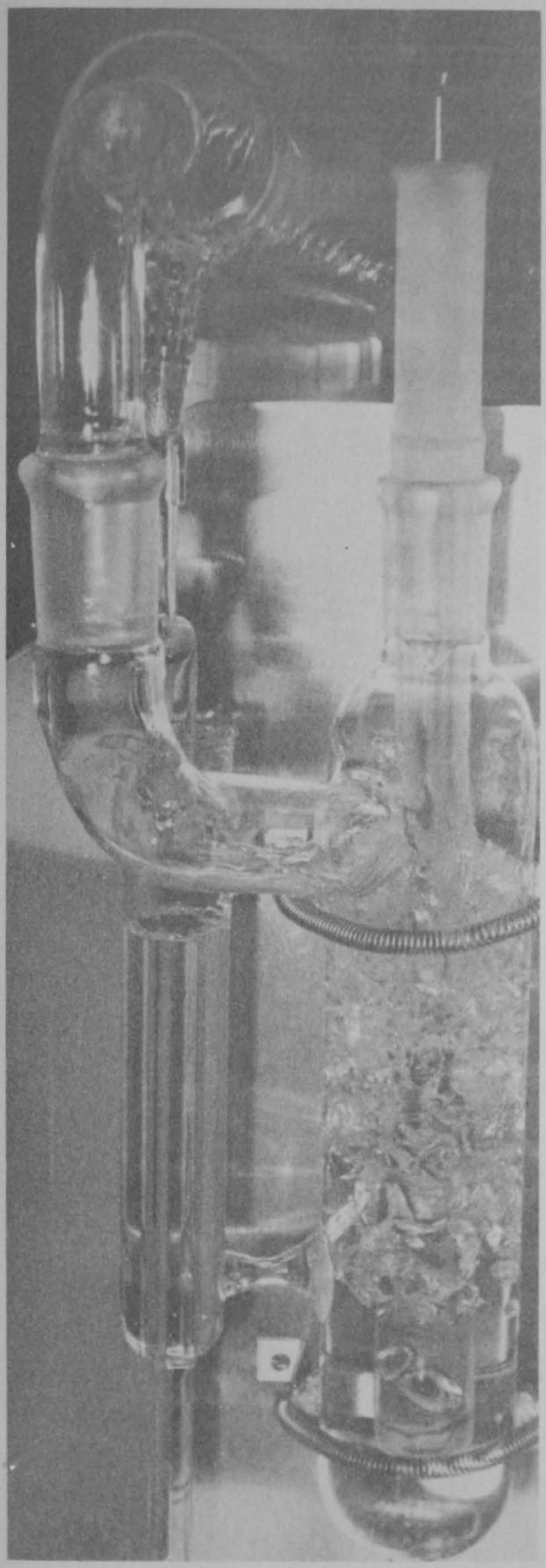

Report
Technological Laboratory
Bureau of Commercial Fisheries
Pascagoula, Mississippi

for fiscal year ending
June 30, 1966

UNITED STATES DEPARTMENT
OF THE INTERIOR

FISH AND WILDLIFE SERVICE
BUREAU OF COMMERCIAL FISHERIES

Circular 262

UNITED STATES DEPARTMENT OF THE INTERIOR

Stewart L. Udall, *Secretary*

Charles F. Luce, *Under Secretary*

Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*

FISH AND WILDLIFE SERVICE, Clarence F. Pautzke, *Commissioner*

BUREAU OF COMMERCIAL FISHERIES, Harold E. Crowther, *Director*

Report
Technological Laboratory
Bureau of Commercial Fisheries
Pascagoula, Mississippi

for fiscal year ending June 30, 1966

By

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

Circular 262

Washington, D.C.

September 1967

STAFF

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

I. CHEMISTRY OF SEAFOODS

Mary H. Thompson, Program Leader

A. Composition and nutritive value of fish and shellfish

Robert N. Farragut, Project Leader
Donald R. Travis, Chemist
John P. Wilson, Chemist

B. Chemical reactions in processed seafoods

Melvin E. Waters, Co-Project Leader
Harold C. Thompson, Co-Project Leader

C. Pesticide residues in fish and shellfish

Robert N. Farragut, Project Leader
Bobby J. Wood, Chemist

II. MICROBIOLOGY OF SEAFOODS

B. Q. Ward, Program Leader

A. Microbiological studies - marine origin

Bobby J. Carroll, Project Leader

B. Microbiological studies - terrigenous origin

Bobby J. Carroll, Project Leader
Gladys B. Reese, Microbiologist

C. A survey of the Gulf of Mexico for Clostridium botulinum

E. Spencer Garrett, Microbiologist

ABSTRACT

Results of research on the composition and nutritive value of seafoods, the processing difficulties encountered with shrimp, and methods for the removal of pesticide residues from seafoods are described. Microbiological studies on microorganisms of public health significance and Salmonella in fishery products are presented, as are the results of the survey of the Gulf of Mexico for the presence of Clostridium botulinum. Other activities of the Laboratory staff are acknowledged as are the staff publications for fiscal year 1966.

Report
Technological Laboratory, Bureau of Commercial Fisheries
Pascagoula, Mississippi

for fiscal year ending June 30, 1966

INTRODUCTION

During 1965, the fisheries in Region 2 enjoyed a prosperous and productive year. The estimated value of the U.S. shrimp catch was $82.6 million--8 percent above the record of $70.4 million set in 1953. A record 97.2 million pounds of breaded shrimp were manufactured and sold. Landing of blue crabs increased to 167.2 million pounds, and spiny lobsters rose to a high of 6.2 million pounds. The menhaden catch of 1.7 billion pounds accounted for 36 percent of the total U.S. catch of fish and shellfish, about half of which was taken within the borders of Region 2. Fisheries of Region 2 were more productive in terms of both poundage and dollar value than were those of any other region.

Figure 1.--Apparatus to remove the shells of sun-dried shrimp by flailing the shrimp in a metal drum.

CONSULTING AND ADVISING

In keeping with the great diversity and change in the fisheries of Region 2, a wide variety of consulting and advising activities was undertaken by the staff of the Technological Laboratory at Pascagoula in fiscal year 1966. A new and growing industry in the region is that of catfish farming and processing. Numerous requests were received during the year for information on growing, processing, and marketing this species. Although most of the products are marketed in the fresh-iced state, several producers are considering marketing a frozen whole fish.

Technological interest has revived in the sun-curing of shrimp, primarily owing to the requirements of the emerging nations for simple, effective means of preserving shrimp. Southern Louisiana has a large sun-dried shrimp industry that provides an accurate source of information. Other fishery products receiving attention, through extension-type service, included canned mullet loins, mullet roe, little tuna, and bonito, as well as enzyme-digested industrial fish.

Contract monitoring service and information are provided to contractors studying a variety of problems connected with the fishing industry. The Laboratory advised and supervised an Economic Development Administration contract to Theodore H. Miller, Marine Chemurgics, concerned with developing processing methods and markets for fishery products of Carteret County, N.C. The fisheries off the North Carolina coast were surveyed in terms of availability and productivity; methods of processing were studied; and market-feasibility studies were undertaken. As a result of the contract, a successful venture in IQF (individually quick frozen) scallops and fish portions was launched.

Public Law 88-309 funds, granted to the Gulf Coast Research Laboratory at Ocean Springs, Miss., financed research on depuration of oysters and a study of the feasibility of rebedding oysters in unpolluted areas.

Reports on the progress of research projects at the Bureau's Laboratory in Pascagoula during the fiscal year follow under the general headings of Chemistry of Seafoods and Microbiology of Seafoods. This report also contains a list of the publications produced by the staff during the year.

