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PROCEEDINGS

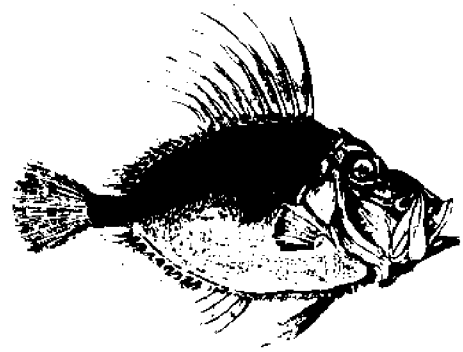
of the

SEVENTH ANNUAL TROPICAL AND SUBTROPICAL

FISHERIES TECHNOLOGICAL CONFERENCE OF THE AMERICAS

January 11-14, 1982

New Orleans, Louisiana



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LONGLINE SHARK PRODUCTION DURING A TRIAL CRUISE IN THE GULF OF MEXICO

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INTRODUCTION

An initial longlining trip for sharks was completed out of Brownsville aboard Captain Carl Gayman's vessel, S.S. Missionary Ridge, from May 20-28, 1981. Specialized shark gear which could be incorporated with swordfish longlining vessels was constructed and evaluated. Fish production relative to water depths, baits, species, and hook numbers was quantified. This report discusses some of the results from this initial effort.

PROCEDURE

Gear

During the first two weeks in May, shark longlining gangions were constructed. Although the cooperating vessel was already rigged for longlining, specialized gangions had to be suppled for shark fishing. Snaps used on the gangions consisted of a heavy duty A/K Longline Snap constructed of .144" wire. An 8/0 swivel was attached to the longline snap. Thirty feet of 500# test monofilament leader was connected to the snap. Three feet of 1x7-1/16" stainless steel leader separated the monofilament line from the hook. An 8/0 swivel was used to attach the monofilament line to the stainless steel leader. Mustad 3½/0 Shark Hooks-No. 34970 were used on all gangions.

Past experiences with stainless leader attached to ringed tin hooks had indicated excessive amounts of electrolysis in the eye of the hook. Some fishermen reported that only 2-3 trips could be performed before the eye of the hook completely corroded away. To control this problem, resulting from dissimiliar metals, strips of alumimum were cut and wrapped around both the hooks and the wire bites adjacent to the swivels. These anodes worked satisfactorily on this first trip; however, electrolysis of the anodes was so rapid that it appears that two trips will be the life expectancy of the material.

Longline Gear Array

Hooks were spaced approximately 150-200' apart. Six to seven hooks were placed between 40" inflatable bouys. Hiflyers with radar reflectors and lights were placed at intervals of every 50 hooks - approximately 1½ n. miles. Buoy drops were 30 feet in length. Mainline consisted of #7 braided line.

Gear Operation

Initial sets were put over at midnight and allowed to drift until 10 or 11 a.m. Although it is known that sharks are not caught in abundance at night, it was anticipated that some swordfish could be caught through a portion of the trip to subsidize the operation. No yields of swordfish were obtained; moreover, currents were such that the drift of the line was too erratic and often

was not in the desired areas in the daylight hours. As a result, sets made in the latter portions of the trip were put over just before daylight so that positioning of the gear could be more closely achieved.

Baits

Several different types of bait were used and evaluated during the trip. It was learned that bait can dictate fishing success. The primary bait utilized was Atlantic mackerel. This was, by far, the most successful. In several sets, large squid were also utilized on portions of the gear. Yields from squid were also satisfactory. Other baits did not provide desirable results. Mullet that was in an inferior state of preservation did not produce shark in quantities. The majority of one set was baited with pieces of unborn shark. No sharks were produced on the gear baited with this cut bait.

RESULTS AND DISCUSSION

Two hundred and fifty-three sharks were landed in seven sets (Table 1). Eviscerated dockside weight was 16,450 pounds. In addition to variables in production due to bait, water depth was also important in shark production. The trip was made in waters east and northeast of Port Isabel. Constant problems were encountered with erratic currents in this portion of the Gulf of Mexico. Seventy fathoms appeared to be a prime area for the silky sharks-*Carcharhinus falciformis*.

Sets made in the seventy fathom area rapidly drifted out past the 100 fathom curve. Throughout the voyage, yields outside of 100 fathoms were not commercially feasible.

Sizes of silky sharks landed suggested a correlation to depth. The larger, mature sharks appeared to be in the 60-80 fathom range. Smaller, immature sharks appeared more abundant in deeper water.

Shark Species

As can be noted in Table 1, silky sharks accounted for the majority of the harvest. Fishing efforts were limited to waters along the continental shelf, which appears to be a principle habitat for this species. Other species of shark were insignificant in the overall catch.

Shark Fins

Dried shark fins from this trip weighed approximately 200 pounds. Fins were trimmed of excess meat, slit about two inches and hung on monofilament line strung above the wheelhouse to dry.

Miscellaneous Observations and Potential Harvesting Improvements

The 1/16" stainless steel leader served well in harvesting sharks. In only three instances was the leader broken. The monofilament portions of the gangions were not as desirable. Several sharks were lost due to chafing and eventual breakage of this leader material. The monofilament breaks were more numerous during the middle and latter portions of the trip as a result of prolonged chafing from contact with sharks. A 3/16 inch hard lay nylon line may be a worthwhile substitution for the monofilament.

Improvement with the type of anode is needed. Although the aluminum works, it obviously will need constant replacement. This is very labor and time consuming. A more durable anode would be desirable.

TABLE 1

LONGLINE SHARK PRODUCTION - BROWNSVILLE CRUISE

DATE	LORAN C COORDINATES (7980) Out	LORAN C COORDINATES (7980) In	DEPTH FATHOMS Out	DEPTH FATHOMS In	HOOK QUANTITY	PRODUCTION* Species	Numbers	Pounds	COMMENTS
5/21/81	11213.3 23925.7 11204.0 23941.4	11202.2 23927.5 11195. 23950.	125	65	309	Silky Finetooth Hammerhead Mako	29 2 3 1	2,741 146 440 400	Inshore drift; Baits- 80% mackerel, 20% squid
5/22/81	11206.4 23960.4 11204.0 23941.4	23954.0 46591.5 23898.6 11203.1	150	100	300	Silky Bull Whitetip Mako	35 3 1 1	2,299 408 150 150	Baits- 80% mackerel, 20% squid
5/23/81	23920.0 46597.0 23915.9 46591.5	23921.6 44619.2 23930.9 44607.2	70	150	315	Silky Dusky Blacktip Bull Tiger	18 2 2 1 1	942 144 138 120 50	Offshore drift- gear probably passed 70-90 fathom depth before sunup. Lost 5 large sharks at boat.
5/24/81	23935. 46557. 23942.2 46579.3	11196.2 23933.4 11209.0 23917.0	75	130	490	Silky Big-eye thrasher Blacktip Dusky	28 1 2 2	959 140 154 120	Silkies are small. 30% of gear baited with poor quality mullet & mackerel did not produce. Used 36 swordfish gangions-hooks tied to mono.
5/25/81	11224.7 23896.5 raw 180° No end reading (Rain squalls)	11200.0 23898.0 11236.6 23879.5	80	45	490	Silky Hammerhead Tiger Dusky	60 1 1 1	5,529 155 148 70	20 of 36 swordfish gangions missing hooks. Inshore drift.
5/26/81	11224.9 23897.6 11233.3 23877.4	11228.5 23898.5 11235.8 23874.7	100	96	360	Silky Hammerhead	12 1	152 59	Majority of line baited with chunks of unborn shark -Yielded 1 foul-hooked shark.
5/27/81	11223.8 23891.6 11209.6 23889.9	11224.5 23917.5 11215.5 23936.2	75 50	190	360	Silky Hammerhead	39 1	1,162 58	Current took gear offshore. Bait all squid.

* Does not include portions of shark that were damaged by other sharks. Weights were taken individually at sea and are of eviscerated sharks.

Much production was lost due to our inability to maintain the line in desired depths. It may be appropriate to anchor sections of the gear so that a constant depth can be maintained. It should be noted that currents off the Port Isabel area appear to be more erratic than in other offshore waters of Texas.

Another production problem which was revealed related to handling. Evisceration of sharks, especially removal of the heads, is very labor intensive. Introduction of mechanical knives or similar equipment may be appropriate to expedite handling. Presently, more crew would be needed to harvest and effectively process sharks than in other longline fisheries. This problem is manifested to a greater extent because of the low value of shark in the Texas markets.

Microbiological and Chemical Analyses

In addition to production, there is also concern regarding the shelf life of fresh shark meat. An initial question was whether sharks could be stored on ice for a reasonable period of time, or whether they would have to be frozen on-board in order to assure a quality product.

Two sharks caught on the first day were randomly selected and set aside on ice in the hold. Samples of meat from the belly cavity and from the dorsal fin area were removed each day and analyzed on-board for aerobic bacterial count (surface counts). The remainder of the meat was stored for chemical analyses to be performed in the Seafood Technology Laboratory at Texas A&M University. Although there were contamination problems associated with on-board bacterial plating, counts did not reach spoilage levels ($10^7/cm^2$) after seven days on ice. Bacterial typing and urease activity are shown in Table 3. The data show a dominant *Moraxella* flora, as well as an increase in the total percentage of urease positive bacteria with increasing storage time. This shift may be a reflection of induced enzyme formation resulting from the relatively high urea content inherent in shark meat. Chemical analysis have not been completed at this time.

Two sample sharks were marked each day and subsequently handled similarly to, and stored with, the rest of the catch. None of these fish had a detectable ammonia-like odor when offloaded. Small sharks four and six days old were taken to the Seafood Laboratory in Corpus Christi, baked with unseasoned butter-baste and distributed to an untrained taste panel for organoleptic evaluation. Results are shown in Table 2.

In summary, it would appear as though sharks can be stored on ice boats for at least seven days. Critical factors in this storage stability include: 1) immediate bleeding by severing the caudal fin; 2) keeping the dressed carcass [headed, gutted and fins removed] in the shade as much as possible, and frequently cooling with the deck hose before placing in the hold. The period of time carcasses are kept on deck will vary depending on catch rate and crew size, but should probably not exceed one hour in the summer; 3) minimal bruising while handling [especially when placing in the hold]; 4) proper icing [including the belly cavity] and 5) deck hose rinse with occasional scrubbing as the product is offloaded.

Plans for the next trip include:

1. Production data (gear, catch rate, baits, etc.)
2. Microbiology (total aerobic counts, urease reaction.)

TABLE 2

ORGANOLEPTIC EVALUATION

TASTE	TEXTURE	WOULD EAT AGAIN	COMMENTS
Good	Good	Yes	Very edible, better than lots of "food fish"
Excellent	Good	Yes	Really impressed
Good	Good	Yes	
Fair	Fair	Maybe	
Palatable	Too spongy	No	
Edible, bland	Soft	Yes	Didn't have an over-powering fish taste which made it more acceptable
Excellent	Excellent	Yes	Rate it above trout, comparable with redfish for flavor
Excellent	Excellent	Yes	
Good	Good	Yes	
Pretty good	Pretty good	Yes	
Very good	Very good	Yes	Very rich
Very good	Very good	Yes	
Good	Good	Yes	Would have preferred that it was fried
Good	Very good	Yes	
Very good	Good	Yes	Mild fish, no strong smell
Good	Good, does not fall apart	Yes	Distinctive taste
Good	Good, better than other fish	Yes	Taste similar to Mako, strong taste that takes getting used to
Poor	Very good	Maybe	Not favorably impressed, would like to try different preparation
Very good	Good	Yes	
Very good	Very good	Yes	Very moist
Good	Too soft	Yes	Taste requires getting used to, a little strong

Silky shark (*Carcharinus falciformis*) was baked in a shallow pan at 350° for approximately 45 minutes. The fish was basted with butter and sprinkled with paprika.

3. Chemical analyses (trimethylamine, trimethylamine oxide, and urea.)
4. On-board controlled atmosphere packaging of whole dressed fish with subsequent chemical and microbiological analyses.
5. Lactic acid and sodium chloride dips on fish protions with subsequent urea analysis.

EFFECT OF ON-BOARD HANDLING ON THE QUALITY OF SHARKS FROM THE GULF OF MEXICO

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INTRODUCTION

Commercial harvesting of sharks in the Gulf of Mexico is not new. During the Second World War, when traditional sources were unavailable, the demand for shark liver oil, a principal source of vitamin A, caused a boom in shark harvesting in the United States and Australia. However, the industry faded when cheaper synthetic sources of vitamins were discovered (Springer, 1963). It has been speculated that the Gulf shark population has increased substantially in recent years due to the intensive shrimp fishery which discards tons of by-catch annually into waters heavily populated by scavenging sharks (Weeks, 1974). In assessing the potential development for fishery opportunities in the Texas sector of the Gulf of Mexico, Hildebrand (1981) states that the highest priority should be given to developmental work on sharks. Bottom linesets made by Texas Parks and Wildlife coastal fishery biologists also indicated a potential for commercial production of demersal species (Cody and Avent, 1980). In recent years, the Gulf shrimp industry has been severely effected by dramatic increase in fuel costs coupled with depressed market prices, as a result, approximately twenty percent of the Texas shrimp fleet have installed longline fishing gear during the past two years. Another ten percent is expected to invest in such equipment next year. Most vessels have concentrated on experimental surface longlining for swordfish and bottom sets for tilefish and yellow-edged groupers. Seafood technologists have received an increasing number of requests for assistance in the development of handling techniques and product forms for species caught incidentally on longlines. Sharks comprise the most common incidental catch and most abundant bottom fish in the Gulf of Mexico (Nelson and Carpenter, 1968; Lopez et al., 1978) with day longlining catches often outnumbered swordfish on surface sets (Graham, 1980). The majority of sharks caught are Silkies, but the Mako has gained acceptance as a quality fishery product. However, with improved quality for all sharks caught, a market for all species of sharks can be gradually developed. Presently two ports are presently accepting freshly caught sharks of any species. While the current price for sharks is not particularly attractive to shrimpers who are accustomed to dollars per pound for their product, any additional revenue to help off-set increasing fuel costs should be most welcome. The potential for a shark fishery in the Gulf of Mexico exists and such a fishery would supplement the sole dependence upon shrimping. Hopefully, the development of this new fishery will offer an opportunity for shrimpers to fish for shark when the shrimp season is closed, thus making better use of their investment and give them a year round income.

Sharks belong to the class of fish known as elasmobranchs which have very unique characteristics, sharks have relatively high concentrations of urea and trimethylamine-oxide (TMAO) (Tsuchiya et al., 1951) which function

as thermoregulator and osmoregulator, respectively. During an extensive project designed to evaluate the potential of sharks for human consumption. Russian scientists concluded that the flavor and quality of products made from sharks depended on preliminary handling (Gordievskaya, 1971), especially on-board handling and frozen storage were critical to eating quality. High concentrations of urea are responsible for poor quality, giving the product a metallic flavor and odor and decreasing its acceptance to consumers. During storage on ice, urea is hydrolyzed to ammonia by the action of urease and TMAO is reduced to trimethylamine by TMAO reductase, both of these enzymes typify the action of psychrotrophic bacteria. Both the degraded metabolites (ammonia and TMA) are the principal contributors to odor and flavor associated with marine fish spoilage. Improved quality can potentially be achieved through the inhibition of microbial growth and/or the removal of these metabolites. This study was performed on-board shrimp boats equipped with longlining or gill-netting gear. Optimum on-board handling techniques as functions of chemical and microbiological characteristics of freshly caught sharks have been determined.

