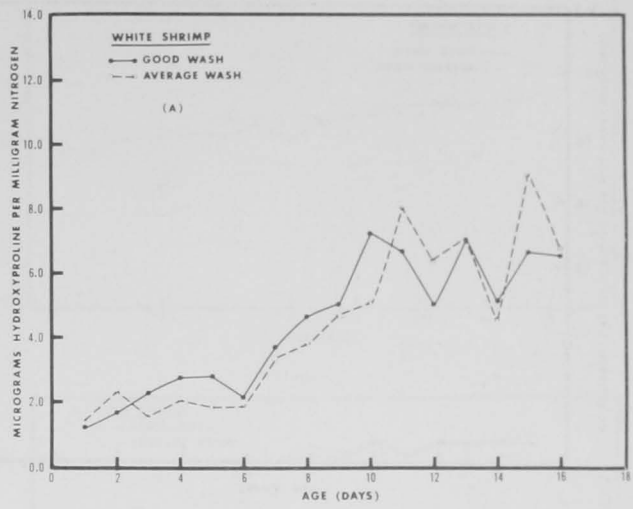
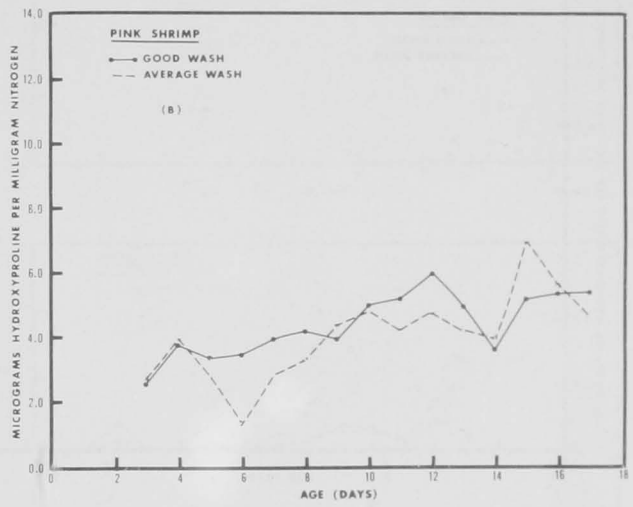


Figure 17.--Change in nitrogen content of the NPN fractions of (A) white shrimp, (B) pink shrimp, and (C) brown shrimp during iced storage.





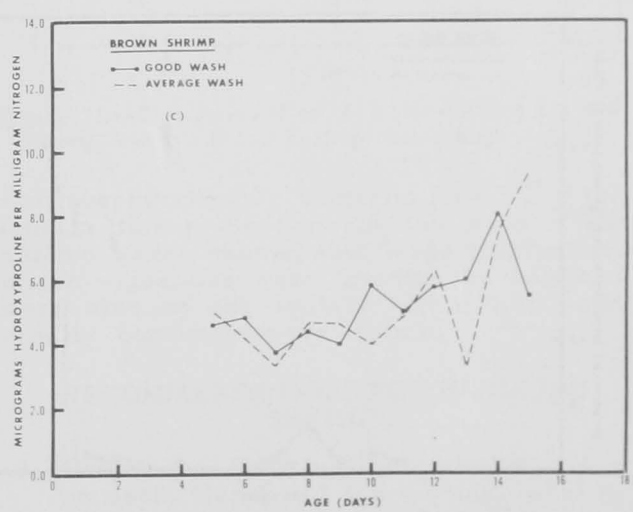


Figure 18.--Change in hydroxyproline content of the citric acid-soluble fractions of (A) white shrimp, (B) pink shrimp, and (C) brown shrimp during iced storage.