STAFF

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

I. CHEMISTRY OF SEAFOODS

Mary H. Thompson, Program Leader

A. Composition and nutritive value of fish and shellfish

Robert N. Farragut, Project Leader
Donald R. Travis, Chemist
John P. Wilson, Chemist

B. Chemical reactions in processed seafoods

Melvin E. Waters, Co-Project Leader
Harold C. Thompson, Co-Project Leader

C. Pesticide residues in fish and shellfish

Robert N. Farragut, Project Leader
Bobby J. Wood, Chemist

II. MICROBIOLOGY OF SEAFOODS

B. Q. Ward, Program Leader

A. Microbiological studies - marine origin

Bobby J. Carroll, Project Leader

B. Microbiological studies - terrigenous origin

Bobby J. Carroll, Project Leader
Gladys B. Reese, Microbiologist

C. A survey of the Gulf of Mexico for Clostridium botulinum

E. Spencer Garrett, Microbiologist

CONTENTS

ABSTRACT

Results of research on the composition and nutritive value of seafoods, the processing difficulties encountered with shrimp, and methods for the removal of pesticide residues from seafoods are described. Microbiological studies on microorganisms of public health significance and Salmonella in fishery products are presented, as are the results of the survey of the Gulf of Mexico for the presence of Clostridium botulinum. Other activities of the Laboratory staff are acknowledged as are the staff publications for fiscal year 1966.

Report
Technological Laboratory, Bureau of Commercial Fisheries
Pascagoula, Mississippi

for fiscal year ending June 30, 1966

INTRODUCTION

During 1965, the fisheries in Region 2 enjoyed a prosperous and productive year. The estimated value of the U.S. shrimp catch was $82.6 million--8 percent above the record of $70.4 million set in 1953. A record 97.2 million pounds of breaded shrimp were manufactured and sold. Landing of blue crabs increased to 167.2 million pounds, and spiny lobsters rose to a high of 6.2 million pounds. The menhaden catch of 1.7 billion pounds accounted for 36 percent of the total U.S. catch of fish and shellfish, about half of which was taken within the borders of Region 2. Fisheries of Region 2 were more productive in terms of both poundage and dollar value than were those of any other region.

Figure 1.--Apparatus to remove the shells of sun-dried shrimp by flailing the shrimp in a metal drum.

CONSULTING AND ADVISING

In keeping with the great diversity and change in the fisheries of Region 2, a wide variety of consulting and advising activities was undertaken by the staff of the Technological Laboratory at Pascagoula in fiscal year 1966. A new and growing industry in the region is that of catfish farming and processing. Numerous requests were received during the year for information on growing, processing, and marketing this species. Although most of the products are marketed in the fresh-iced state, several producers are considering marketing a frozen whole fish.

Technological interest has revived in the sun-curing of shrimp, primarily owing to the requirements of the emerging nations for simple, effective means of preserving shrimp. Southern Louisiana has a large sun-dried shrimp industry that provides an accurate source of information. Other fishery products receiving attention, through extension-type service, included canned mullet loins, mullet roe, little tuna, and bonito, as well as enzyme-digested industrial fish.

Contract monitoring service and information are provided to contractors studying a variety of problems connected with the fishing industry. The Laboratory advised and supervised an Economic Development Administration contract to Theodore H. Miller, Marine Chemurgics, concerned with developing processing methods and markets for fishery products of Carteret County, N.C. The fisheries off the North Carolina coast were surveyed in terms of availability and productivity; methods of processing were studied; and market-feasibility studies were undertaken. As a result of the contract, a successful venture in IQF (individually quick frozen) scallops and fish portions was launched.

Public Law 88-309 funds, granted to the Gulf Coast Research Laboratory at Ocean Springs, Miss., financed research on depuration of oysters and a study of the feasibility of rebedding oysters in unpolluted areas.

Reports on the progress of research projects at the Bureau's Laboratory in Pascagoula during the fiscal year follow under the general headings of Chemistry of Seafoods and Microbiology of Seafoods. This report also contains a list of the publications produced by the staff during the year.

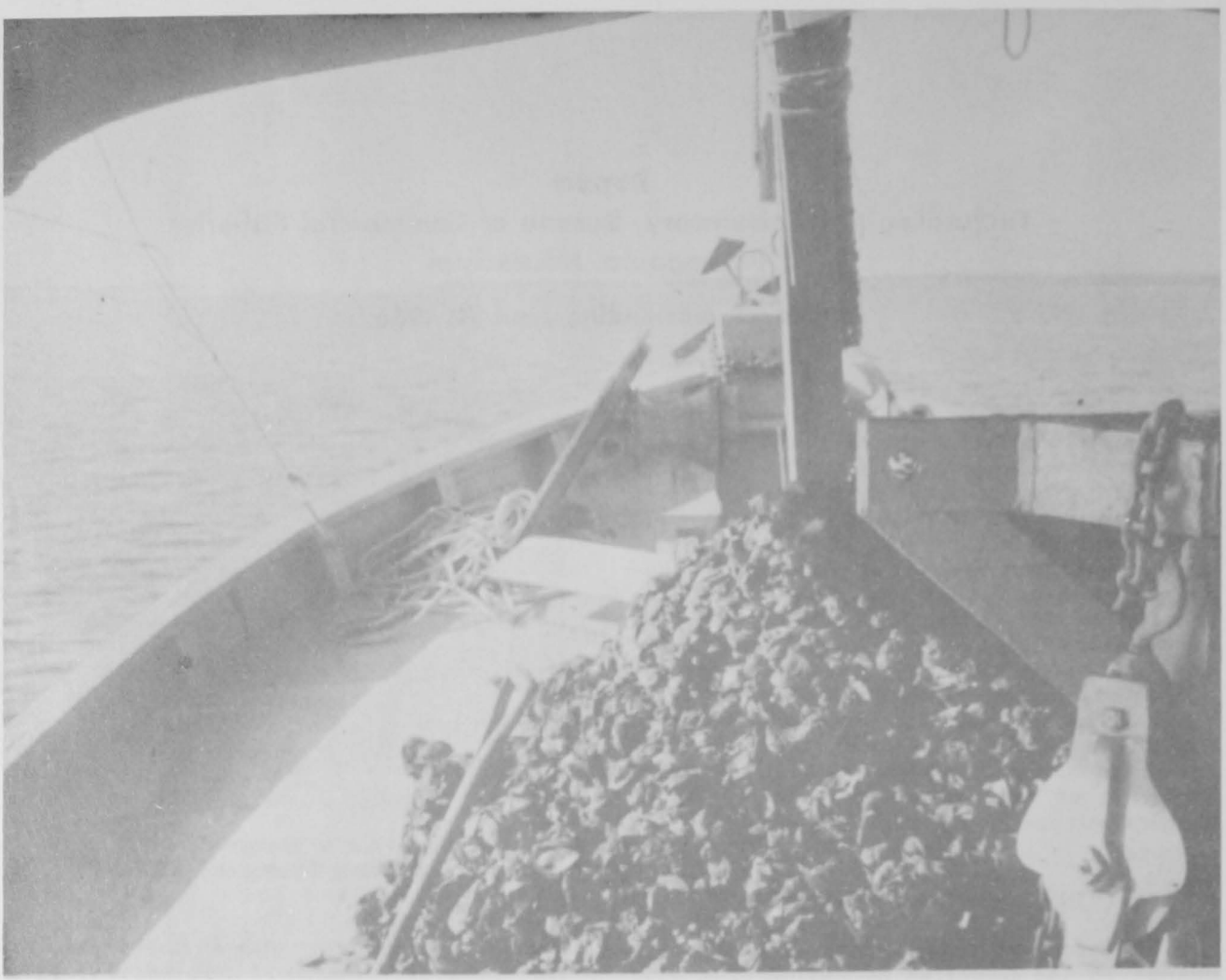

Figure 2.--Oysters to be rebedded in the Mississippi Sound.

CHEMISTRY OF SEAFOODS

In keeping with the spirit of the future, several members of the Chemistry Program actively participated in both short- and long-range training programs during the year. One of the staff members was awarded a year's graduate study at the University of Georgia under the Regional Training Program, and several others attended short courses at the Hormel Institute, University of Wisconsin, and Louisiana State University.