The main objective of this study was to develop on-board handling methods to improve shark quality by reducing the levels of urea and TMAO or retarding the rates of urea and TMAO decomposition. Eventually the improved quality product will gain greater consumer acceptance and increase revenue to fishermen.

PROCEDURE

On-board treatment and slaughtering procedures

The initial phase of the project was performed on-board vessels (one longline, the other gill net). Two ten-day trips were conducted, the first trip was devoted to test methodology and longistics. During the second trip, tested methodology was applied and samples were collected. Sharks brought on-board after a longline or gill net set were given an identification number, weighed and species identified. The caudal fin was cut immediately to facilitate rapid bleeding, a considerable amount of urea and TMAO should be eliminated during this process. After bleeding and rinsing, the head was cut off between the neckbones to remove a large percentage of TMAO-reductase producing bacteria in the gill region. The fins were removed and the shark was thoroughly washed with seawater before being placed in the hold on ice.

On-board experimental design and sample collection

Ice storage treatment - Dressed sharks were stored on ice in the hold. Five samples, of approximately one pound each, were taken at specific locations of the carcass (at the neck, center, tail, center back and belly flap) every other day for a period of 20 days. These samples were frozen and stored in a chest freezer for later analyses (chemical and physical).

Controlled atmosphere treatment - Dressed sharks were stored inside double laminated polyester bags containing a gas mixture of 30% O_2:70% CO_2. This controlled atmospheric environment was constantly maintained via a flow-through system. The bags were maintained in the hold at refrigeration temperature (4°C). Five samples of approximately one pound each were taken as previously described.

Sodium chloride and lactic acid treatments - In order to test the reduction in urea and ammonia contents, dressed sharks were cut into chunks (approximately three-pound pieces) and divided into two lots. One lot was soaked in a 2% sodium chloride solution while the other in a 1.5% lactic acid solution for a period of 48 hours. Samples were taken at three-hour intervals during soaking, and frozen for subsequent urea and ammonia analyses.

General Procedures

On-board - The on-board samples stored on ice and in the CO_2-enriched atmsophere were analyzed for total number of surface bacteria and surface pH every other day. Samples of both treatments were also frozen and analyzed for urea, ammonia and TMAO after landing.

Analyses of frozen samples - The zero day samples were analyzed for protein, fat, moisture and ash. Additional samples taken during the soaking treatments with sodium chloride and lactic acid were analyzed for urea and ammonia.

Analytical Procedures

Proximate analysis - Protein determination was based on Kjeldahl nitrogen content x 6,25 (AOAC, 1975) or the biuret procedure (AOAC, 1975). Moisture, fat and ash were determined by AOAC methods (1975).

Microbial analysis - Total microbial surface counts were determined according to "Compendium of Methods for the Microbiological Examination of Foods" (APHA, 1976).

Physical analysis - Surface pH of the muscle was determined by a surface pH electrode probe connected to a Corning Digital 110 pH meter (Acton, 1972).

Chemical analyses

Trimethylamine-oxide - TMAO content of the muscle was determined by the Collected Methods of Pacific Technologists (Liston, 1962), using titanous trichloride as a reducing agent for TMAO.

Urea and ammonia - Urea and ammonia were determined enzymatically in a single assay based on the principles by Talke et al., (1965) and the method of enzymic food analysis published by Boehringer Mannheim Biochemical (1980).

RESULTS AND DISCUSSION

Ice Storage Treatment

The quality of sharks is mainly influenced by two factors: the rate of urea being hydrolyzed to ammonia and TMAO being reduced to TMA. Both reactions occur rapidly at the early stages of ice storage once the sharks are landed (Tsuchiya et al., 1951). The results obtained are shown in Figures 1, 2 and 3. Figure 1 indicates that the sharks were of prime quality (10^3), which was likely due to optimum on-board handling. Rapid bleeding and the immediate removal of the head had profound effects on eliminating the high concentrations of TMAO and urea in the blood and the bacteria present in gill and gut, thus decreasing the probability of bacterial attack on the available substrates. The urea content (1.8 percent) was relatively low

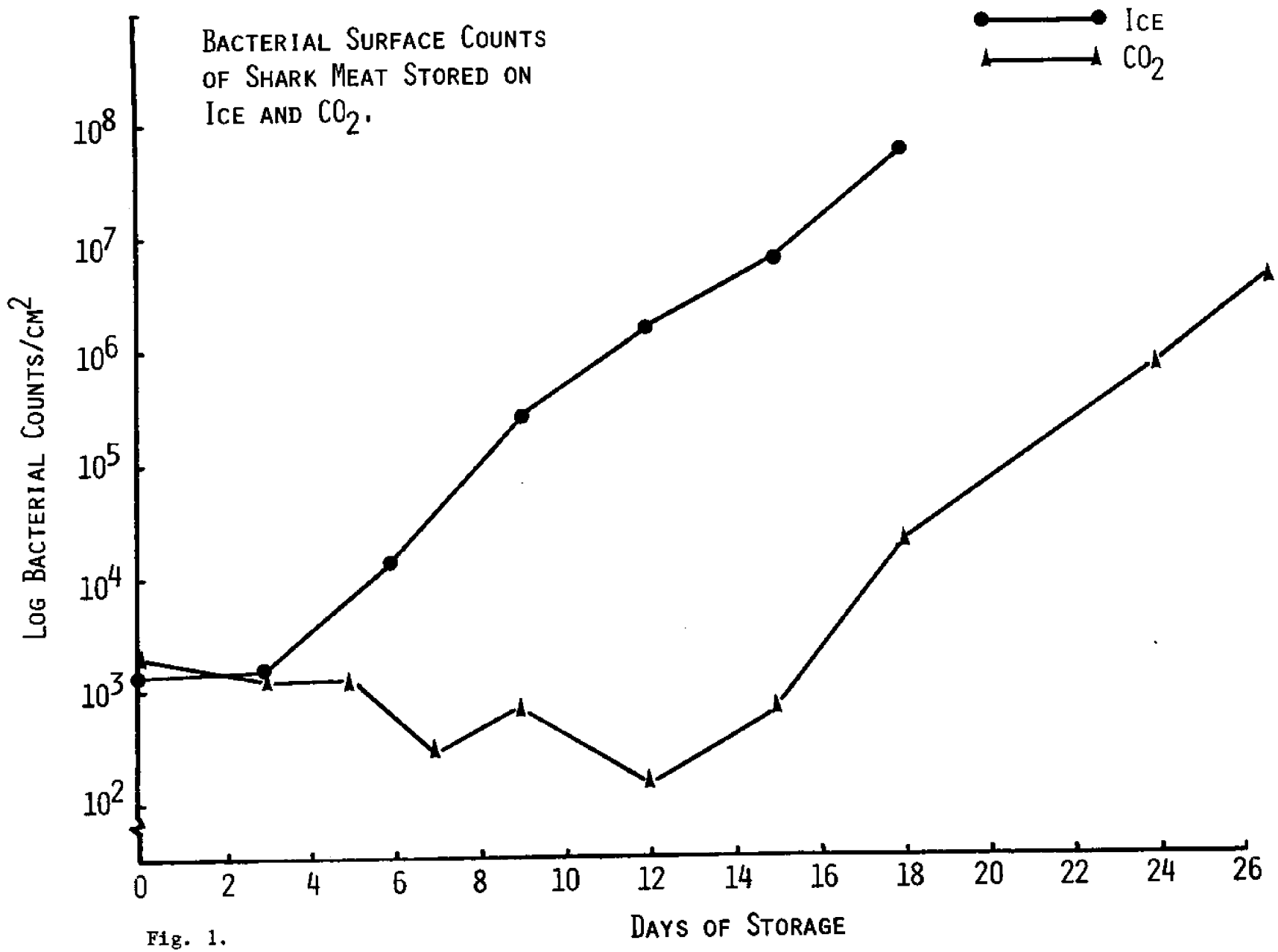
Bacterial Surface Counts of Shark Meat Stored on Ice and CO_2.
Ice
CO_2
Log Bacterial Counts/cm^2
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Days of Storage

Fig. 1.

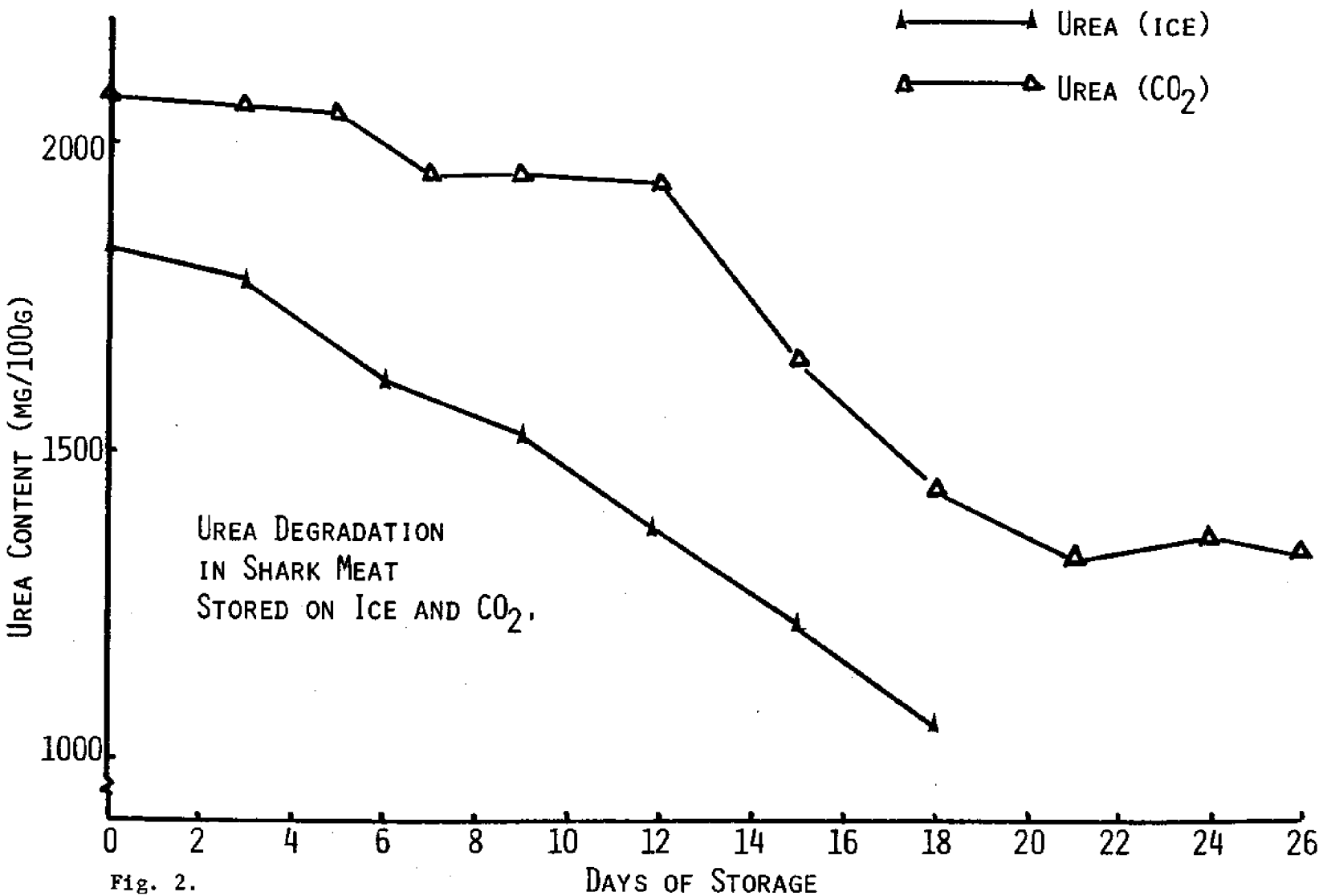
Urea (ice)
Urea (CO_2)
Urea Content (mg/100g)
2000
1500
1000
Urea Degradation
in Shark Meat
Stored on Ice and CO_2.
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Days of Storage

Fig. 2.

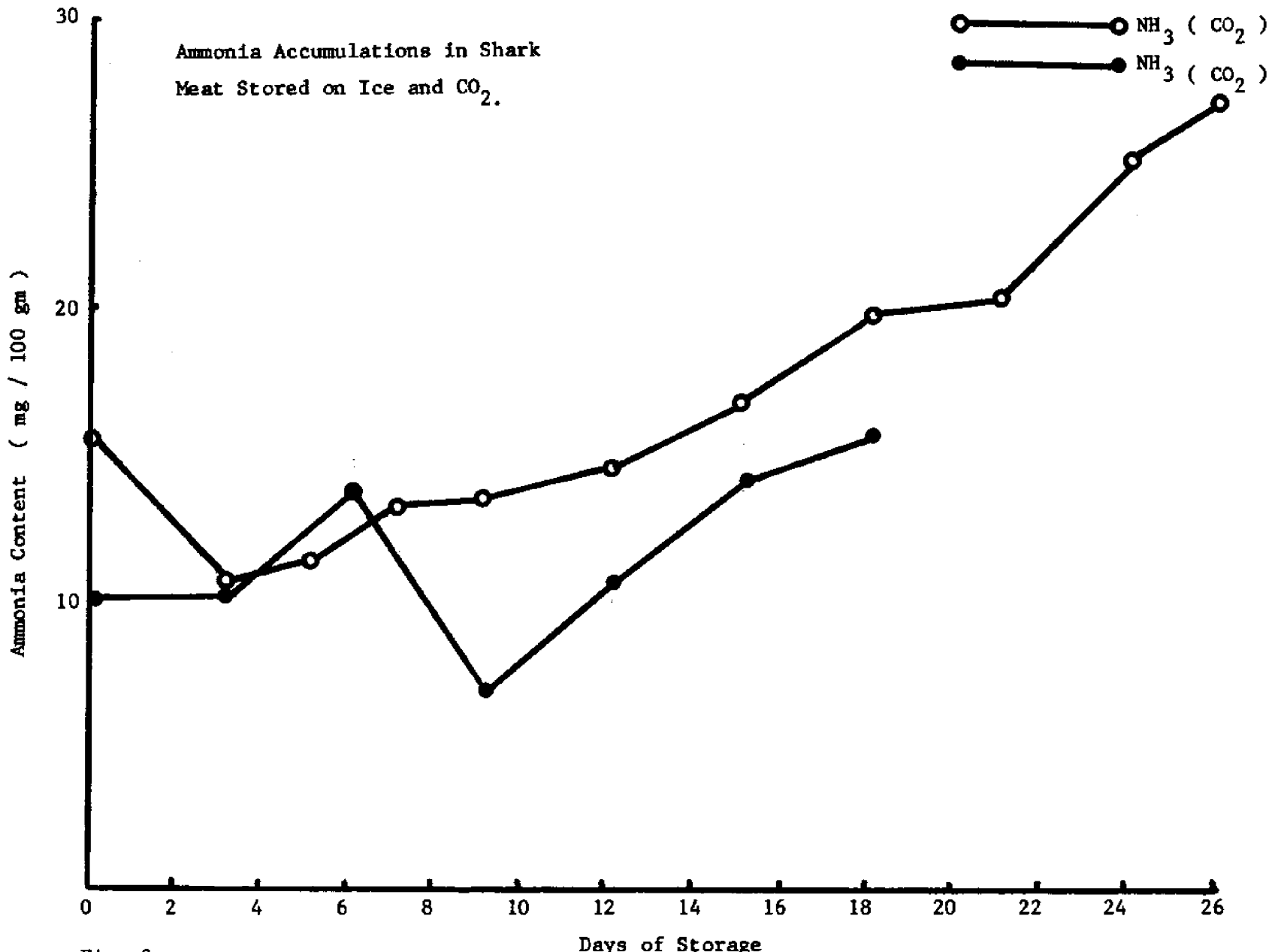
Ammonia Accumulations in Shark
Meat Stored on Ice and CO_2.
NH_3 (CO_2)
NH_3 (CO_2)
Ammonia Content (mg / 100 gm)
30
20
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Days of Storage