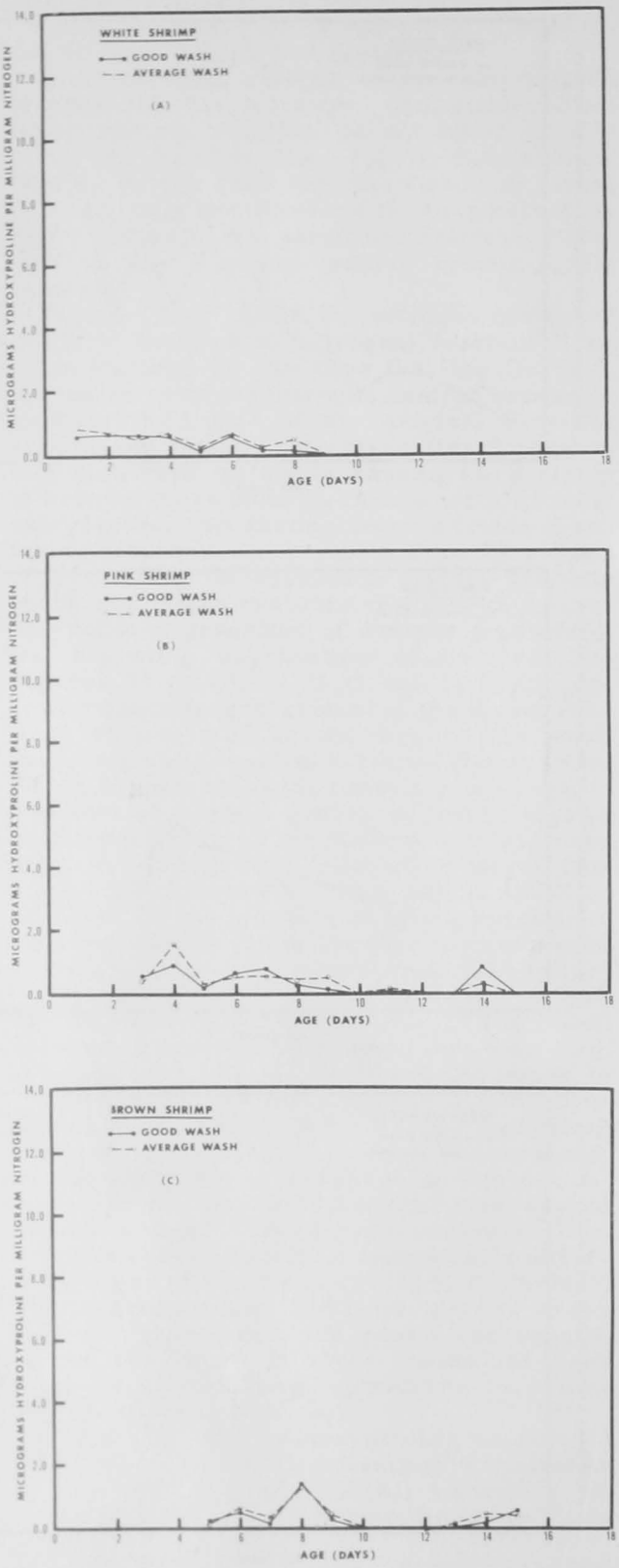


Figure 19.--Change in hydroxyproline content of the salt-soluble fractions of (A) white shrimp, (B) pink shrimp, and (C) brown shrimp during iced storage.

water, there is again produced the same sort of pattern as was indicated above in the acid-soluble fraction (fig. 20(A)). There is a rise on the fifth to the sixth day, a more dramatic increase of fourfold by the 10th day, a further increase on the 12th day of sixfold over the first day. This fraction, too, follows the same general pattern as the bacterial growth pattern. The patterns are the same in all species studies (figs. 18(B,C), 19(B,C), and 20(B,C)).

The only notable difference in pattern is in the salt-soluble hydroxyproline content of the pinks and browns. Here there is a slight rise on the 12th to the 13th day due to the appearance of hydroxyproline in the salt-soluble fraction. The bacterial curve that the chemical curves have been related to is the one that shows the growth in salt-water medium at 25° C.--the estuarine forms previously indicated as having a high proportion of collagenase. This series of hydroxyproline and nitrogen experiments was also performed upon several samples from the aseptically collected lot of shrimp. The chemical picture obtained was exactly the same as previously indicated. The nitrogen content of the aseptic lot fell as low as the nitrogen content of the commercially treated lots, and the hydroxyproline content of the acid and NPN fractions rose as high. There is a good indication that the estuarine bacteria, present on the shrimp as it is caught, do the damage to the actual structure of the shrimp.