Among the national scientific meetings attended were those of the American Chemical Society, Association of Official Analytical Chemists, and the Atlantic Fisheries Technological Conference.

Composition and Nutritive Value of Fish and Shellfish

A project to provide information about the composition and nutritive value of several species of fish and shellfish is being continued. Species under study include Chesapeake Bay blue crab (Callinectes sapidus), Dungeness crab (Cancer magister), brown shrimp (Penaeus aztecus), ocean perch (Sebastes marinus), alewife (Alosa pseudoharengus), Atlantic croaker (Micropogon undulatus), and striped mullet (Mugil cephalus). In general, data are being collected on the proximate composition as well as the amino acid, lipid, mineral, steroid, and vitamin contents of these species. Efforts are also being made to ascertain how seasonal changes affect the composition and nutritive value of the samples and to what extent these changes are related to other known factors.

Samples of ocean perch fillets were divided into four groups according to size and sex of the fish. The low-weight groups were composed of fish having a total fillet weight of less than 100 g.; whereas the high-weight groups were composed of fish having a total fillet weight of more than 100g. Amino analysis

Table 1.--Significant[1] seasonal variations in amino acid content of ocean perch fillet samples

(Month of year in which the amino acid content increases)

Amino acid	Females		Males	
	High weight	Low weight	High weight	Low weight
	Month	Month	Month	Month
Alanine	Feb., Sept.	No variations	Feb.	Sept.
Arginine	No variations	No variations	Jan., Feb.	Sept.
Aspartic acid	Feb., Sept.	--	Feb.	--
Glutamic acid	Sept.	--	Feb.	--
Glycine	Feb., July	Sept.	Feb., Oct.	Sept.
Histidine	No variations	--	Feb.	--
Isoleucine	Sept.	--	Feb.	--
Leucine	Feb., Apr., Sept.	--	Feb. Feb.	--
Lysine	Apr.	--	Jan., Feb.	--
Serine	Feb.	--	Feb.	--
Valine	No variations	--	Feb.	--

[1] 5 percent level of probability.

revealed very little difference in content between fillets from males and females in either the low-weight group or the high-weight group. Further, very few seasonal changes in amino acid level occurred in either the low-weight female or the low-weight male groups; however, significant changes in both the high-weight male and the high-weight female groups were observed. Ocean perch is a late maturing species and bears live young. Development of the gonads begins some 2-1/2 years prior to the first viviposition. It appears then that the smaller ocean perch analyzed have not yet become mature, whereas the larger ones have. A general increase in amino acid content occurs during February, the period of fertilization, and during the late summer, the period of viviposition. Other viviparous species have not been analyzed at the laboratory, but the pattern of amino acid increase prior to ripening of the gonads and decrease during spawning has been noted for several other species. Interestingly enough, if the average values of the amino acids of all the samples are plotted, only one amino acid--glycine--shows a significant seasonal variation. Table 1 indicates the month of the year in which the amino acid content of the four groups increases when group averages are not used.

Fishery products are well known for the balance of essential amino acids contributed to the diet. Since, as a whole, fishery products are extremely digestible, most of their amino acids are readily available to the human. A comparison of the average concentration of essential amino acids in several species of fish and shellfish is shown in figure 3.

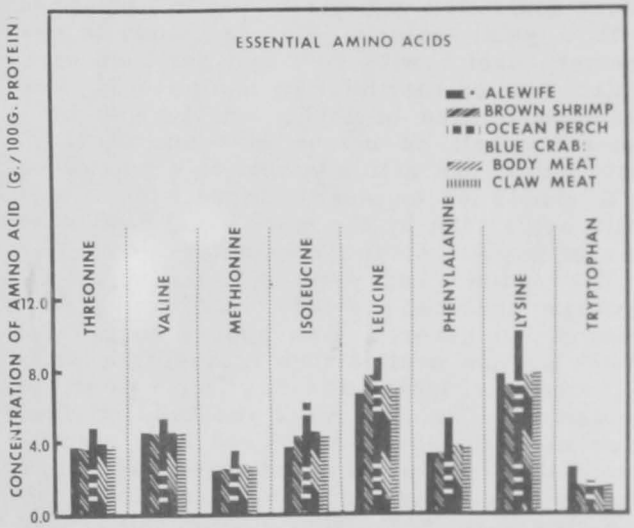

Figure 3.--Essential amino acid concentration in the edible portion of various fishery products.

Analysis of the lipid components of the various species received considerable attention during the past year. In cooperation with scientists at the Hormel Institute, a method for separating and collecting fatty acids in groups according to molecular chain length was devised. A large sample of fatty acid methyl esters is placed on a cyclodextran valerate column in a thermal conductivity gas chromatograph. The chromatograph separates the material into classes of equal chain length, which are collected by condensation onto the walls of a glass tube inserted into the effluent port. The material is then

Table 2.--Concentration of sodium and potassium in various fishery products

Species	Sample parts	Concentration of:			
		Sodium		Potassium	
		Range	Average	Range	Average
		Mg./100 g.		Mg./100 g.	
Alewife	Whole	93-132	110	291-326	311
Blue crab	Body meat	252-354	299	263-438	316
	Claw meat	342-429	375	195-318	282
Brown shrimp	Tails	142-228	197	315-396	357
Dungeness crab	Body meat	154-304	239	359-393	372
	Claw meat	177-478	352	324-367	336
Ocean perch	Fillets	91-125	111	311-359	345

redissolved and analyzed by injection into a flame-ionization gas chromatograph equipped with a polar column. This technique is extremely useful with fish and shellfish oils, which are extraordinarily complicated with small quantities of highly unsaturated fatty acids as well as numerous branched-chain fatty acids. We estimate that this procedure will enable us to identify three times more fatty acids than by the more usual procedure of simple polar column chromatography.

The sodium and potassium content of all species analyzed varied significantly with season. In general, fish appear to have a lower sodium content than do shellfish (table 2), whereas both have the same level of potassium. The claw meat and body meat of blue crab and Dungeness crab have significantly different levels of sodium and potassium at any one sampling time. In all instances the claw meat has higher sodium values than does body meat. Potassium values do not show any distinct pattern in the blue crab; whereas, in the Dungeness crab, potassium values are higher in the body meat than in the claw meat. Sodium values tend to be significantly higher during reproductive periods, whereas changes in potassium value are not correlated with the reproductive cycle.

During the past year we obtained a double-beam atomic absorption spectrophotometer to make quick and accurate analyses for trace minerals. We devised methods for digesting the samples and standardizing the instrument, and gathered data on the precision of the method for measuring copper, iron, cobalt, molybdenum, manganese, magnesium, calcium, zinc, and selenium. Preliminary results indicate that this technique will increase sensitivity, accuracy, and precision of analysis many times over that ordinarily obtained by other techniques.

Microbiological assay methods were developed for four water-soluble vitamins--thiamine, biotin, riboflavin, and niacin. A sample of alewife showed concentrations of 0.395 μg. (micrograms) per gram of thiamine, 0.0248 μg. per gram of biotin, 1.91 μg. per gram of riboflavin, and 22.9 μg. per gram of niacin. The level of thiamine recorded is not significantly different from that of other similar species. Alewives are known to have the antithiamine factor--thiaminase; however, it appears that this species is able to maintain its own level of thiamine.

The proximate composition of Atlantic thread herring (Opisthonema oglinum) from the west coast of Florida was determined in response to a request from industry. Thread herring are a potentially valuable alternate resource species for the fish-reduction industry. The oil content is reasonably similar to that of menhaden and tends to reach a peak when menhaden are not commercially fished. In addition, the high protein content makes a high-value meal possible. The analyses of samples from the middle west Florida coast are shown in table 3. The higher oil values in January and the first part of February suggest that this more southern group of thread herring might have a higher oil content in November and December, falling midway in time between samples previously taken in the middle Atlantic and those taken in the northern Gulf of Mexico. Sampling early in the winter of 1966 should determine the truth of this supposition.