Fig. 3.

as compared to those reported in Australian and Japanese studies which reported from 2.0 to 2.5 percent (James and Olley, 1971; Tsuchiya, 1951). Low urea levels parallel low ammonia levels (10 mg/100 gm sample). As a result of low concentrations of urea and ammonia, and the initial prime quality, the APC of shark stored on ice did not reach the borderline of acceptability (10^6-10^7) until fourteen days. The concentration of the precursor of ammonia, urea, decreased sharply from 1800 mg/100 gm to 1100 mg/100 gm while ammonia accumulation never reached 20 mg/100 gm. When comparing these figures for urea degradation and ammonia accumulation, it can be concluded that the leaching action of melting ice is much stronger than the degradation of urea to ammonia when the samples were stored on ice. The reduction of TMAO to TMA was evident in the ice-stored samples (Figure 4). The 50% reduction indicated a large amount of TMA accumulation in the muscle tissue after two weeks of storage. Interestingly, the reaction never went to completion which is in agreement with those findings reported by Elliots (1952) who demonstrated the feedback inhibition of the enzyme (TMAO reductase) by the reduced TMAO product (TMA) which increased the muscle pH away from the optimum pH of the enzyme (pH 7.2 to 7.4) during spoilage.

Controlled Atmosphere Treatment

The CO_2 enrvironment extended the shelf-life by more than one week when compared to the ice storage samples (Figure 1). The surface bacterial counts did not exceed 10^7 after 25 days. The rate of urea degradation and ammonia accumulation were quite similar to those stored on ice, so the CO_2 envrionment seems ineffective in inhibiting the urea hydrolysis (Figures 2 and 3). However, CO_2 does have some inhibitive effect on TMAO-TMA conversion. The TMAO content remained relatively stable throughout the storage period, part of the reason this may account for the improved shelf-life of the shark. (Figures 1 and 4).

Sodium Chloride and Lactic Acid Treatments

The other alternative for improving the quality of shark is to eliminate as much urea and ammonia as possible to avoid off-odor and off-flavor. As shown in Figures 5 through 8, there were up to 60% of urea and ammonia reductions in sodium chloride solution treatment and approximately 40% reductions in lactic acid solution treatment. However, no data is available on the influence of chunk size and penetration of these chemicals into the meat. Judging from the texture and the degree of protein denaturation in the muscle, sodium chloride solution seemed to be a more thorough treatment.

CONCLUSION

On-board handling techniques employed in this study assured a prime quality product. Urea and ammonia levels were relatively low when compared with earlier reports. As a result, sharks stored on ice had an extended shelf-life of about three to four weeks when coupled with a controlled atmospheric environment. CO_2 seems effective in inhibiting the reduction of TMAO to TMA but not the hydrolysis of urea to ammonia. There are about 60 to 40 percent reduction in urea and ammonia when samples were soaked in sodium chloride solution and lactic acid solution, respectively. Sodium chloride solution is a better treatment than lactic acid. Finally, it is recommended that good on-board handling techniques coupled with CO_2 enviroment storage and sodium chloride solution treatment can assure good quality products from a shark fishery in the Gulf of Mexico.

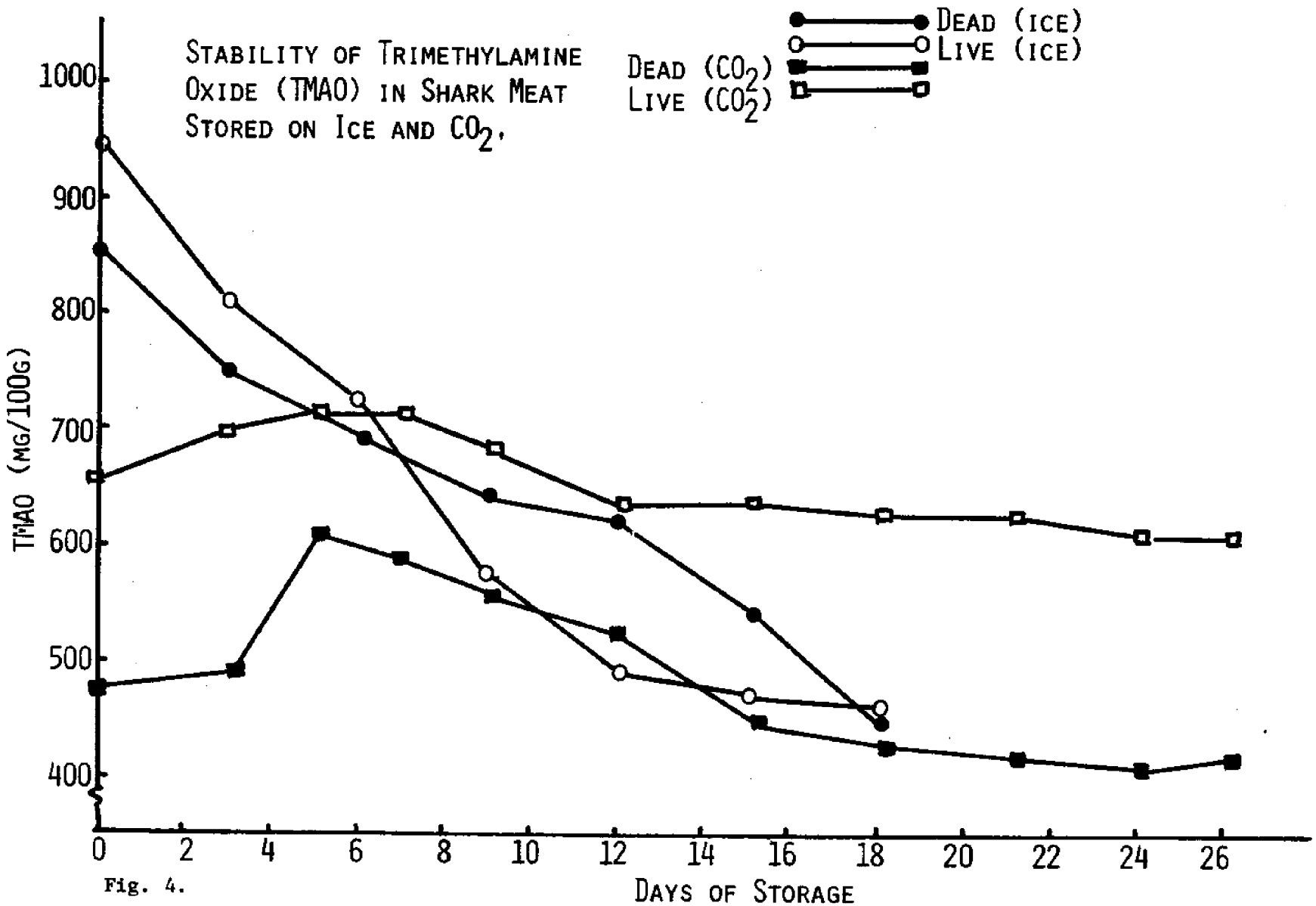
STABILITY OF TRIMETHYLAMINE OXIDE (TMAO) IN SHARK MEAT STORED ON ICE AND CO_2.
DEAD (ICE)
LIVE (ICE)
DEAD (CO_2)
LIVE (CO_2)
TMAO (MG/100G)
1000
900
800
700
600
500
400
0
2
4
6
8
10
12
14
16
18
20
22
24
26
DAYS OF STORAGE

Fig. 4.

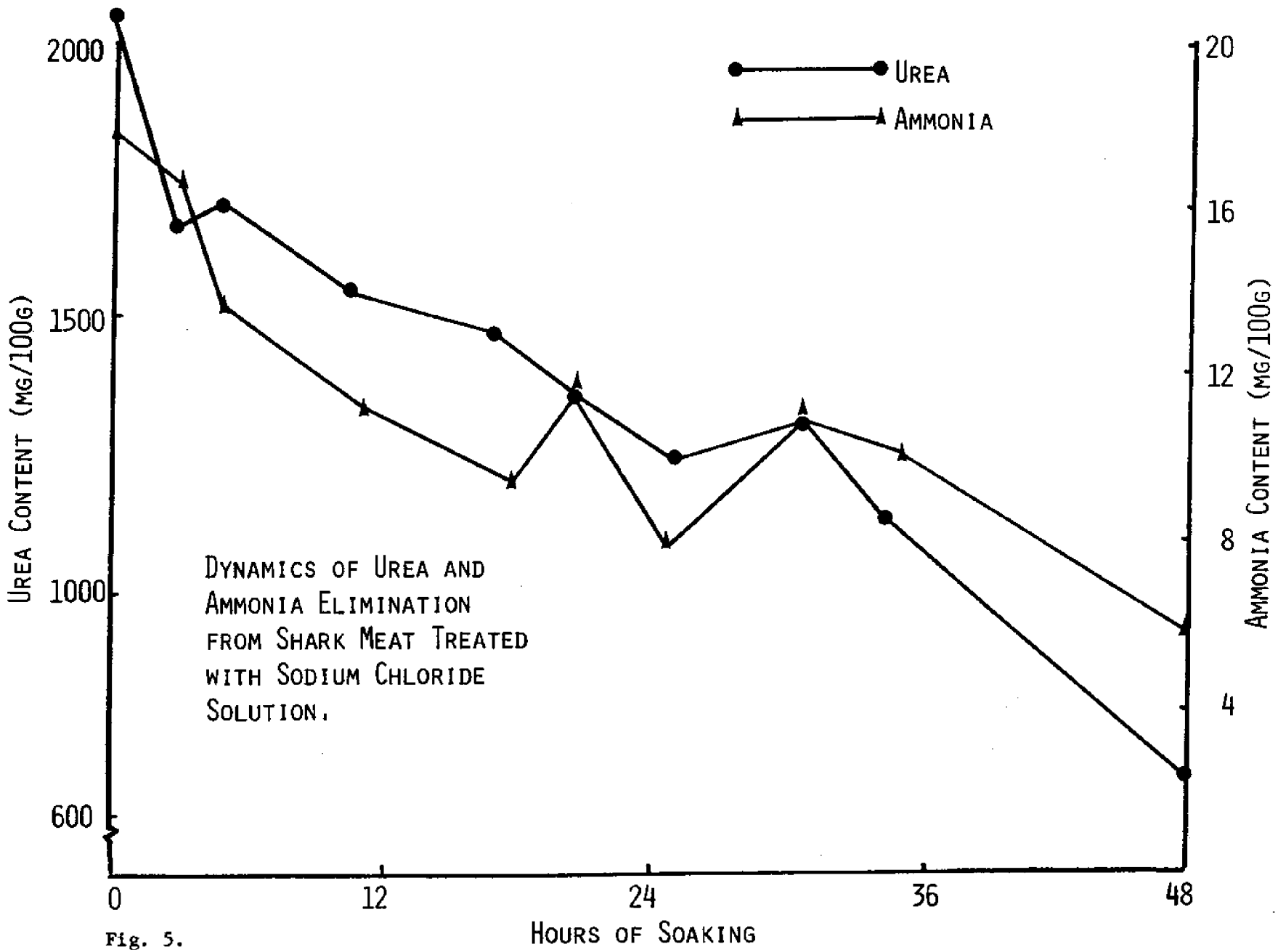
Urea
Ammonia
Urea Content (mg/100g)
Ammonia Content (mg/100g)
2000
1500
1000
600
20
16
12
8
4
0
12
24
36
48
Hours of Soaking
Dynamics of Urea and Ammonia Elimination from Shark Meat Treated with Sodium Chloride Solution.

Fig. 5.

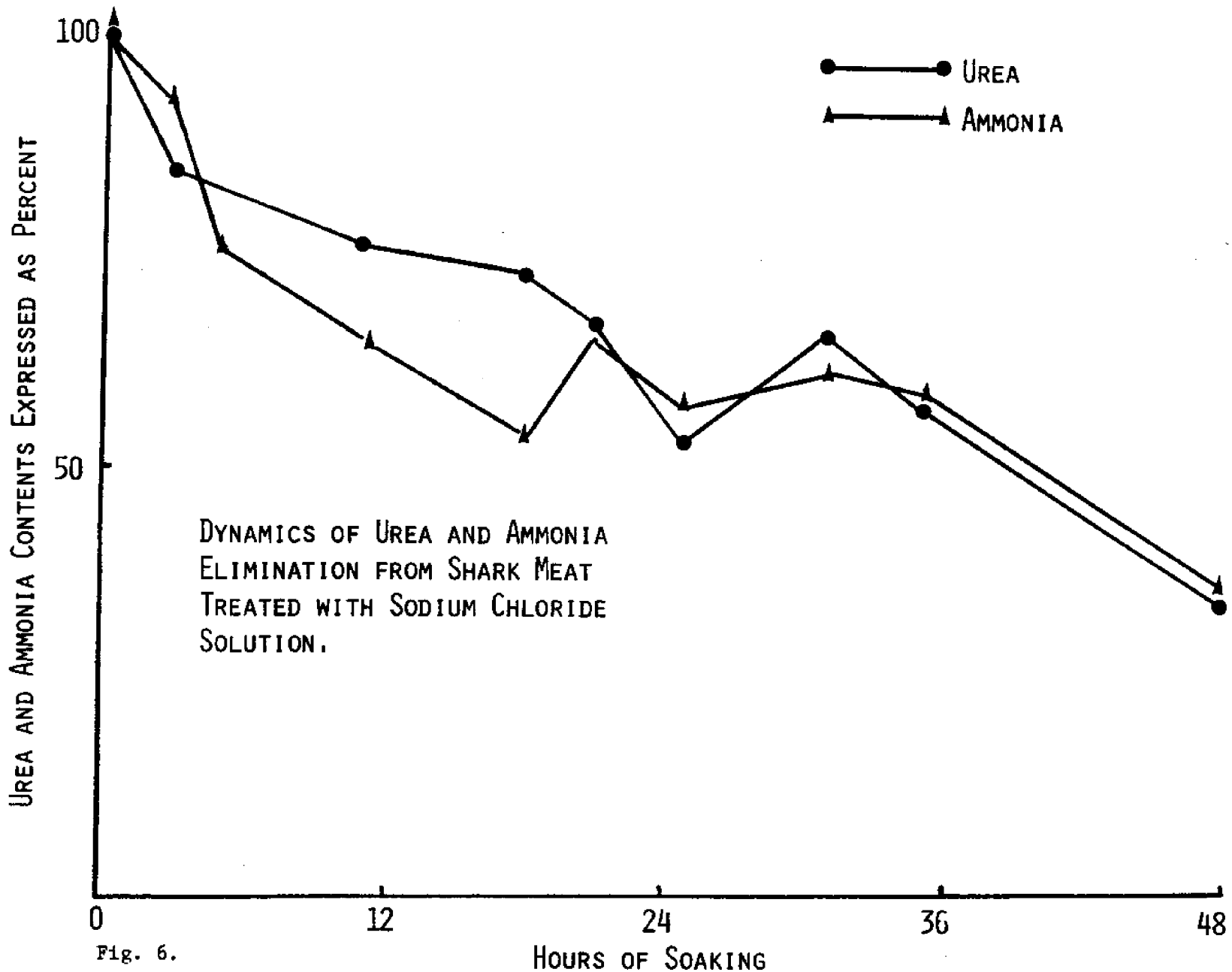
100
50
Urea and Ammonia Contents Expressed as Percent
Urea
Ammonia
Dynamics of Urea and Ammonia Elimination from Shark Meat Treated with Sodium Chloride Solution.
0
12
24
36
48
Hours of Soaking

Fig. 6.