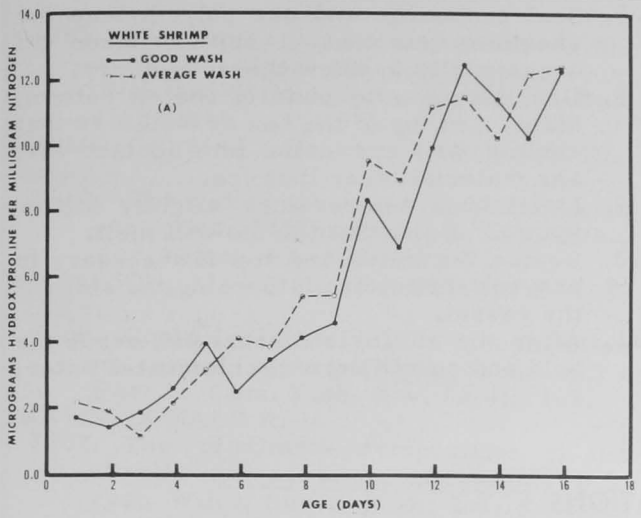
The initial pH of the good-washed whites was almost 7.4 and that of the average-washed was 7.3 (fig. 21(A)). The pH of the good-washed white remains lower than that of the average-washed until the ninth day, when both are equal. Both then follow generally the same pattern throughout the remainder of the storage period.

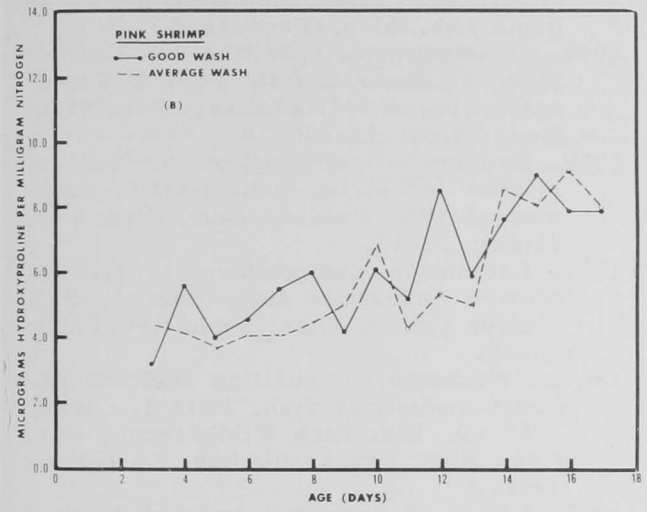
The initial pH of the pinks was somewhat lower; by the third day the good-washed pinks had a pH of 7.1 and the average-washed pinks a pH of 6.9. In this instance washing seemed actually to cause a rise in pH (fig. 21(B)).

The browns had reached a pH of 7.1 for the good-washed and 7.4 for the average-washed by the fifth day (fig. 21(C)). Once again the average-washed shrimp seem to have the advantage in pH until the 10th day when both lots become equal.

Histological Results

Histological sections were stained and studied at intervals to point out the amount of deterioration of connective tissue at various times during the population shifts. Considerable destruction of the epithelium and swelling of the connective tissue were noted as early as the seventh day. By the 14th day, the connective tissue was sloughing and disrupted and