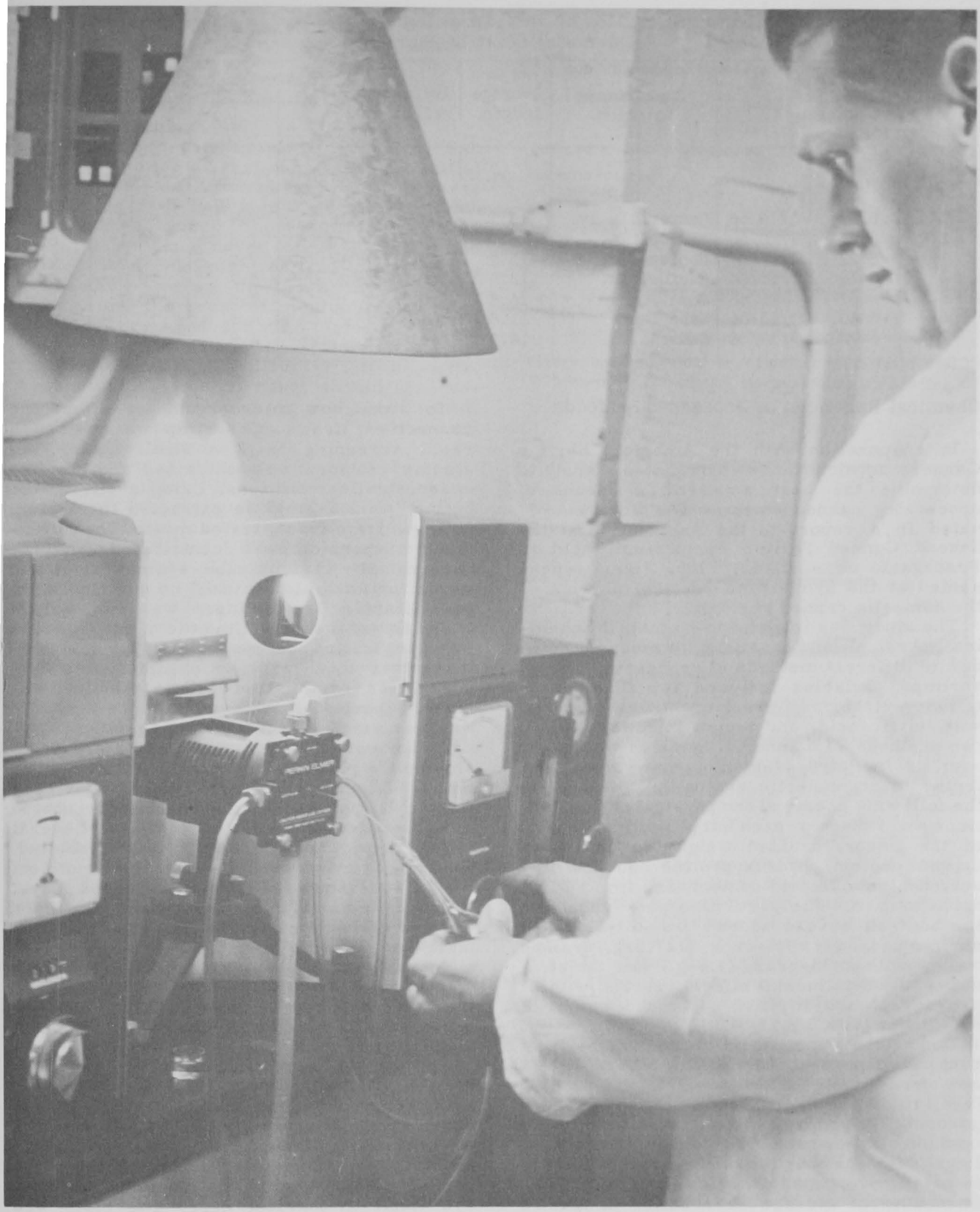

Figure 4.--Analysis of essential trace minerals through the new technique of atomic absorption spectrophotometry.

Table 3.--Data on proximate composition of thread herring (Opisthonema oglinum) obtained from the lower west coast of Florida

Sample	Bowers station	Date	Approximate location	Average length	Average weight	Average concentration of:			
						Protein	Oil	Moisture	Ash
				Cm.	G.	Percent	Percent	Percent	Percent
1	6317	1/19/66	Gasparilla Is.	16.5	71.1	18.2	7.7	69.9	5.15
2	6328	1/20/66	Sanibel Is.	14.9	54.3	20.1	7.8	70.1	6.15
3	6322	2/1/66	Sanibel Is.	15.2	55.8	17.3	7.2	68.9	6.08
4	6419	2/25/66	Sanibel Is.	13.9	40.0	20.0	5.7	70.9	5.44
5	6427	2/26/66	Cape Romano	14.8	50.3	18.2	7.5	70.2	4.77
6	6446	3/15/66	Cape Sable	15.8	64.9	20.6	4.5	70.0	5.01
7	6472	3/19/66	Sanibel Is.	14.6	48.4	17.2	4.8	71.5	4.38

Chemical Reactions in Processed Seafoods

In cooperation with the American Shrimp Canners Association we completed a study to determine the best available means of processing canned shrimp. The work culminated in a report to the Association at the Second Canned Shrimp Symposium, held in Pascagoula on April 15, 1966. Firms represented at the Symposium pack 98 percent of the domestic canned shrimp.

The study was intended to evaluate (by chemical, physical, and organoleptic means) a number of different methods of processing canned shrimp. Variables included two fill-of-container weights, different quantities of added salt, citric acid, and lemon juice, as well as two methods of blanching. Samples withdrawn over a simulated 24-month period were subjected to organoleptic evaluation. In addition, the following chemical and physical determinations were made on each lot: pH, optical density of the liquor, drained weight, texture, dissolved protein, hydroxyproline content, dissolved titratable acid content, and iron sulfide discoloration. The particular pack receiving the best all-around scores was salt-blanched shrimp packed at a 4.5-ounce (127.4-g.) drained weight with a 75-grain (4.5-g.) salt tablet. A noticeably detrimental effect occurred with all packs designed to have a drained weight of 3.7 ounces (104.7 g.).

During the year we processed another test pack of canned shrimp in the laboratory. A new food additive was added to the pack at levels of 0.25 percent, 0.50 percent, and 1.0 percent. We processed the pack in the normal commercial manner but stored it under refrigeration. Although the test period of storage is not yet over, samples withdrawn after 3 and 6 months of storage were better in texture and showed less struvite formation than untreated shrimp canned from the same lot. With further experimentation, this food additive may prove valuable in increasing the shelf life of canned shrimp.

To study how microorganisms affect the connective tissue of shrimp we devised a rapid screening test. A small quantity of shrimp collagen was extracted and purified under sterile conditions. Using a citric acid buffer (pH 3.5-3.6), we extracted the shrimp collagen from fresh, tested meats. The buffer-meat suspension was centrifuged, and the supernatant was dialyzed against deionized water for several days until no more collagen precipitated. The collagen was redissolved and dialyzed a second time the same as before for purification purposes. The purified collagen was dyed with an organic azide dye, which was synthesized with a sterile technique. A portion of the dyed collagen "azocoll" was then suspended in nutrient agar.

A homogenate was made of 10 percent shrimp in physiological saline solution. The homogenate was plated on nutrient agar containing 0.1 percent skimmed milk and incubated at room temperature for 5 days. Several different proteolytic bacteria were isolated. These cultures were transferred to nutrient broth and incubated at 37° C. for 2 days. Nutrient agar slants were made for each species of bacteria and held in a refrigerator until they could be used.