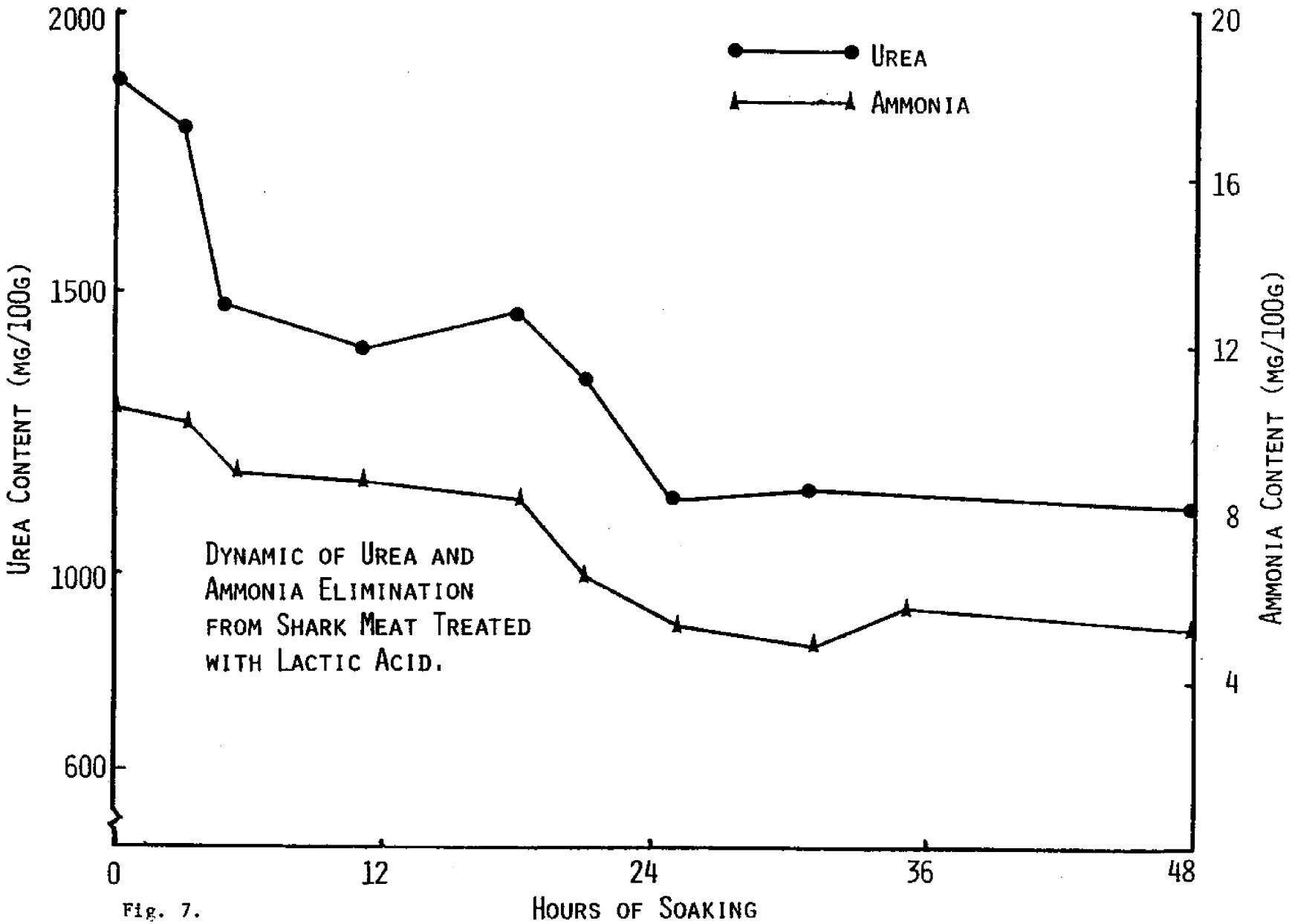
Urea
Ammonia
Urea Content (mg/100g)
Ammonia Content (mg/100g)
2000
1500
1000
600
20
16
12
8
4
0
12
24
36
48
Hours of Soaking
Dynamic of Urea and
Ammonia Elimination
from Shark Meat Treated
with Lactic Acid.

Fig. 7.

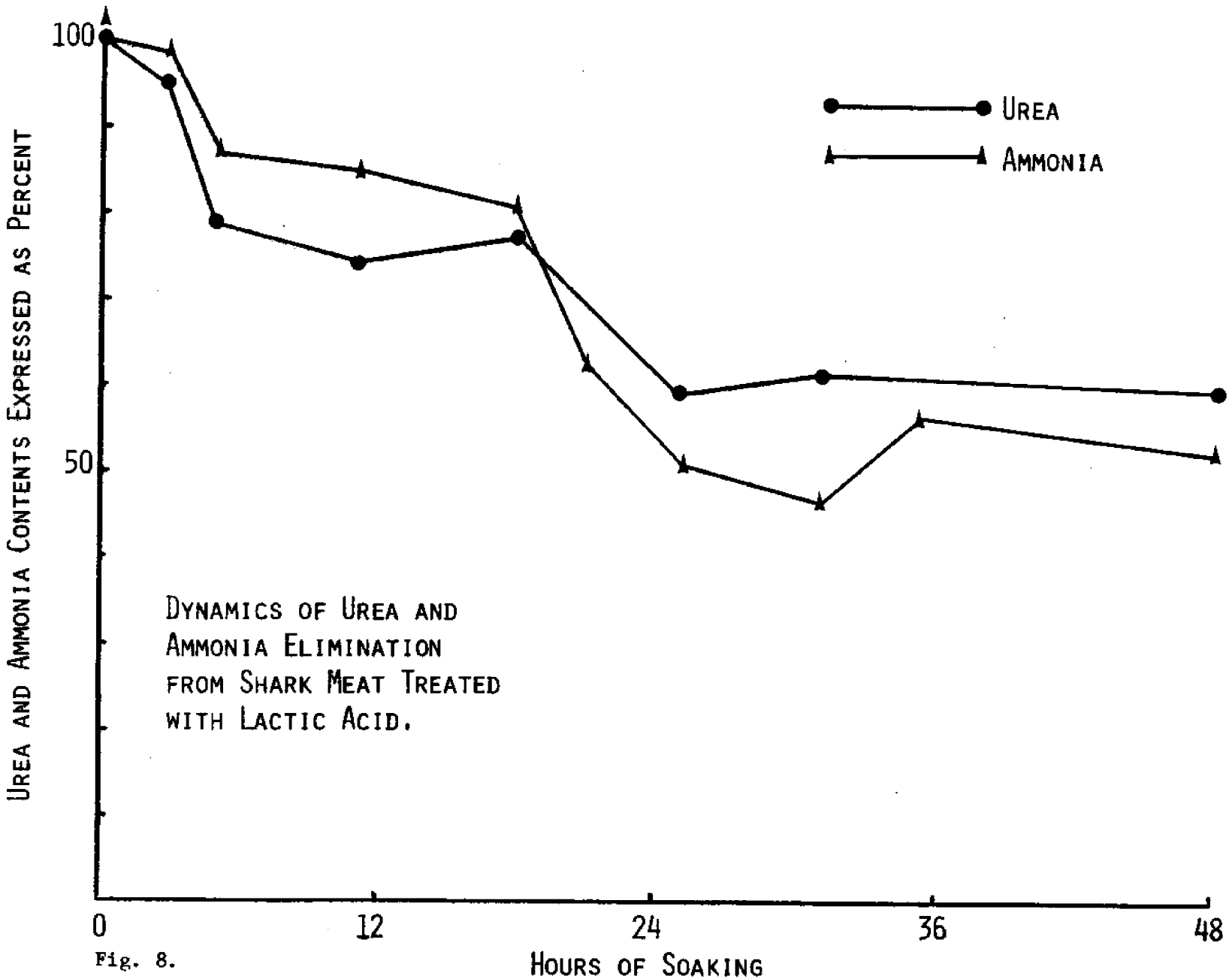
100
50
Urea and Ammonia Contents Expressed as Percent
Urea
Ammonia
Dynamics of Urea and
Ammonia Elimination
from Shark Meat Treated
with Lactic Acid.
0
12
24
36
48
Hours of Soaking

Fig. 8.

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SEAFOOD TECHNOLOGY AND SPORT FISHING

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INTRODUCTION

Saltwater sport fishing is a valuable and growing industry in the United States. In 1975, 16 million saltwater fishermen spent over $3 billion for fishing and related activities. Texas saltwater recreational fisheries is currently valued at over $300 million.

Fishing tournaments are an important part of sport fishing. As organized short-duration events, tournaments provide revenue to coastal communities from sales to out-of-town and out-of-state participants. For example, the participants of the 1971 Narragansett Tuna Tournament spent an estimated $211,000 (Fallell, 1972). The combined economic impact of the 1973 Biloxi Rodeo and the Mississippi Deep Sea Fishing Rodeo was estimated to be over $915,000 (Daniel, 1974). The 1981 Milford World Championship Weakfish Tournament, in Milford, Delaware resulted in $172,000 additional revenue for the state of Delaware (Graefe et al., 1981).

As the popularity of tournaments has grown so has the value of the prizes awarded. Of fifteen scheduled tournaments in Texas during 1980, the seven major tournaments awarded over $3 million in prizes. In 1981, the Poco Bueno Tournament awarded $240,000 to the first place vessel and a total of $500,000 in prizes; but the increasing bounties have necessitated close monitoring of the catch. As prizes have increased, so has the competion for those prizes and the incidence of tampered-with fish and shellfish.

A recent Salmon Derby in Washington state yielded three illegal entries that would have won their owners first, second, and third places. Prizes were valued at $50,000 and included a 17-foot boat. Fortunately officials were alerted and the laboratory tests run showed that the fish had been frozen before entry in the contest.

As exemplified by the above incidents, a need has developed for rapid and reliable tests to determine the freshness of the catch. During the past five years, methods for determining freshness have been investigated by the Texas A&M Marine Advisory Service at fishing tournaments along the Texas coast and at the Texas A&M University Seafood Quality Laboratory in College Station, Texas. The main problem examined has been differentiating between a freshly caught and a frozen and freshly thawed fish.

PROCEDURE

One test that has proven very reliable for identifying frozen versus fresh fish is examination of the eye lens. If the fish has been frozen, the eye will appear sunken and the pupil cloudy. Removal of the lens will reveal a solid white or opaque sphere. The opaque lens is the result of protein denaturation in the lens. In a fresh fish the eye will appear normal, not sunken or cloudy, and the eye lens is clear.

The fish eye lens test can also be used to distinguish between a frozen fish and a fish that has been poorly handled, such as being left on the deck of a boat all day. The eye of a fish that has been exposed to the sun may also appear cloudy. Removal of the eye lens of the sun-exposed fish will show only a partial or one-side coloration. The lens will appear white or cloudy on the side that has been exposed to the sun because the heat generated by the sun will have begun the denaturing of the lens. The lens from a frozen fish will be solid white or uniformly opaque because the freezing has subjected the lens to the same temperature of all sides.

Another test initiated to determine freshness was measurement of the refractive index of expressible muscle fluid. This test has been used to determine biological condition and texture classification of halibut by Declerck and Vyncke (1974). The test involves removing a plug of fish tissue and squeezing the tissue in a handpress to extract a few drops of fluid for reading on a refractometer. Declerck and Vyncke reported that sample size and pressing time had no marked influence on the refractive index readings.

RESULTS AND DISCUSSION

Results obtained did indicate that the average refractive index value of fresh fish tissue fluid was higher than the average value for the same species frozen (see Table 1). The lower refractive index value for frozen fish was initially predicted by Declerck and Vyncke and may be a result of the quantity of drip lost during the thawing process. When frozen the cell membrane ruptures and cellular fluids are released. These fluids may add additional water to the muscle fluid causing dilution and resulting in lower refractive index values.

TABLE 1. Refractive Index Average Values

SPECIES	FRESH	FROZEN
1. Speckled Sea Trout	5.4	4.55
2. Red Snapper	10.4	5.8
3. Flounder	9.5	8.3

Statistical analysis of the effect of number of hours stored and handling treatment on refractive index values did not prove significant. Handling treatment included storing the catch (i) in a live well (ii) on ice (iii) on a stringer in the water or (iv) left on deck of boat.

Extract release volume (ERV) can be used to express changes in hydration capacity of protein. ERV decreases and water holding capacity increases as spoilage occurs in meat (Jay and Konton, 1964). Tests were run to determine the ERV of fish at varying degrees of freshness and spoilage. This test has been shown effective for evaluating spoilage in beef and chicken protein but not shrimp (Vanderzant and Nickelson, 1971). The data for fish ERV showed wide variation within species when subjected to the same handling treatment. No consistent differences were identified for different storage times.

The torry meter has been shown to be a useful tool in measuring freshness of fish. It is most effective when used in determining fresh from frozen fish (Hegen, 1981) with fish that has been frozen always registering 0-3 on the meter's scale of 0-16. Visual observation of the fish can, with little practice, be correlated to an approximate torry meter reading with good accuracy. Because values for freshly caught and promptly chilled fish vary from species to species and scores can remain constant for several consecutive days with proper icing, the usefulness of an expensive machine like the torry meter is diminished for fishing tournaments. Checking fish color, eye clarity, flesh firmness and gill appearance can give an equally reliable indication of freshness and handling.