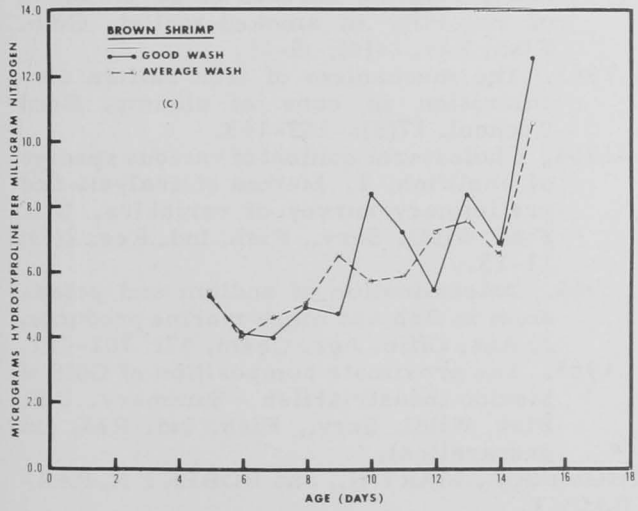
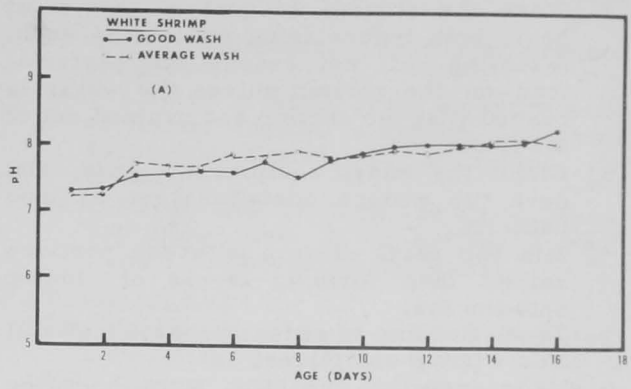
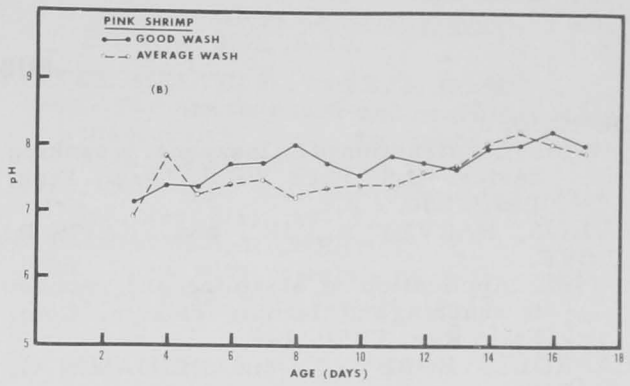


Figure 20.--Change in hydroxyproline content of the NPN fractions of (A) white shrimp, (B) pink shrimp, and (C) brown shrimp during iced storage.





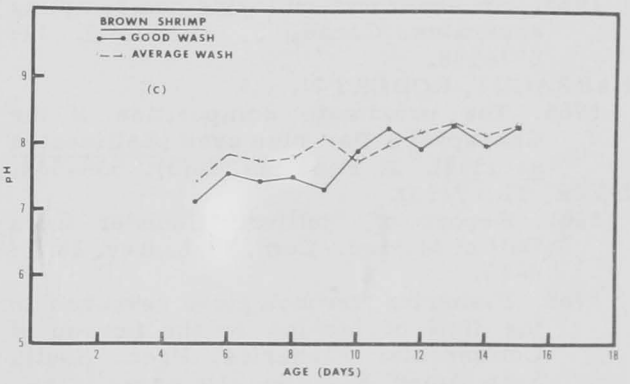


Figure 21.--Change in pH of (A) white shrimp, (B) pink shrimp, and (C) brown shrimp during iced storage.

was accompanied by bacterial invasion of the muscle tissue. Sections of the wood of the shrimp boxes showed that large numbers of micro-organisms were invading the wood and were also on the surface where they could readily contaminate the shrimp.

RECOMMENDATIONS FOR HANDLING SHRIMP

1. Handle the shrimp quickly while they are on deck. Carry out the sorting, heading, washing, and icing in the shade if possible (consider rigging some sort of tarp for shade).

2. Wash the shrimp adequately with a jet hose, both before icing and at the dock. (Fluming will not reduce the bacterial load on the shrimp unless the water is rushed past the shrimp and drained out of the flume end).
3. Clean the hose, equipment, hands, and deck, to reduce contamination by land bacteria.
4. Mix two parts shrimp with one part ice rather than forming layers of shrimp between ice.
5. Treat flooring to reduce pockets that will hold shrimp and juices.
6. Make sure that the bilge pump operates efficiently.
7. Seal penboards and use polyethylene for sheathing so that it can be taken off occasionally to allow the wood to dry.
8. Use adequate amount of ice on bottom, sides, and top of the box or holds so that shrimp will not come into contact with any material other than ice.
9. Maintain a temperature slightly higher than 32° F. so that the ice will melt.
10. Re-ice the sides and top if necessary to prevent shrimp from touching the sides of the vessel.
11. After the cargo is discharged, scrub the hold and equipment with chlorinated water.

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