Several azocoll-nutrient agar plates were poured, and the plates were streaked with the various bacteria. Some of the species released the dye after 24 hours incubation at 37° C., thus showing positive collagenase action on the azocoll. Several of the proteolytic bacteria, and these were weaker collagenase producers than others, had slower enzymatic action on the azocoll. Two of the nine colonies, although proteolytic on the milk substrate, did not affect the collagen. We poured one plate with a suspension of the azo-dye itself in nutrient agar. The plate was streaked with the two most active proteolytic bacteria in an attempt to observe whether the bacteria or the enzymes they produced could utilize the dye itself. The plate was incubated at 37° C. over 60 hours, with no indication

that the dye was being utilized or affected by the bacteria or enzymes that they produced.

Pesticide Residues in Fish and Shellfish

Such simple techniques as preparing fish and shellfish for market can reduce the initial pesticide residue content by a factor as large as 10. Figure 5 indicates the degree to which merely filleting cod (Gadus morhua) can control the residue level of DDT, DDD, and DDE.[1] Pesticide residues tend to congregate in the fatty tissues, the brain, the liver, the kidney, and the heart of most animals. When fish are prepared for the table, it is customary to fillet them, thus removing all the centers of concentration but the fatty deposits. Theoretically, this removal should also remove a large portion of the pesticide content. Analysis of several species of fish proved this hypothesis correct.

Cleaning also eliminated much of the pesticide in shellfish, in particular the lobster (Homarus americanus) from Maine (fig. 6). A whole Maine lobster contains 0.450 p.p.m. (parts per million) DDE and lesser amounts of DDD and DDT. The edible portion has less than half this concentration. Although this reduction is considerable, some residue is left.

Because even the smallest amount of residue concerns us and because we have no guarantee that residues will not increase over the next few decades, the laboratory has turned its attention toward various processing procedures and their effects on the remaining residues. We hope in this manner to be able to discover techniques that will enable industry to control the amount of residue if such control should become necessary.

The first processing method we investigated was the application of heat. The investigation of the effects of heat processing at the plant level does not lead to an easily understood picture, for sampling difficulties are encountered. In the case of pet food, one complication is that the amount of pesticides in Gulf of Mexico trash fish, used principally in the production of pet food, seems to be seasonal. The increase in pesticide residues usually can be related to crop-dusting activity and rainfall over the Mississippi River watershed.

To check quickly whether heat reduces the amount of residues, we took samples from various places in the processing line at a local pet food cannery and analyzed these samples for pesticide residues. Some of the samples came from a single boatload of fish

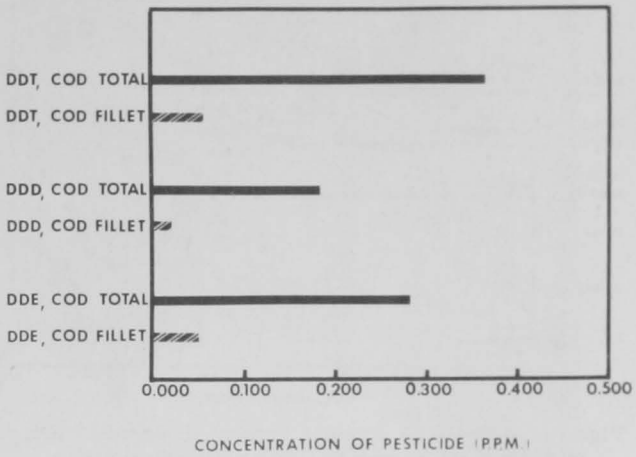

Figure 5.--Reduction of chlorinated pesticide residue present in cod (Gadus morhua) following filleting.

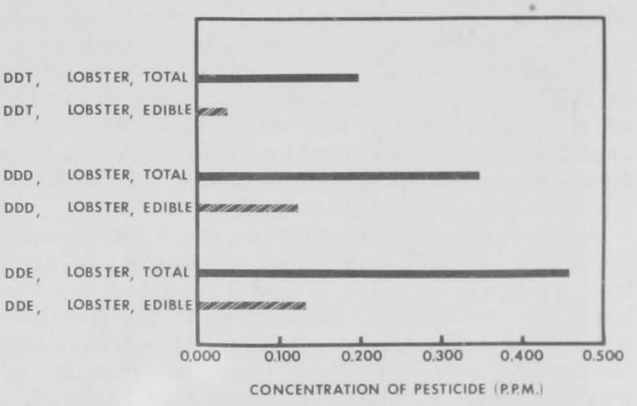

Figure 6.--Reduction of chlorinated pesticide residue present in lobster (Homarus americanus), following cleaning.

captured at the mouth of the Mississippi River. The other ingredients of the pet food such as the cereal, water, and oil were also sampled. The largest amount of pesticide in raw fish was DDE, followed by DDD and DDT (fig. 7). After the fish had been ground and mixed, the largest amount of pesticide was still DDE, but the concentration was reduced. Cooking before retorting apparently altered the concentration greatly. The decrease of DDT from the raw fish to the ground, mixed, and cooked product was so great that the concentration of DDT was almost undetectable. DDD increased in concentration in much the same manner as DDT decreased. Further heat processing in a retort for 1-1/2 hours did not decrease the pesticide by any appreciable amount. The data showed no reduction or changes in any of the pesticide residues resulting from the retort process. Heat did, however, appear to affect the amount of pesticide residues in canned pet food, for we found a

[1] DDT = Dichlorodiphenyltrichloroethane
DDD = 2,2-bis-(p-Chlorophenyl)-1, 1-dichloroethane
DDE = 2,2-bis-(p-Chlorophenyl)-1, 1-dichloroethylene

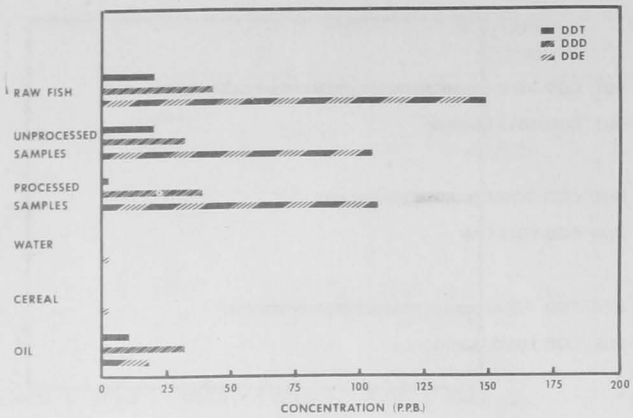

Figure 7.--Pesticide residue content of samples taken along the production line in a pet food cannery.

considerable decrease during the cooking process.

To ascertain the effect of another type of heat processing on pesticide residues, we made a study at a menhaden plant near Pascagoula. Samples for analysis were taken at the boat. Further samples of the same lot of fish were taken immediately after steam cooking, after pressing, and after drying. The first-cycle oil, the second-cycle oil, and the finished oil were also sampled.

Analyses of the pesticides were performed on extracted oil and, when possible, related to 100 g. of sample. We did not consider the loss of water from whole fish to the finished meal. Sample contamination from existing facilities was possible, but no means of evaluating this source of error was devised.

Table 4 shows the concentration of five pesticides throughout the menhaden reduction process. The most obvious loss in pesticide residues occurs when the water and oil are pressed from the cooked fish. About 70 percent of the total pesticide is removed by this process. The pesticides are extracted with the water and oil and show up again in the press liquor. The figures shown in table 4 for press liquor and oil are calculated on fat content, whereas figures for whole fish and cooked fish are shown on the basis of a 100-g. sample.

The effect of heat processing on pesticide residues can best be evaluated by comparing whole fish, cooked fish, press cake, and meal separately from the liquor and the oil. Heat is first applied as live steam and then as dry heat in the dryers. A loss of pesticides due to heat is observed after the whole fish are steam cooked. Sometimes this loss has been very small, as in the case of DDT, but other times it has been as much as 27 percent. Pesticides lost in this heating do not reappear in the following operations. A small increase of pesticide residue from press cake to fish meal occurred on several occasions. All data were calculated on a 100-g. basis. The loss of moisture from press cake is in the order of 40 percent. Thus, there is a true loss of pesticides in the drying process.