It is recommended that each tournament stress the importance of handling the fish as an edible and, hence, valuable marine product. By incorporating this recommendation into the tournament rules, a fish would be disqualified if it has not been properly handled.

During a tournament large fish can be kept cool by covering and spraying with water, while smaller fish can be kept in fish bags or ice chests. This practice not only maintains quality but reduces dehydration. Because dehydration can be responsible for a 10-15 percent weight loss, recommending proper handling is especially valuable to the size and weight conscious tournament participant.

In order to reduce the incidence of illegal and tampered with entries in fishing tournaments, close monitoring must be undertaken. Examination of catch does provide some amount of deterrent but efforts to find a reliable, inexpensive and exacting method for judging fish freshness should be continued.

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VERIFYING "FRESHNESS" OF TOURNAMENT FISH

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INTRODUCTION

Recreational fishing tournaments are increasing in popularity and value. The exact value of this business is difficult to assess, but the economic impacts influence specific sales, promote recreational facilities, and attract tourism. The tournaments are usually supported by advertising, sponsorship and entry fees (Milon et al., 1981). Tournament 'purses' often exceed 100 to 200 thousand dollars with individual prizes in excess of 10 to 20 thousand dollars.

As a consequence of the valuable tournaments, there is a high temptation for illegal entries. The most common illegal entry is a previous caught gamefish which has been properly frozen, then thawed for entry during the tournament. Tournament officials and recreational fishing organizations often request the assistance of an 'expert' judge to monitor the weigh-ins and to declare the fish as a legal catch-of-the-day. Unfortunately, most judgements are based on simple sensory assessments, i.e., eye clarity, gill color, flesh firmness, and odors. The assessments are limited and can confuse the interpretation of a poorly handled 'fresh' catch with that of a properly frozen and thawed illegal catch.

Thus a variety of simple objective techniques were investigated as possible tests to verify the 'freshness' of gamefish caught during fishing tournaments. The primary concern was to differentiate fresh and previously frozen fish. Criteria for dockside techniques required simple application with immediate results. Selection of laboratory techniques, to support dockside results, emphasized simplicity and potential application to a variety of fish species.

MATERIAL AND METHODS

Sensory, Torrymeter, histological and chemical techniques compared samples from fresh catch-of-the-day and previously frozen king mackerel, *Scomberomorus cavalla*. All king mackerel were caught with common recreational gear trolling in waters 2 to 3 miles off Jacksonville Beach, Florida during July and August, 1981. Whole fish were either iced or abused immediately after catch. Iced fish were stored in ample amounts of flake ice. Whole fish stored on deck in ambient conditions represented an improperly iced or abused catch. After 12 to 24 hours in ice a portion of the iced fish was washed, tightly wrapped in plastic bags, then slowly frozen in a walk-in freezer (-20°C). Additional king mackerel for sensory and Torrymeter assessments were sampled during an actual fishing tournament, Greater Jacksonville Kingfish Tournament, July 29-August 1, 1981.

Visual assessments included rating for eye clarity, gill color, and flesh firmness. The ratings were based on a 10 point visual scale with 9

representing initial conditions immediately after catch and lower ratings denoting deterioration relative to the initial conditions. Three judges provided the ratings. Visual ratings were recorded for iced and abused mackerel immediately and 1, 2, 4, 8, 12 and 24 hours after catch. Internal body temperature in the thickest portion of the fish was determined with a simple internal dial type thermometer. Visual conditions were also noted for frozen king mackerel. Simultaneously with visual ratings, Torrymeter readings were recorded for iced and abused fish. The probe head of the meter was positioned in three separate locations above and parallel to the lateral line of the fish. Each location was centered in one-third portions of the body length. Three readings were averaged per location to provide nine readings per fish. Similar meter readings were recorded for previously frozen king mackerel which had been thawed slowly in refrigeration. All meter readings were based on the manufacturer's programmed 16 point scale; 16 indicating superior quality and freshness. The Torrymeter was built by GR International Electronics Limited, Perth, Scotland. This hand-held instrument was designed to measure changes in the dielectric properties of fish skin and muscle tissue which result during storage and initial spoilage.

A basic histological procedure was used to compare the microstructure of muscle tissue from iced and frozen mackerel. Small pieces of the body musculature were taken from three places along the left side of each fish; an anterior sample from just behind the head at the level of the anterior base of the first dorsal fin; a mid-body sample from the level of the second dorsal fin, and posterior sample from near the caudal fin. Slices 2 to 3 mm thick were cut from each sample to give both cross and longitudinal sections through the muscle fibers. The tissues were then fixed in either 10 percent neutral buffered formalin, Sanfelice or Bouin's fixative (Humason, 1979). After fixing for 24 to 48 hours, the tissues were washed overnight in running water and dehydrated in a series of ethyl alcohol solutions of increasing concentration (50% - 24 hrs, two changes each of 70, 95, and 100% - 2 hours per change). Xylene was used as the clearing agent (two changes - 2 hours per change) and the tissues were infiltrated and embedded in paraffin (Paraplast Plus, melting point 56-57^{0}C). Embedded samples were sectioned at 5 to 8um on a rotary microtome using a steel knife. Sections were mounted on glass slides and stained using the regressive hematoxylin and eosin technique (Luna, 1968). Photomicrographs were made using Kodak Panatomic X film.

Determinations for cook drip loss and protein extractablility were used to assess chemical properties of fish muscle tissue. Samples from catch-of-the-day fish (12 to 24 hours on ice) were compared with samples stored in ice for 1 to 7 days and samples stored frozen for 1 to 7 days, and 2 or 6 weeks. To determine cook drip loss a 40g sample of muscle tissue was pressed to uniform thickness over the bottom of an Erlenmeyer flask. The flask was sealed with aluminum foil, then placed in a 90^{0}C water bath for 30 minutes. The total volume (ml) of liquid exudate and condensate was measured as cook drip loss. To determine protein extractability, a 25g sample of muscle tissue was blended with a refrigerated salt solution (0.6 M KCl + 0.01 M $NaHCO_3$) in a ratio of 12:1 (solution to sample). Blending conditions were 7000 rpm for 30 seconds in refrigeration. The blend was centrifuged for 1 hour at 9000 rpm. Three 15 ml

samples from the supernate were used in Kjeldahl determinations of percent nitrogen and percent protein (6.25 multiplier for conversion). Protein extractability was expressed as grams of protein recovered per 100 grams of muscle tissue.

RESULTS AND DISCUSSION

King mackerel caught specifically for this study had an average total body weight of 6.6 kg (14.6 lbs.) and fork length of 91.4 cm (36 in.). Each fish appeared in excellent health, free of noticeable, external parasites and fought with vigor before entering the boat. Three fish were stored in ice and three were purposely abused by storing in ambient conditions (90% relative humidity and 35°C) on a shaded deck. Average internal body temperature for all fish immediately after catch was 30°C (86°F). This temperature gradually decreased to between 1 and 2°C within 8 hours ice storage, but remained between 30 and 35°C in abused fish.

The visual assessments for iced and abused catch-of-the-day fish are shown in Figure 1. Deterioration in appearance of the abused fish was noticeable within 2 hours after catch. The most noticeable change was decreased eye clarity which progressed to cloudy eye appearance after 8 hours. Similarly, deterioration in the flesh of the abused fish was first noted as surface dehydration and cracking after 2 hours and softened flesh after 4 hours. Finger pressure would leave permanent indentations in the surface flesh after 8 hours. The gill color of abused fish decreased from the original rich red-purple color to a dull, pink coloration after 4 hours, followed by an accumulation of slime at 8 hours. In contrast, the appearance of the iced fish remained unchanged for 4 hours after catch. After 8 hours in ice there was a slight decrease in eye clarity. After 24 hours of ice storage, the flesh remained relatively firm and resistant to indentation and the gill color faded to a red-pink color with some noticeable accumulation of slime. Visual distinction between iced and abused fish was obvious, but the visual appearance of thawed fish was similar to the abused fish. Freezing caused thick clouding of the eyes, dehydration and cracking on the surface, softened flesh, and faded gill color. Thus the visual distinction between abused and previously frozen king mackerel was not as obvious.

The Torrymeter readings from iced and abused fish were substantially different at 2 hours after catch (Figure 1). The initial readings for fresh caught fish (12.0) were similar to initial readings recorded for other southeastern marine fish species (Hegen, 1981). Within 24 hours after catch, readings from properly iced fish did not fall below 12.0, but readings for abused fish gradually decreased to 7.0. The difference in readings from iced and abused fish is probably due to differences in initial spoilage, autolytic activity, and internal body tempertures. Since recreational fishermen do not always provide proper icing, judges can anticipate Torrymeter readings to range from 16.0 to 7.0 for catch-of-the-day king mackerel. In contrast, readings from previously frozen king mackerel never exceeded 4.0. The average meter reading for previously frozen fish was 1.0. For iced, abused or frozen fish there was no appreciable difference in meter readings for the three body locations.

The histological comparison between iced catch-of-the-day and previously frozen king mackerel reveals dramatic differences in both cross and

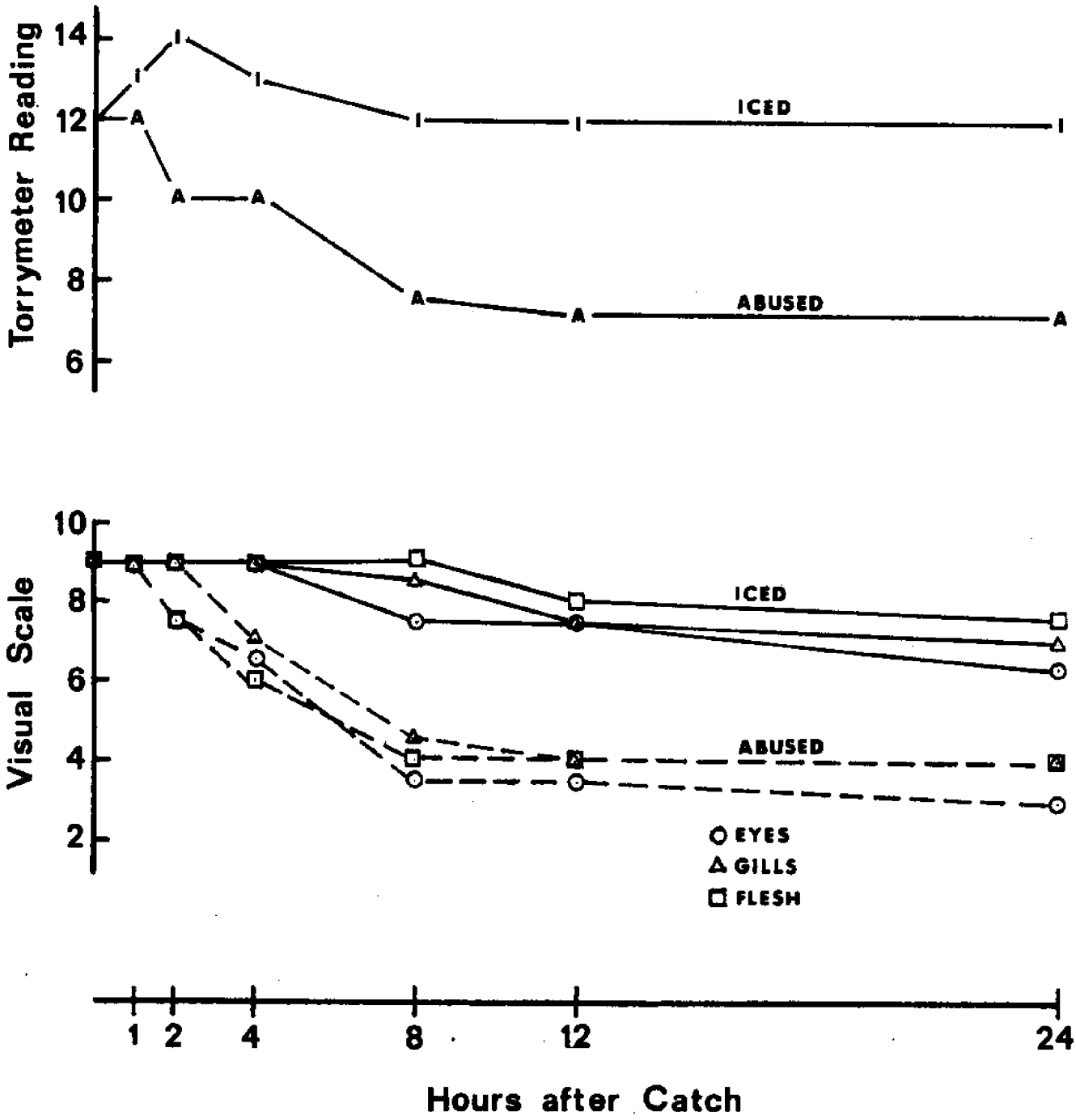

Figure 1. Average visual assessments and Torrymeter readings for catch-of-the-day king mackerel stored in ice or abused for 24 hours. The standard deviation for any mean visual assessments (n=3 observations per hour of measurements) did not exceed 0.5. The standard deviation for any mean meter reading (n=9 observations per hour of measurements) did not exceed 0.5.

longitudinal sections (Figs. 2 and 3). Iced muscle tissues show distinct myofibers, intact sarcolemmas, and only minor intracellular artifacts (Figs. 2a and b). Extracellular spaces are regular and show no sign of distortion other than shrinkage resulting from histo-processing. The previously frozen tissues reveal many irregularly shaped spaces about and within the myofibers, and the sarcolemmas are disrupted and distored (Figs. 3a and b). Structural alterations caused by freezing are thought to result from intra- and extracellular ice crystal formation as reported by Bello et al. (1981). Thus a simplified histological procedure can be used to distinguish catch-of-the-day and previously frozen tournament fish.

Great care had to be taken during the histological preparation of king mackerel skeletal muscle, particularly during the cutting of the sections, in order to insure production of good quality slides. There did not seem to be any qualitative difference in ease of preparing samples from the anterior and mid-body samples. Tissues taken from the posterior region, however, exhibited a greater tendency to cut poorly. The major problems were excessive tissue hardening to a brittle condition and a tendency for the muscle fascicles to separate when slices were floated in a water bath. This is probably due to the greater amount of connective tissue found in the body musculature in the posterior region. The problems were more pronounced in frozen tissues. Bello et al. (1981) sought to overcome these and other problems by developing an improved method for fish muscle preparation. Their procedure included a double embedding technique utilizing nitrocellulose. The techniques used in the present study are simpler and sufficient for differentiating catch-of-the-day and previously frozen tournament fish. Tissues fixed in 10 percent buffered formalin gave the best results. Samples fixed in Sanfelice and Bouin's tended to crumble or shred when sectioned.