The press liquor contains the major portion of the pesticide residues. The press liquor is extracted from the press cake in the pressing process and then washed three times with water to obtain the finished oil. The pesticides seem to be concentrated in the first or second washing, but as the oil is finished it begins to contain a smaller amount of pesticides than does the press liquor (fig. 8).

Some seasonal differences in content of pesticides were noted in this study. The data represented in table 4 are from samples collected in the last week of June. Although this collection date is rather late in the spring or

Table 4.--Pesticide residues in the menhaden reduction process, June 1966

Sample	Concentration of:				
	DDE	DDD	DDT	Dieldrin	Endrin
	P.p.m.	P.p.m.	P.p.m.	P.p.m.	P.p.m.
Whole fish	0.14	0.15	0.12	0.06	0.02
Cooked fish	0.12	0.14	0.11	0.06	0.02
Press cake	0.05	0.06	0.05	0.02	0.01
Fish meal	0.06	0.07	0.06	<0.01	<0.01
Press liquor	0.57	0.75	0.69	0.20	0.12
Oil:					
First-cycle oil	0.64	0.86	0.64	0.23	0.21
Second-cycle oil	0.49	0.59	0.53	0.17	0.03

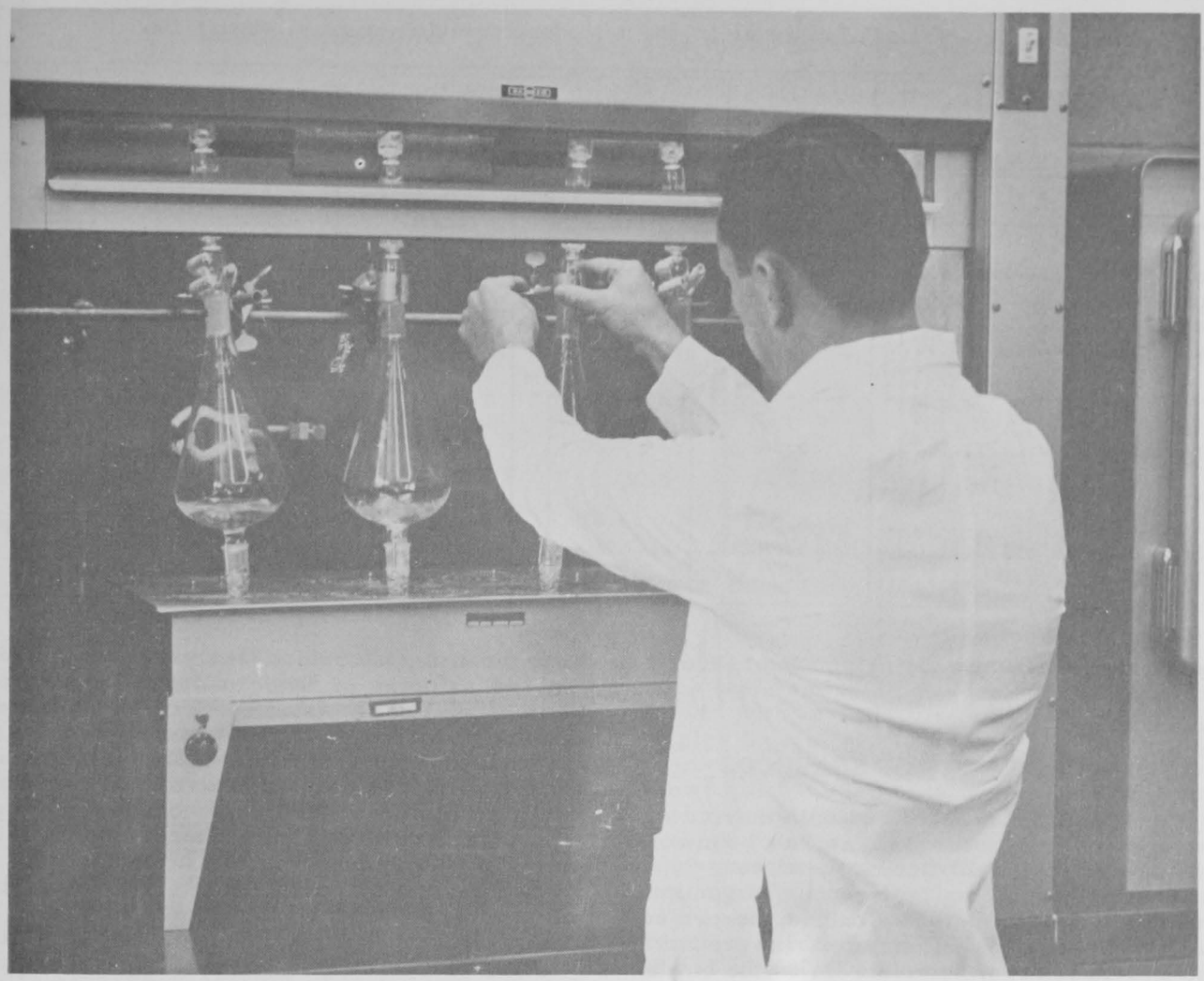

Figure 8.--Evaporation of samples in preparation for the gas-liquid-chromatography analysis of fishery products for chlorinated pesticide residue levels.

very early in the summer, the concentration of dieldrin and endrin[2] is still fairly large. Additional samples were obtained in August 1966. Although this work was completed in another fiscal year, it is included in this report for comparative purposes, since this phase of the work ended. Table 5 shows that by August dieldrin and endrin have been reduced to a trace. A rise in dieldrin and endrin is observed in the spring for most fish caught near the Mississippi River Delta. This rise is probably due to spring rains and runoff from the sugar cane fields. This group of samples contained considerably more DDT, DDD, and DDE--DDE was the highest at 1.14 p.p.m.

In this second sample group, a decrease in pesticide concentration also appears after cooking. Increases in the process appear to be of about the same proportion as in the first set of samples. The changes in the pesticide concentrations noted seem to be similar to the previous set of samples for all steps in the menhaden reduction process. The meal in the August sample contains a much larger amount of DDT, DDD, and DDE than was noted previously. The larger concentration of pesticides in the raw product understandably contributes to this increase in the meal.

The loss of pesticides due to heat processing and oil finishing is not completely understood. The pesticides could be changed to another form or destroyed, or they may be volitalized and removed in the steam. Laboratory

[2] Dieldrin = 1,2,3,4,10,10-Hexachloro-6, 7-epoxy-1, 4,4a,5,6,7,8,8a-octahydro-1, 4-endo, exo-5, 8-dimethanonapthalene

Endrin = 1,2,3,4,10,10-Hexachloro-6, 7-epoxy-1, 4,4a,5,6,7,8a-octahydro-1, 4-endo, endo-5, 8-dimethanonapthalene

Table 5.--Pesticide residues in the menhaden reduction process, August 1966

Sample	Concentration of:				
	DDE	DDD	DDT	Dieldrin	Endrin
	P.p.m.	P.p.m.	P.p.m.	P.p.m.	P.p.m.
Whole fish	1.14	0.81	0.24	Trace	Trace
Cooked fish	0.84	0.59	0.22	Trace	Trace
Press cake	0.33	0.19	0.05	Trace	Trace
Fish meal	0.42	0.23	0.08	Trace	Trace
Press liquor	5.38	3.20	1.33	Trace	Trace
Oil:					
First-cycle oil	4.94	3.41	1.44	Trace	Trace
Second-cycle oil	5.89	4.10	2.15	Trace	Trace
Polish oil	3.97	2.31	1.06	Trace	Trace

experiments are now being planned to clarify these points.

MICROBIOLOGY OF SEAFOODS

A program of research on both marine and terrigenous[3] microorganisms has been submitted and approved. During fiscal year 1966, only the project on terrigenous organisms was funded and operative. Since the project deals with seafoods and other marine products, the research does, however, lie on the border between marine and terrigenous types. Marine, estuarine, and fresh-water sediments and fish viscera provided samples for Clostridium botulinum studies. Fish meal and estuarine sediments near fish meal plants provided samples for Salmonella studies. Thus, although these projects are dealing with organisms commonly recognized as of terrigenous origin, the organisms are being found in an estuarine environment of rather high salinity. As a good example, C. botulinum has been isolated from sands on the outside beaches of both the Gulf and Atlantic waters.