The chemical properties of the fish muscle tissue investigated in this study did not provide clear distinction between catch-of-the-day and previously frozen king mackerel. Due to a large variation in results the cook drip loss and protein extractability from samples of iced catch-of-the-day and fish iced one additional day was not significatly different from fish stored frozen after catch through 6 weeks (Figs. 4 and 5). As anticipated, a sharp increase in protein extractability was observed in the fish muscle obtained from one day frozen storage. This increase might be due to damage of protein bonds and cell walls of fish muscle. The decreased protein extractability after three days frozen storage may result due to further protein denaturation. Numerous researchers (Reay, 1934; Dyer, 1951; Dyer and Fraser, 1959; Miyaucki et al., 1963; Mahon and Schneider, 1964; and Chalken and MacCallum, 1965; and Kato et al., 1974) have noted a gradual increase in drip loss and protein extractability after prolonged frozen storage. Connell (1975) and Mills (1975) agree with this general trend but caution about the drawbacks of applications and interpretation of these chemical/physical tests. Similarly, our results should be viewed with caution due to their extreme variability. Further investigation is necessary before these tests can be recommended for assessing the freshness of tournament fish.

Torrymeter readings recorded from fish caught during an actual king mackerel tournament are presented in Tables 1 and 2 and in Figure 6. The average tournament fish measured 108 cm (42.5 in.) and weighed 11 kg (24 lbs.).

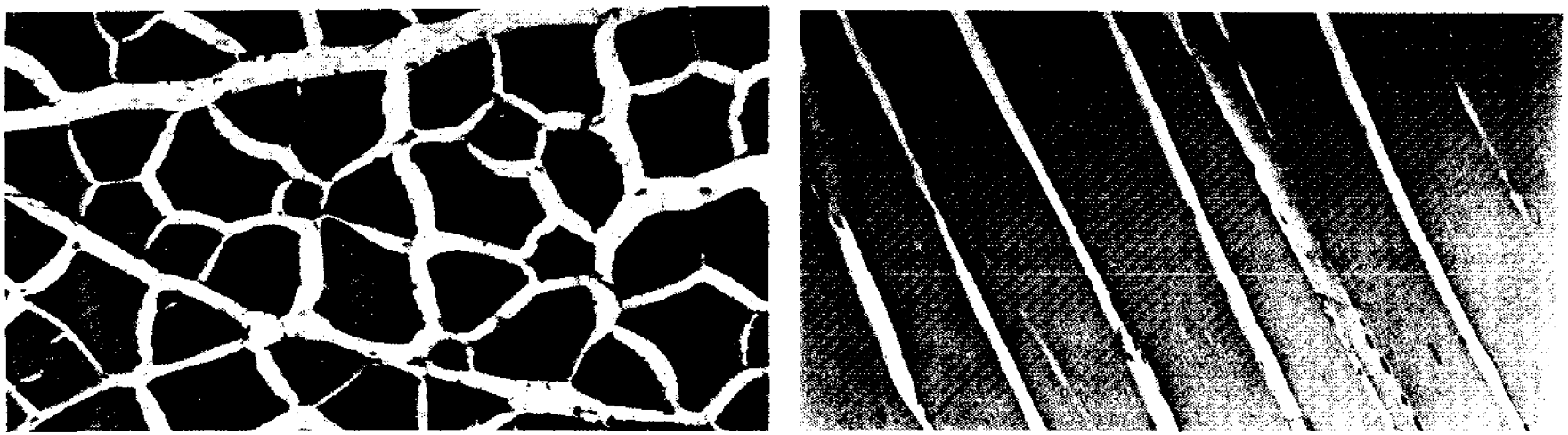

Figure 2. (a) Cross and (b) longitudinal sections of iced catch-of-the-day king mackerel fixed in 10 percent neutral buffered formalin and magnified 160X.

Figure 3. (a) Cross and (b) longitudinal sections of previously frozen king mackerel stored at -20^{0}C then thawed and fixed in 10 percent neutral buffered formalin and magnified 160X.

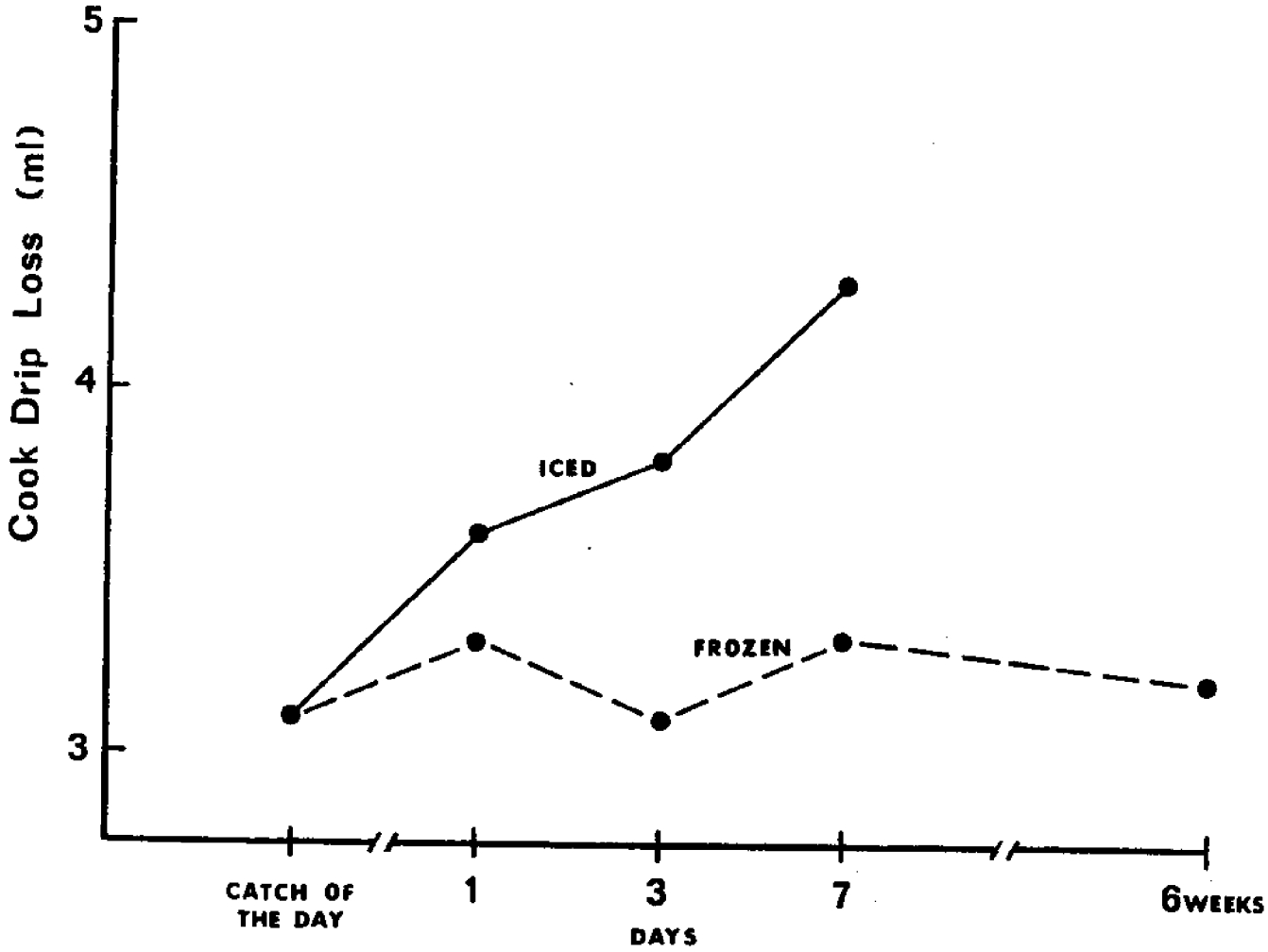

Figure 4. Cook drip loss (ml) from 40g of muscle tissue of iced and frozen king mackerel. Catch-of-the-day represents fresh caught fish held in ice between 12 and 24 hours.

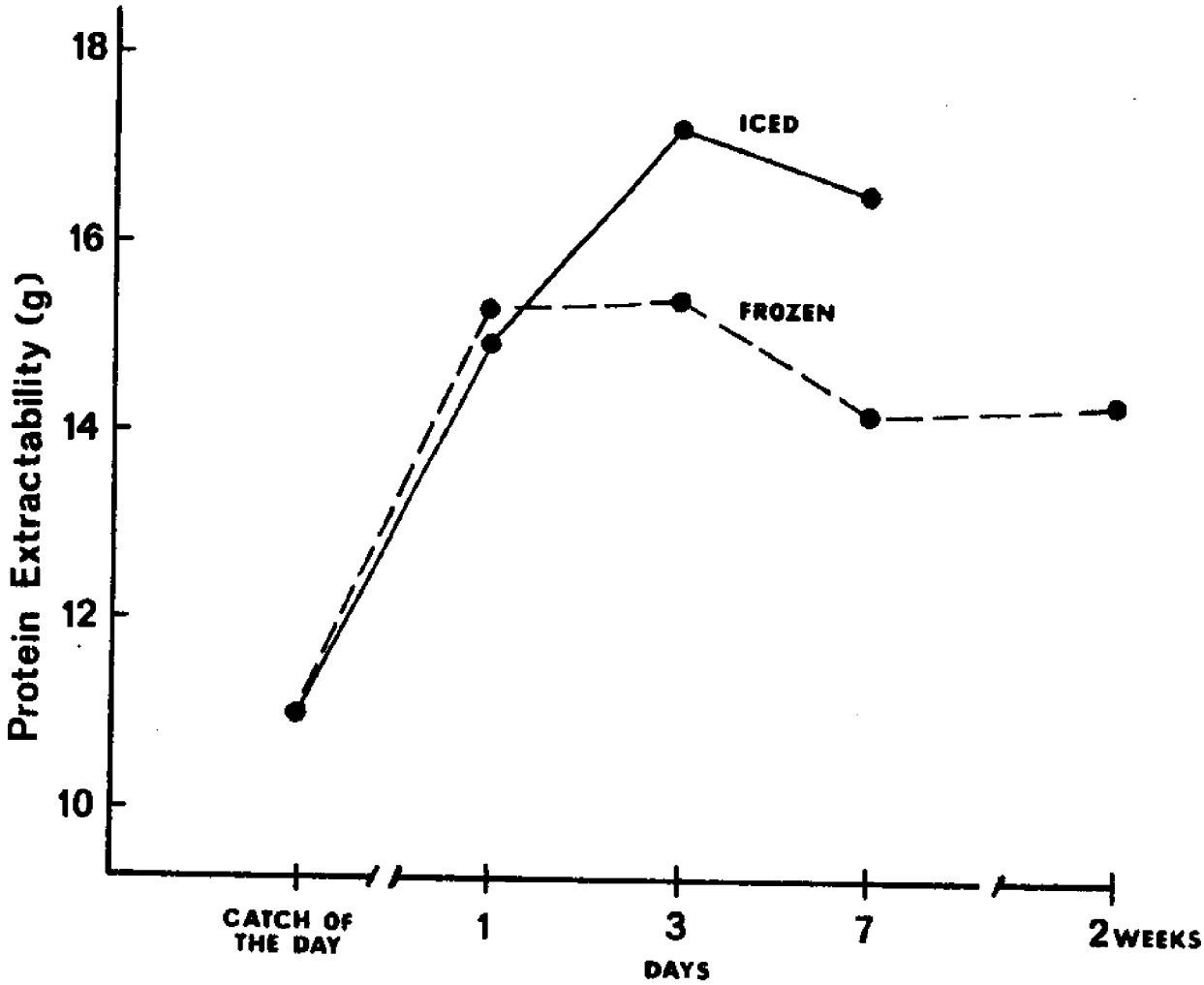

Figure 5. Protein extractability (g) expressed per 100g of muscle tissue of iced and frozen king mackerel. Catch-of-the-day represents fresh caught fish held in ice between 12 and 24 hours.

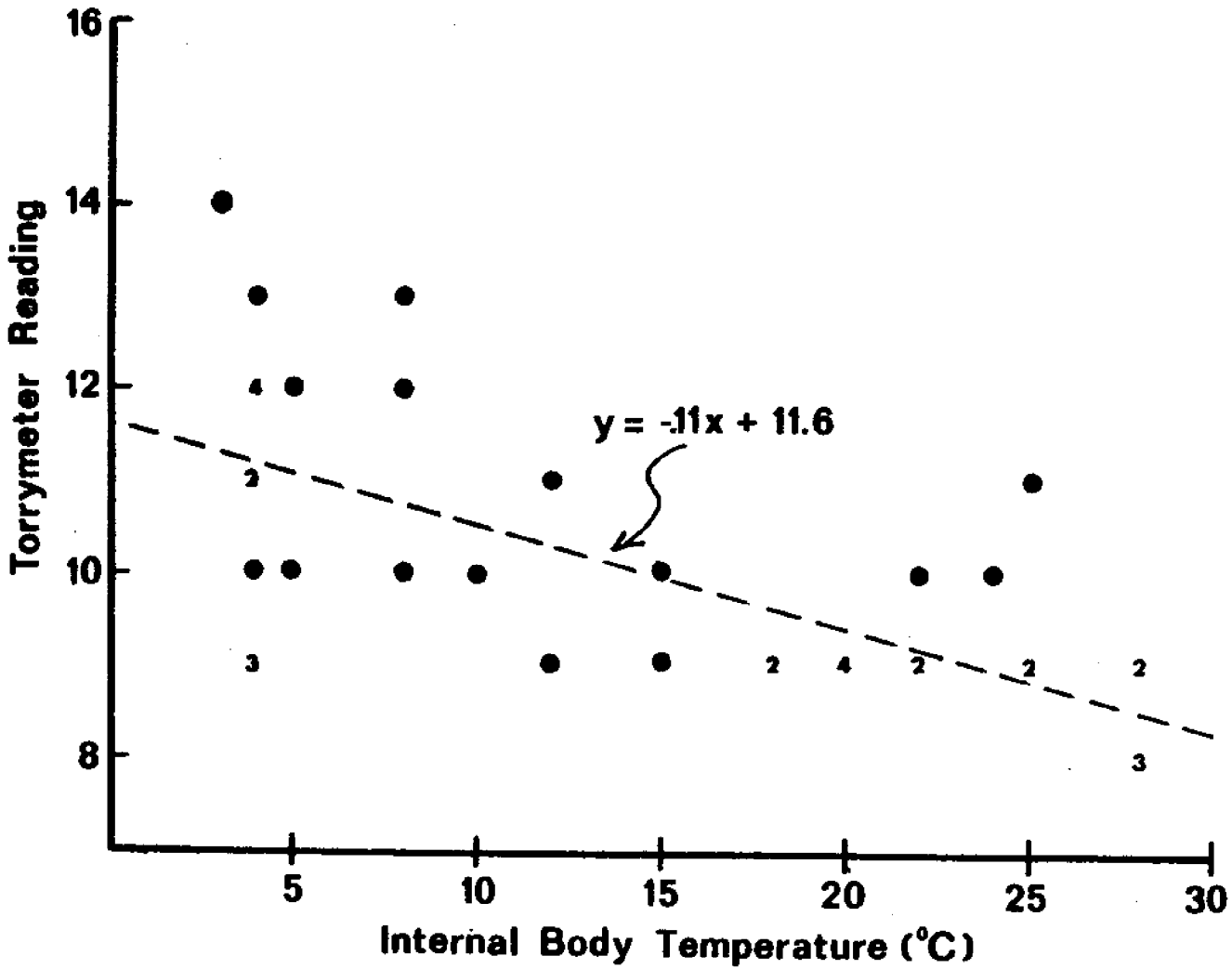

Figure 6. Torrymeter readings per the internal body temperature for forty king mackerel caught during a fishing tournament. Single fish are represented by o. Numerals represent duplicate readings from different fish. For the regression equation, y= meter readings and x= internal body temperature.

Table 1. Torrymeter readings and size for forty king mackerel caught during an actual fishing tournament.

	Mean	Std. Deviation	Range
Torrymeter reading	10.1	1.5	8-14
Fork Length cm.	108	14.2	69-135
(in.)	(42.5)	(5.6)	(27-53)
Body Weight kg.	11	4.3	2.5-22.8
(lbs.)	(24.4)	(9.4)	(5.5-50.2)

Table 2. Torrymeter readings by sex for king mackerel caught during an actual fishing tournament.

Sex	No. Fish	Mean	Std. Dev.	Range
Male	10	10.5	0.9	8-14
Female	10	10.3	1.4	8-14

Table 3. Recommended interpretation for Torrymeter readings for marine fish caught during a recreational fishing tournament.

Range	Interpretation
16-12	Fresh catch-of-the-day
12-8	Probable catch-of-the-day
8-4	Suspect catch-of-the-day Product abuse
4-0	Previously frozen fish Spoilage

The range (8 to 14) and mean (10.1) Torrymeter readings were within the experimental range (7-16) noted earlier for iced and abused catch-of-the-day fish. Sex did not influence the meter readings, but readings were influenced by the internal body temperature. Approximately one-third of the fish were properly iced and had an internal body temperature below 5^0C (41^0F) but higher body temperatures as a result of abuse and improper icing depressed the meter readings (Fig. 6). A simple linear regression of meter readings (y) as influenced by internal body temperature was calculated to be:

$$y = -.11x + 11.6; \; R = -.620$$

During the tournament two king mackerel in excess of 13.6 kg (30 lbs.) were encountered which had a meter reading below 4.0. These fish were declared previously frozen and rejected from the tournament without protest from the individual fishermen.

CONCLUSION

The Torrymeter can be used to support visual assessments to verify catch-of-the-day fish in recreational fishing tournaments. Further support to verify field results can include the simple histological procedure outlined in this report. These results apply specifically to king mackerel, but offer potential application to other recreational species.

A general interpretation of meter readings for recreational tournaments is listed in Table 3. Fishermen should be encouraged to provide proper icing to control internal body temperature and avoid abuse to assure higher meter readings. In abused fish cloudy eyes, softened flesh, and low meter readings near 4.0 could be misinterpreted as signs of a previously frozen fish. Judges should remember that the Torrymeter can not effectively distinguish between catch-of-the-day fish and fish caught and properly iced beyond one day. For multi-day tournaments rules would have to be established to prevent carry-over from a previous days catch.

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EVALUATION OF THE TEXTURAL ATTRIBUTES OF MINCED FISH PATTIES MADE FROM FISH (TURBOT AND POLLOCK), SOY FLOUR, SOY PROTEIN CONCENTRATE AND SODIUM ALGINATE

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INTRODUCTION

Recent emphasis on expanding use of domestic seafood resources has focused attention on utilization of nontraditional fish species, and total recovery and utilization of fish flesh (Bello and Pigott, 1980). The seafood industry faces the challenge of producing wholesome, attractive fishery products from the flesh of nontraditional fish, and from the trimmings of fish used for common fillet production. Many approaches have been taken to meet this challenge, in particular, to solve the textural problems associated with fishery products formulated with minced fish. The textural attributes of minced fish flesh are poor in comparison to intact flesh because of the difference in their physical and chemical nature. Shenouda (1980) reviews these differences and their consequence on texture, and Rizvi (1981) describes the adverse effects that the mincing process has on fish flesh.

Many researchers have attempted to solve the problem of objectionable minced fish texture through product formulation. Carver and King (1971) developed fish cakes composed of recovered whiting flesh, potatoes, onions and seasonings. King and Flick (1973) blended minced fish flesh with ground beef to produce a product they label "beefish" patties as a method to solve color and texture problems. Bello and Pigott (1979) blended minced fish flesh with structured protein fiber, modified tapioca starch and sodium chloride in an attempt to produce a satisfactory fish cake. Deng and Tomaszewski (1980) monitored consumer acceptance for fish patties made from croaker, sodium chloride, sodium trypolyphosphate and sodium alginate. Rockower et al. (1982) studied the textural attributes of minced fish patties made with 4 species of fish blended with and without soy protein. The diversity in researched formulations reflects the complexity of the textural problem.

Our work has focused on developing a fish patty made from fish bits and pieces remaining after cutting frozen blocks of pollock (*Pollachius virens*) and Greenland turbot (*Reinhardtius hippoglossuides*). These two fish species, a lean and fatty species respectively, were blended in varying formulations with soy protein concentrate, soy flour and sodium alginate. Objective and subjective characteristics, composition, and cost parameters were studied as a function of ingredient levels. The purpose of this work was to develop a marketable product based on sensory, economic and compositional characteristics. This study varies from previous work because

of the two specific fish species used, selection of soy flour rather than potato, wheat or rice flour, and the specific levels and combinations of ingredients studied.

METHODS AND MATERIALS

Pollock (Pollachius virens) and Greenland turbot (Reinhardtius hippoglossuides) were purchased in the frozen fillet blocks from the Rich Sea Pack Corporation. The frozen blocks were cut and minced to exemplify fish bits and pieces. Structured protein fiber (SPF-200), soy flour (Bontrae 2101), sodium alginate (Keltone), dehydrated onions, dehydrated celery, sodium chloride and light density sodium tripolyphosphate were supplied by the Ralston Purina Company, the Central Soya Company, the Kelco Company, the Foremost-Gentry Company, California Vegetables Concentrates, the Diamond Crystal Salt Company and the FMC Corporation, respectively. Host Favorite batter and breading products of the North American Food Service Corporation, and peanut oil were obtained from the Hi Neighbor Wholesale Company, a local distributor located in Gainesville, Florida.

PATTY PREPARATION

The minced fish patties were prepared by placing the combination of fish and soy proteins required for a particular treatment into an aluminum hobart bowl. Rehydrated onions and celery, sodium tripolyphosphate and sodium chloride were then added as fixed level ingredients along with the final experimental variable, sodium alginate. The sodium alginate was used at 0.2, 0.3 and 0.4% levels based on the total ingredients weight per patty formulation. Pollock and turbot blocks were reduced to pieces of approximately 1 cubic inch dimensions prior to the mixing process. Before the soy flour (50% protein on a moisture free basis) was added to the mix, it was hydrated at 1 part soy flour to 1.6 parts water. Frozen soy protein concentrate (93% protein on a moisture free basis hydrated to 65% moisture) was thawed at 35^{0}F before mixing. The dry onions and celery pieces were rehydrated in cold (35^{0}F), distilled water for 5 minutes. The ingredients were blended at hobart speed No. 2 for 10 minutes in a 35^{0}F refrigerated room to prevent heating the mixture.

Eightfive grams of the mixture was weighed into one petri plate, precoated with soybean oil. Nine plates were packed per experimental treatment. The circular shaped patties were then removed from the petri plates and placed in a 7% solution of calcium chloride for 30 seconds. Interaction of sodium alginate with calcium caused a thin gelatinous film to form on the patty surface in a mechanism described by Morris (1973). The patties were subsequently battered, breaded and deep fat fryed in peanut oil at 375^{0}F for 45 seconds. After frying the patties were immediately wrapped in wax paper and aluminum foil to protect them from freezer burn during frozen storage (-30^{0}F). All processing steps except cooking were executed at 35^{0}F. This was done to prevent adverse protein-protein interactions from contributing to textural deterioration.

EXPERIMENTAL DESIGN

Six combinations of fish, soy flour, soy protein concentrate and sodium alginate were formulated once with turbot as the fish component, again using pollock and finally with a 1:1 ratio of turbot and pollock. These 18 combinations were replicated at 3 sodium alginate levels 0.2, 0.3 and 0.4% for a total of 54 treatments. The levels of fish were chosen at 100, 85, and 70% of the total proteinaceous ingredients. Likewise, levels of soy flour and soy protein concentrate varied at 10, 15 and 30% of the total proteinaceous ingredients. These levels were chosen to fit a simplex-lattice mixture design (Cornell, 1981).

Mathematical models were fitted to data collected on each treatment to predict values for eight responses (Y_i = Breakpoint value, Firmness Score, Flavor Score, Overall Acceptability Score, Cost Using Pieces, Cost Using Fillets, Protein and Fat Content). Experimental model;

$$Y_i = \beta_1 T + \beta_2 P + \beta_3 B + \beta_4 S + \beta_5 A + \beta_{12} TP + \beta_{13} TB + \ldots$$

$$+ \beta_{45} SA + \beta_{123} TPB + \ldots + \beta_{345} BSA + \beta_{1234} TPBS + \ldots +$$

$$\beta_{2345} P_2 B_3 S_4 A_5 + \beta_{12345} T_1 P_2 B_3 S_4 A_5$$

where for the ingredient variables, T = turbot (range 0 to 100%), P = pollock (range 0 to 100%), B = the soy flour Bontrae (range 0 to 30%), S = the soy protein concentrate SPF-200 (range 0 to 30%) and A = alginate (range 0.2 to 0.4%) (Cochran and Cox, 1957). The β's are coefficient values which when estimated indicate the effects of various ingredient combinations of the response. When using this model to predict the response, the sum of the independent variables T, P, B and S must equal 100%. The first portion of the model containing β's with single subscripts represents the linear blending effects of the mixture components. Model terms containing β's with double subscripts represent the nonlinear blending effects of component pairs. The portion of the equation containing the β terms with triple subscripts represents the nonlinear blending effects caused by blending three components. Similar explanations pertain to the model terms containing β's with four and five subscripts.

Not all 31 model terms (β's) were required to adequately describe the effect of the five ingredient variables on the eight responses in each of the eight models generated to predict their respective responses. Two statistical methods were used to determine the inclusion of a specific term in the model. A t-test was performed on the individual terms in the model using the ratio of the estimated β coefficient value and its standard error, and a test was performed on competitive models to determine whether or not there was an improvement in the models adjusted multiple correlation coefficient (R_A2) as a result of the inclusion of the model term (Cochran and Cox, 1957). The closer a model's R_A^2 value is to 1, the better the model fits the observed responses. Model R_A^2 values should not be confused with the values of the simple correlation coefficients (r) that were calculated to compare the relation between responses.

RESPONSE MEASUREMENTS

Breakpoint measurements of patty firmness were obtained by using an Instron Universal Testing Machine. The Instron was equipped with a large CCTM load cell to measure deformation force resulting when a 1.9 cm diameter probe was pushed into the patty surface at 2 cm/min crosshead speed. Patty thickness encountered by the plunger was 2 cm with a standard deformation distance of 1.8 cm beyond the surface. The resulting deformation pattern was recorded at 5 cm/min chart speed. Frozen patties were prepared for the Instron by immediately baking at 400^{0}F for 30 min. and then allowing the patties to cool at room temperature for at least 1 hour before testing. Three patties per treatment were tested for breakpoint response and 4 firmness readings were recorded per patty for a total of 12 response values per treatment. Increasing breakpoint values in g/cm^2 denoted increased patty firmness.

To form the sensory panel, eight judges were selected from twenty volunteers based on their ability to distinguish patty texture levels within the range of the treatments studied. A sequential analysis procedure was used to screen the prospective judges (Amerine et al., 1965). Each of the eight selected panelists were to evaluate the 54 treatments described earlier on a scale from 1 to 9 for firmness (1 = extremely softer than the reference to 9 = extremely firmer than the reference). A similar scale was used to rate flavor and overall acceptability as a combination of flavor and texture. Flavor and acceptability were rated on a 9 point scale (1 = extremely poorer than the reference to 9 = extremely better than the reference). All 54 treatments were evaluated 3 times by each panelist for each of the 3 sensory attributes scored per treatment. Four, one-quarter treatment patty slices were presented to each panelist per sitting along with one-quarter slice of a reference patty having a breakpoint value approximately equal to the grand average of all 54 treatment breakpoint values. An attempt was made to evenly balance the texture level of the 4 treatment patties presented per sitting based on prior breakpoint response.

COST

The cost of each of the patty treatments was taken as the weighted sum of each ingredient. Table 1 lists the prices of each ingredient figured into the cost. Price was based on a constant 23.33% level of batter and breading, 75% of this constant factor from batter and 25% from breading. The costs of dehydrated celery and onions were adjusted based on 188 and 120% rehydration, respectively, as determined through hydration studies.

COMPOSITION

Table 2 lists the proximate analysis of turbot and pollock as determined by standard methods (AOAC, 1980). Six replicate samples were tested from each component per fish species. Composition of other ingredients was provided by the manufacturer. The protein and fat content for each patty per treatment was based on the amount of the various ingredients found in

Table 1: Summary of the ingredient unit prices used to calculate the cost of all the minced fish patty treatment formulas.

Proteinaceous Ingredients	($/lb)	Additional Ingredients	($/lb)
aTurbot	$1.54	S. Alginate	$5.52
aPollock	1.05	Celery	1.35
SPC	.61	Onions	.75
bSF	.32	NaCl	.04
		NaTPP	.38
		Batter	.42
		Breading	.41

[a]The price of filleting leftovers for both fish species was calculated at $.30/lb.
[b]Hydrating soy flour at the level of 1 part soy flour to 1.65 parts water reduces this ingredients' cost to $.12/lb.

Table 2: Proximate analysis of the two fish species, turbot and pollock.

Fish	Moisture	Protein	Fat	Ash
Turbot	72.3 (0.3)*	14.7 (0.7)	12.8 (1.0)	1.1 (0.0)
Pollock	83.1 (0.7)	16.2 (0.5)	0.03 (0.1)	1.2 (0.0)

[a,b]Mean values in each column designated by the same letter were not significantly different as determined by the Duncan's Multiple Range test at the =0.01 level of significance.

*Values in parenthesis are standard deviation.

the specific raw patty formulas. Batter, breading and frying contributions were assumed constant over all treatments.