Gladys Reese, Microbiologist, presented a paper, "Examination of Precooked Frozen Seafoods for the Presence of Microorganisms of Public Health Significance," before the Annual Meeting of the American Society for Microbiology at Los Angeles.

Microorganisms of Terrigenous Origin

We completed a phase of the examination of precooked frozen seafoods to determine the number of microorganisms of public health significance. Microbial analyses were made on 144 samples of three different precooked frozen seafoods (shrimp creole, breaded shrimp, and fish sticks). We determined total aerobic plate counts, and counts for the coliform group, fecal types of Escherichia coli, fecal types of Streptococcus faecalis, coagulase-positive Staphylococcus, and Salmonella (table 6).

Differences in counts among various types of precooked products may have been due to their processing. Both precooked breaded shrimp and precooked breaded fish sticks are cooked at a high temperature and are immediately packaged. In most plants, some of the vegetables for the shrimp creole are precooked, cooled, and added to the hot creole tomato sauce. Fried breaded products are cooked at about 375° F. (191° C.); whereas, the creole ingredients are boiled at a temperature of only 212° F. (100° C.).

Comparative studies were also made on various media and methods for the isolation of the microorganisms. Results show that considerable differences in effectiveness for seafoods exist among media and methods used in research on other products. A careful comparative study apparently is needed before a medium is selected for each different product. Results from this study of 144 samples may be summarized as follows:

1. Precooked breaded shrimp had the lowest total plate counts; precooked breaded fish, the second lowest total plate counts; and shrimp creole, the highest total plate counts.
2. KF Streptococcus agar and KF Streptococcus broth tended to isolate fecal streptococci reliably from the seafood products even when they were present in small numbers.

[3] Terrigenous = of land environment.

Table 6.--Results of the microbial analyses of precooked seafoods

Sample type	Samples	Average total plate count	Average coliforms	Average *Escherichia coli* Types I & II	Average fecal streptococci	Average staphylococci	Average *Salmonella*
	Number	No./ 100 g.	No./ 100 g.	No./ 100 g.	No./ 100 g.	No./ 100 g.	No./ 100 g.
Precooked breaded shrimp	92	566	23.4	10	[1] 25.8 (4.3)	[1] 57.5 (9.8)	0
Precooked breaded fish (sticks and portions).....	24	26,626	1,606	12.8	5,853	[1] 16.2 (4.0)	[1] 0 (0)
Shrimp creole..	28	127,000	16,217	0	25,778	0	0

[1] Numbers in parentheses represent percent samples from which isolations were made.

3. Samples on plate count agar had higher total aerobic plate counts.

4. Tellurite Polymyxin Egg Yolk (TPEY) agar and Staphylococcus Medium 110 isolated coagulase-positive staphylococci with about equal reliability, although during this investigation the TPEY showed a slightly higher percentage of isolation where small numbers of staphylococci are present.

Salmonella in Fishery Products

Studies were started to determine the extent of Salmonellae in marine products. Other studies have shown that these microorganisms occur in all food and feedstuffs; therefore, the heat resistance of the microorganism during processing and any subsequent pasteurization is of vital importance. The laboratory made determinations that warranted the following conclusions:

1. Time-temperature variables, with regard to Salmonellae in fish meal, vary from one meal to another. Differences in fat content, moisture content, particle size, and numbers and types of Salmonellae present reflect different thermal resistance pictures.

2. The length of time between contamination and thermal destruction determinations influences the temperature and time necessary to inactivate all Salmonellae consistently. Our investigations have shown, as have those of other workers, that data derived from artificially contaminated meals do not give consistent results when applied to naturally contaminated meals. We have also observed that *S. senftenberg* 775W is not the most heat-resistant strain tested.

Numerous thermal destruction determinations were made on several meals contaminated with a mixture of five *Salmonella* strains (*cerro*, *senftenberg* 775W, *montevideo*, *oranienburg*, and *thomasville*). These tests showed that the temperature and the time necessary to consistently destroy all Salmonellae present varied considerably. The following combinations proved sufficient for the inactivation of *Salmonellae* in one meal containing 10.6 percent oil: 150° F. (66° C.) for 60 minutes; 160° F. (71° C.) for 35 minutes; 165° F. (74° C.) for 28 minutes; 170° F. (77° C.) for 20 minutes; or 180° F. (82° C.) for 14 minutes. On the other hand, another meal with 10.8 percent oil containing the same number of *Salmonella* cells (430 per gram) required the following combinations of temperatures and times: 150° F. (66° C.) for 50 minutes; 160° F. (71° C.) for 30 minutes; 165° F. (74° C.) for 20 minutes; or 180° F. (82° C.) for 9 minutes. A third meal with a relatively high concentration of oil (12.5 percent) was studied to determine whether the increased oil content would protect the *Salmonella* somewhat. Processing at 190° F. (88° C.) for 5 to 10 minutes was necessary to produce *Salmonella*-free meal consistently. The difference in thermal destruction rate between the first and second meal cannot be explained at this time. A higher oil content (third meal) does have an effect, however.

Some meals were subjected to various temperature-time treatments--150° F. (66° C.), 30 minutes; 165° F. (74° C.), 20 minutes; 180° F. (82° C.), 5 minutes; and up to 230° F. (110° C.), followed by immediate cooling--and were submitted to the Bureau of Commercial Fisheries Technological Laboratory at College Park, Md., for assessment of

Table 7.--Geographic locations of samples found positive for the presence of Clostridium botulinum

Type	Date	Sample number	Origin	Sampling station
E	8/4/65	37	Speckled trout	Snake Bight Bay, Fla.
C	8/4/65	48	Sand and shell	Grassy Key, Fla.
C	8/10/65	91	Sand	Lynn Haven, Fla.
E	8/26/65	100	Croaker	Mobile Bay Channel, Ala.
E	8/27/65	116	Croaker	Mobile Bay Channel, Ala.
E	8/27/65	125	Croaker	Mobile Bay Channel, Ala.
E	8/27/65	133	Croaker	Perdido Bay, Fla.
E	8/27/65	136	Croaker	Perdido Bay, Fla.
E	8/27/65	142	Croaker	Perdido Bay, Fla.
E	8/27/65	143	Croaker	Perdido Bay, Fla.
E	8/27/65	148	Croaker	Perdido Bay, Fla.
E	8/27/65	150	Mullet	Perdido Bay, Fla.
E	8/27/65	158	Mullet	Perdido Bay, Fla.
E	8/27/65	161	Catfish	Perdido Bay, Fla.
E	8/27/65	165	Catfish	Perdido Bay, Fla.
E	8/26/65	183	Mud	Bayou la Batre, Ala.
E	8/26/65	195	Mud	Fanstinas Beach, Mobile Bay, Ala.
E	8/27/65	200	Sand	Fort Morgan, Mobile Bay, Ala.
E	8/27/65	205	Sand	Pensacola Bay Bridge, Fla.
E	9/26/65	237	Flounder	Port Isabel, Tex. (Laguna Madre)
E	9/26/65	246	Flounder	Port Isabel, Tex. (Laguna Madre)
E	9/26/65	249	Snapper	Port Isabel, Tex. (Laguna Madre)
B	9/26/65	250	Snapper	Port Isabel, Tex. (Laguna Madre)
E	9/26/65	268	Sand	Padre Island, Tex. (seaside)
E	9/26/65	270	Sand	Padre Island, Tex. (bayside)
E	9/26/65	286	Sand	Padre Island, Tex. (seaside)
E	9/27/65	298F	Shrimp	Aransas Bay, Tex.
E	9/27/65	390	Drum	Galveston, Tex.
E	9/27/65	295	Spot	Galveston, Tex.
E	9/27/65	404	Spot	Galveston, Tex.
E	9/27/65	431	Speckled trout	Rockport, Tex.
E	9/27/65	440	Speckled trout	Rockport, Tex.
E	10/12/65	583B	Croaker	Galveston, Tex.
C	10/12/65	591C	Croaker	Galveston, Tex.
D	10/12/65	598	Turtle	Petit Bois Island, Miss.
E	10/12/65	607	Mullet	Mississippi River Mouth, La.
D	3/5/66	29	Shell	Tavernier Key, Fla.
E	3/7/66	44	Mud and sand	St. James City, Fla.
C	3/6/66	75	Trout	Key West, Fla. (Florida Bay)
C	3/6/66	76	Trout	Key West, Fla. (Florida Bay)
B	3/7/66	87	Drum	Naples, Fla.
B	3/8/66	100	Sand and shell	Clearwater Beach, Fla.
C	3/8/66	106	Mud	Net Spread Key, Fla.
A	3/9/66	253	Sand	Bayou la Batre, Ala.
C	3/24/66	345	Sand	Corpus Christi Bay, Tex.
D	3/25/66	385	Mud	Brazos River, Tex. (tidal)
A	3/26/66	399	Mud	Rollover Junction (E. Galveston Bay), Tex.
A	3/27/66	419	Mud	Vermillion Bay (Weeks), La.
F	3/28/66	470	Buffalo	Atchafalaya River, La. (freshwater)
E	3/29/66	489	Mud	Bayou Terrebonne (Montegut), La.
E	3/29/66	490	Mud	Wonder Lake, La.
D	5/18/66	521	Mud, shell, and gravel	Tickfaw River, La.
C	5/19/66	525	Mud	Mississippi River (Pilottown), La.
D	5/19/66	531	Croaker	Mississippi River (Empire), La.
E	5/19/66	539	Croaker	Mississippi River (Empire), La.
E	5/19/66	553	Mud	Pointe la Hache, La.
C	5/20/66	555	Shell and sand	Shell Beach, La.
D	5/20/66	557	Crab	Shell Beach, La.

nutritive value. Analyses at that Laboratory showed that the nutritive value of the meals was not damaged as a result of the above-mentioned heat treatments.

Survey of the Gulf of Mexico for Clostridium botulinum

In April 1965, the AEC (Atomic Energy Commission) and the Bureau's Technological Laboratory at Pascagoula completed a research agreement for a survey of C. botulinum in the Gulf of Mexico. AEC interest was based upon the possible presence of C. botulinum in seafoods undergoing radiation pastuerization. By this agreement, Pascagoula bacteriologists were to sample marine sediments and fish viscera from Key West, Fla., to Brownsville, Tex. The mouse-assay method was used to analyze the samples for the presence of the microorganism.

Upon satisfactory completion of the Gulf of Mexico studies, AEC extended the contract for the Atlantic coast from Staten Island, N.Y., to Key Largo, Fla. We collected cold-weather samples for this Atlantic survey during the last quarter of fiscal year 1966.

We have examined 1,414 samples to date. In sediments and animals collected during warm-weather months between Key West, Fla., and Brownsville, Tex., we found C. botulinum, predominantly type E (table 7). Incidence was higher in the eastern Gulf animals, but East vs. West incidence figures for sediments were essentially equal (4.6 percent vs. 4.4 percent). The organism was present to the southmost limits of both Texas and Florida. Types A and F were never detected in the Gulf of Mexico. In samples collected during colder weather, the East-West incidence differential in animals was minimized, and the overall levels were lower than were those of summer. In sediments, however, C. botulinum was found more often during colder months, especially in the West. All known types of C. botulinum were detected in winter samples, and type E no longer predominated. It seems that when C. botulinum increased in fish, it was reduced in sediments, and vice versa, but at this point only a generalized statement can be made to the effect that seasonal variations apparently occurred. We recommend a thorough study of the ecology of the organism.

During the progress of our Gulf Coast study, we tested a number of South American samples obtained by the vessel Oregon. C. botulinum--predominantly types A and C, but inclusive of types B and E--is present in animals of the Gulf of Venezuela and the Gulf of Darien (Carroll, Garrett, Reese, and Ward, 1966).

An additional aspect of the research agreed upon in the contract was the evaluation of existing fluorescent antibody procedures. Numerous agencies and research firms have considered the detection of toxins by such methods. Thus, we attempted to detect cells and spores, using the Wellcome somatic antibody[4], the only commercial product available. Data indicated that at this time the fluorescent antibody technique is not effective in a screening operation. We feel that it has great potential but that its antigenic composition is not sufficiently inclusive. We encountered no obvious false positives, but mouse-inoculation tests indicated numerous positives that could not be verified by fluorescent staining.

PUBLICATIONS

CARROLL, BOBBY J., TRAVIS D. LOVE, BENJAMIN Q. WARD, and MELVIN E. WATERS.
1966. Microbial analyses of frozen raw breaded shrimp. U.S. Fish Wildl. Serv., Fish. Ind. Res. 3(3): 5-11.

CARROLL, BOBBY J., EPHRAIM S. GARRETT, GLADYS B. REESE, and BENJAMIN Q. WARD.
1966. Presence of Clostridium botulinum in the Gulf of Venezuela and the Gulf of Darien. Appl. Microbiol. 14(5): 837-838.

FARRAGUT, ROBERT N., and MARY H. THOMPSON.
1966. Proximate composition of Pacific Coast Dungeness crab (Cancer magister). U.S. Fish Wildl. Serv., Fish. Ind. Res. 3(3): 1-4.

LOVE, TRAVIS D. and MARY H. THOMPSON.
1966. Annual report, Bureau of Commercial Fisheries Technological Laboratory, Pascagoula, Mississippi, fiscal year 1965. U.S. Fish and Wildl. Serv., Circular 251, 25 p.

LOVE, TRAVIS D., and MELVIN E. WATERS.
1967. Utilization of mullet (Mugil cephalus) as a canned or smoked product. Com. Fish. Rev. (in press).

THOMPSON, JOHN R., MARY H. THOMPSON, and SHELBY DRUMMOND.
1966. A method for restoring dried crustacean specimens to taxonomically usable condition. Crustaceana 10(1): 109.

THOMPSON, MARY H.
1966. Proximate composition of Gulf of Mexico industrial fish. U.S. Fish Wildl. Serv., Fish. Ind. Res. 3(2): 29-67.

[4] Use of trade names is merely for the purpose of description; no endorsement is implied.

THOMPSON, MARY H., and R. N. FARRAGUT.
1966. Amino acid composition of the Chesapeake Bay blue crab Callinectes sapidus. Comp. Biochem. Physiol. 17(4): 1065-1078.
1965. The amino acid composition of the alewife (Alosa pseudoharengus). U.S. Fish Wildl. Serv., Fish. Ind. Res. 3(1): 47-53.

TRAVIS, DONALD R.
1966. The proximate composition of the alewife (Alosa pseudoharengus). U.S. Fish and Wildl. Serv., Fish. Ind. Res. 3(2): 1-4.

WARD, BENJAMIN Q., and BOBBY J. CARROLL.
1966. spore germination and vegetative growth of Clostridium botulinum type E in synthetic media. Canad. J. Microbiol. (in press).

WARD, BENJAMIN Q., and BOBBY J. CARROLL.
1967. Effects of sulfa drugs on spore germination, vegetative growth, and toxin formation. Canad. J. Microbiol. (in press).
1967. Production of toxin by Clostridium botulinum type E in defined media. Canad. J. Microbiol. (in press).

WARD, BENJAMIN Q., BOBBY J. CARROLL, and EPHRAIM S. GARRETT.
1965. Study of Clostridium botulinum type E (AT(49-7)2777). In Conf. 651024, p. 124-130. AEC, Division of Technical Information, Washington, D.C.

WOOD, BOBBY J.
1966. Elution of dieldrin and endrin from Florisil. J. Assoc. Anal. Chem. 49(2): 472-473.

MS. #1640