RESULTS AND DISCUSSION

The simple correlation coefficients (r's) between each of the specified responses are listed in Table 3. Breakpoint values and sensory panel firmness scores were highly correlated ($p<0.01$) suggesting that the objective measurement might be used with confidence as a predictor of the subjective response. The use of a screened panel may have contributed to such a high correlation. Deng and Tomaszewski (1980) found that an objective breaking force measurement was a useful tool in predicting sensory firmness scores in their experiment with minced croaker patties. Rockower et al. (1982) made similar conclusions in the evaluation of minced fish patties made from four fish species. Breakpoint values and firmness scores were negatively correlated with flavor scores indicating that as the patties became firmer, flavor scores in general declined. Thus, when selecting patty formulations to control firmness, the flavor of the patty could be adversely affected. Flavor and overall acceptability scores were highly correlated ($p<0.1$), while texture and overall acceptability measurements were not significantly correlated ($p<0.05$). This indicates that flavor serves as a better predictor of acceptability than does texture, however, this does not preclude texture from being a major factor in product acceptability. Since our objective texture response was not correlated with acceptability, breakpoint values cannot be used to predict acceptability. Thus, a sensory panel cannot be eliminated in the process of product development. Flavor and overall acceptability scores were positively correlated with percent fat and negatively correlated with percent protein. Since the main reason for protein and fat content variation in the patties was the fish component the fatty turbot was associated with increased flavor and acceptability.

Table 4 shows that regardless of soy protein and alginate levels, patties made from pollock are significantly firmer than those made from a turbot:pollock (1:1) blend or from turbot alone. Patties made from turbot yielded significantly higher flavor scores than those made from the turbot:pollock blend and these in turn drew significantly higher flavor and overall acceptability scores than patties made from pollock alone. There was no difference in the price of patties made from pieces of turbot and pollock; however if fish fillets are used as the initial raw material source, the cost of the patties increases as the ratio of turbot to pollock increases because tubot fillets are more expensive than pollock fillets. Patties prepared with pollock had significantly higher protein contents and lower fat contents than those formulated with the turbot:pollock blend and these in turn were characterized by significantly lower fat and higher protein percentages than patties made with turbot. The general trends show increased acceptability with increasing amounts of fat and these trends are associated with an increased turbot to pollock ratio. These results suggest that the addition of a stable fat to the formula of patties dominated by pollock might be a means of increasing the acceptability of patties composed of pollock. The amount of fat added should increase with increasing amounts of pollock in the blend.

Table 3--Correlations between the specific response variables determined for the set of 54 minced fish patty treatments prepared from various combinations and levels of turbot, pollock, soy flour, soy protein concentrate and sodium alginate.

Responses	Firmness Score	Flavor Score	Acceptability Score	Percent Protein	Percent Fat
Breakpoint Value	0.939**	-0.728*	-0.602	0.553	-0.530
Firmness Score		-0.824**	-0.683*	0.754*	-0.736*
Flavor Score			0.951**	-0.938**	0.931**
Acceptability Score				-0.887**	0.886**

*Significant at $\alpha = 0.05$. **Significant at $\alpha = 0.01$.

Table 4--The effects of fish combination and alginate level on the average specified responses for the set of 54 minced fish patty treatments prepared from various combinations and levels of turbot, pollock, soy flour, soy protein concentrate and sodium alginate.

Ingredient	Break-point (g/cm^2)	Firmness Score	Flavor Score	Accept-ability Score	Cost Using Pieces ($/lb)	Cost Using Fillets ($/lb)	Protein (%)	Fat (%)
Turbot	871[a]	4.21[a]	5.35[c]	4.98[b]	0.36[a]	1.09[c]	15.98[a]	9.83[c]
Pollock	1242[b]	5.88[b]	4.87[a]	4.54[a]	0.36[a]	0.80[a]	17.17[c]	0.30[a]
Turbot: Pollock (1:1)	946[a]	4.93[a]	5.19[b]	4.87[b]	0.36[a]	0.95[b]	16.57[b]	4.93[b]
Alginate Linear Trend	*	*	N.S.	N.S	*	*	*	N.S.

[abc]Mean values in the same column followed by the same letter are not significantly different as determined by the Duncan's Multiple Range test at the $\alpha = 0.05$ level of significane.

* Linear trend significant at $\alpha = 0.05$ level; N.S. - not significant.

Holding all variables constant except for the alginate level, there was a linear decrease in patty firmness as the alginate level increased from 0.2 to 0.4 percent (Table 4). Although patty cost increases and protein content decreases as the the alginate level increases from 0.2 to 0.4 percent, these changes are small and of no essential consequence. The use of alginate despite its expense was justified for two reasons. First, preliminary studies demonstrated that sodium alginate was required in the mixture to impart coherence to the patties. Second, alginate is needed to form a thin gelatinous film on the patty surfaces, via interaction with CaCl after extrusion. The gelatinous film would prevent patties from sticking to conveyor belts or other surfaces during processing.

Mathematical models were developed from the data to predict the magnitude of each response within the range of the ingredient levels studied. These models were used to evaluate the potential of a particular combination of pollock, turbot, soy flour, soy protein concentrate and sodium alginate based on the sensory, economic and compositional responses studied. Figure 1 was provided as an example to illustrate the rationale behind choosing a potential formula. This figure contains mixture response surface contour plots predicted with the models to reflect breakpoint values, overall acceptability and cost responses. In these examples ingredient variability was limited to combinations of turbot, soy flour and soy protein concentrate at the 0.2 percent alginate level. Other variables were not included in Figure 1 in order to limit the complexity of the illustration. The mathematical models used to generate these plots are as follows:

$$\text{Breakpoint value (g)} = 1716.9T + 4333.3B + 4600S$$

$$\text{Overall accept. score} = 5.125T + 4.375B + 4.833S + 1.833TB + 0.583TS + 2.75BS$$

$$\text{Cost (\$/lb)} = 0.349T + 0.31B + 0.417S$$

where T = (%Turbot -70)/30, B = %Soy Flour (Bontrae) /30 and S = %Soy Protein Concentrate/30 and for all combinations, T + B + S = 1. The R_A^2's for the breakpoint value and cost models were both greater than 0.85. An R_A^2 for the acceptability model could not be calculated because all degrees of freedom were used in fitting the model and none were left to compute an R_A^2. All ingredient combinations along the same contour lines are slanted toward the S vertex, the soy protein concentrate tends to have a greater firming effect than the soy flour. Breakpoints increase from the minimum value of 606 g/cm^2 for the 100% turbot and 0% soy protein mixture to the maximum value of 1622 g/cm^2 for the 70% turbot and 30% soy protein concentrate combination. Overall acceptability scores increase to a maximum as the turbot level decreases from 100 to 79%, soy flour component increases from 0 to 13% and soy protein concentrate decreases from 30 to 17%. The combination of ingredients that yielded the maximum overall acceptability score contained 80% turbot, 11% soy flour and 10% soy protein concentrate. A comparison of the firmness and acceptability plots showed that acceptability scores were maximized with increasing patty firmness subject to the condition that the increase in patty firmness came from an increase in the level of a nearly 1:1 ratio of soy flour to soy protein concentrate.

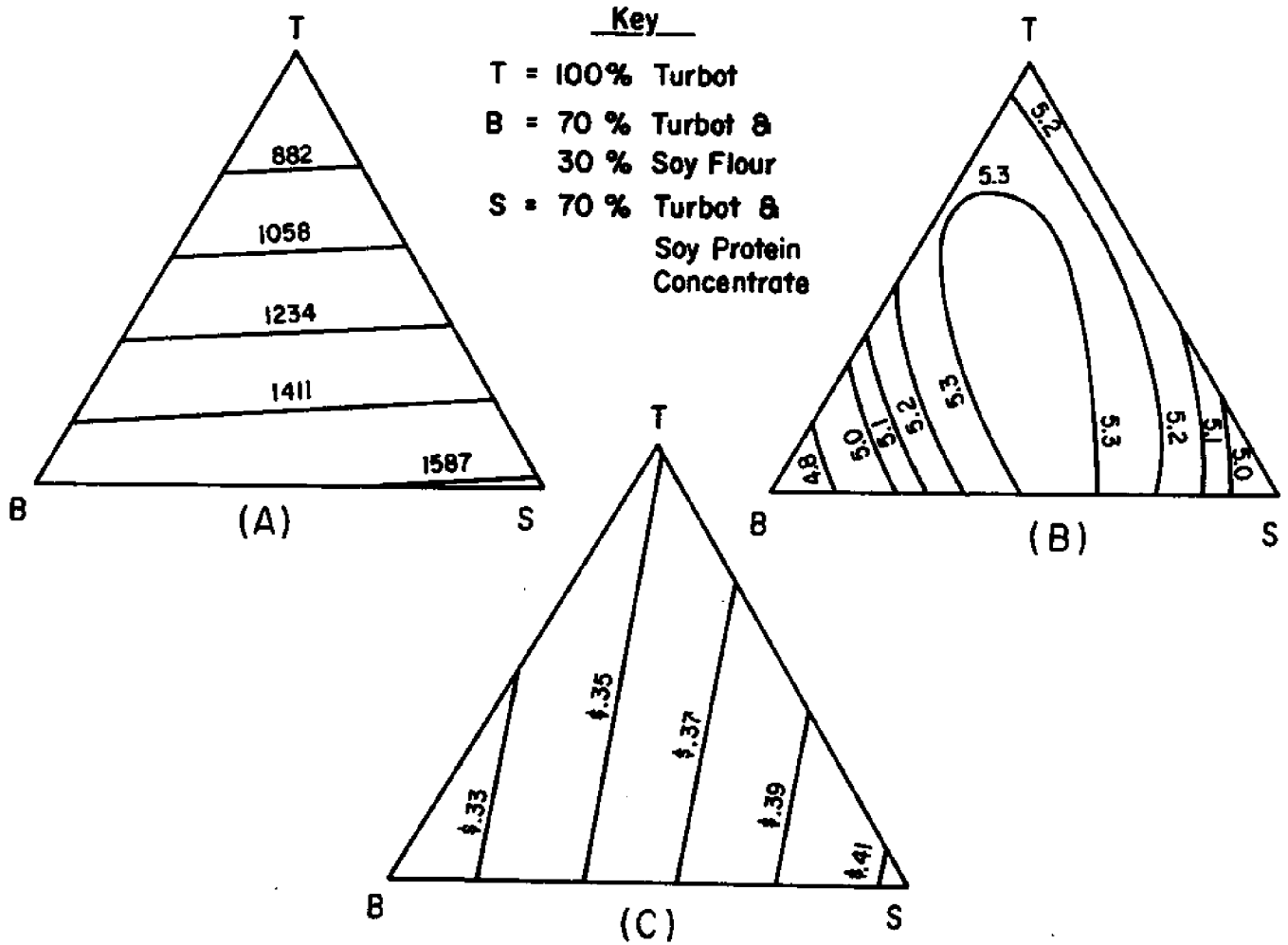

Fig. 1--Mixture response surface contour plots showing the (A) breakpoint response (g/cm^2), (B) sensory panel overall acceptability scores and (C) cost ($./lb) of minced fish patties made from various mixtures of turbot pieces, soy flour and soy protein concentrate at the 0.2% alginate level.

The cost plot indicated that the prices of the patties increased as the level of soy protein concentrate increased from 0 to 30% for patties made from turbot pieces. Turbot level did not affect cost. When our cost and acceptability plots were compared we found that both the least expensive combination (70% turbot and 30% soy flour) as $.31/lb and the most expensive combination (70% turbot and 30% soy protein concentrate) at $.42/lb were among the least acceptable. The most acceptable combination (70% turbot, 13% soy flour and 17% soy protein concentrate) was intermediate in cost (ca. $.36/lb). This example demonstrated that the most expensive product is not always the most acceptable. Discoveries such as this justify the expense and time expended to do product development research similar to this study.

NOTE

This preliminary paper will be presented in complete form, to include the specific model parameters, at the Annual Institute of Food Technologist meeting in Las Vegas, Nevada, June 1982, and subsequently should be published in the Journal of Food Science. This work was supported with funds from the National Fisheries Instititute, Washington, D.C., and the University of Florida's Institute of Food and Agricultural Sciences.

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SUITABILITY OF SEAFOODS TO RETORT POUCH PROCESSING

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INTRODUCTION

Current research and commercial efforts for retort pouch utilization in the United States are centered around two sizes of retort pouches. One market is the retail area where a six to twelve ounce pouch is positioned against frozen entree items. A large segment of the U.S. processing industry feels that an additional market may result from the development of the institutional-size retort pouch (Badenhop and Milleville, 1980). The larger retort pouch has distinct quality and economic advantages over existing No. 10 can products. Regardless of size, the flavor and nutritive quality of a pouched product is retained due to its decreased heat penetration distance to the cold spot of the container, which results in shorter cook times and less peripheral over-heating. Minimal brining levels are necessary in the pouches resulting in decreased ingredient and transportation costs (Badenhop and Milleville, 1980) and less flavor dilution (Andres, 1979).

The development of a retort pouch operation for seafoods could minimize the impact of rising utility and transportation costs in their marketing of energy intensive frozen or refrigerated products (Olabode et al., 1977). Concurrently, the establishment of a 200-mile fishing limit by the Federal Fisheries Conservation Act of 1976, offers an enormous potential for exportation of seafood items to countries which had previously harvested these items from U.S. waters. This Act gave the United States fisheries control of over 20 percent of the world's seafood supply. A major portion of this new supply can be directed to large seafood markets in Japan and Europe. The advantage of the retort pouch and the emerging development of packaging materials and equipment represent a major opportunity to tap this export market. Also, these cultures have demonstrated a growing interest in "non-traditional" seafood species (i.e., eels, conch, squid, various fish species, etc.) as specialty products which draw a premium return. These economics could certainly accelerate the commercialization of the pouch in this area.

Interest in the retort pouching of seafoods has been reflected by various operations throughout the United States. In Anacortes, Washington, Speciality Seafoods has obtained a pouch processing patent and is currently marketing smoked salmon and oysters in retort pouches. This company has expressed an interest in producing dungeness crab products (Anon., 1978). Likewise, discussions with shrimp and crab companies in the southeast have stirred regional interest.

It is recognized that seafoods processed in retort pouches cannot match the quality of fresh items. It is believed, however, that there are some seafood items when pouched can match or exceed the quality of frozen

items which are subsequently fully cooked. Certainly, the retort pouch can provide the processed product superior to current canned items. The institutional sized pouch can provide a product which provides convenience, ambient storage, precooked state and high flavor attributes to a mass feeding operation. The institutional customer may not be seeking replacement items for common entrees, but it could be looking for quality seafood products ready for combination with sauces or soup stocks, or for use in the growing salad bar and buffet operations.

The purpose of this project was to test the suitability of three classes of seafoods to retort pouch processing (fish, shrimp, crab meat). From this initial study it is believed that one or two seafood product concepts will show exceptional promise from both a functional and economic viewpoint to justify further work.

PROCEDURE

A series of experiments were designed to compare the products from canned and institutional-sized retort pouch processes. Small cans (307 x 113) were used in the experiment. It was recognized that this size can holds only 7 oz. and was not the normal size for most products, but it was used to simplify procurement requirements and to provide a container which would heat at a rate close to that of the pouches. A reasonable can product composition was assumed in accordance with 21CFR part 161.170 and/or 161.190 for fish, and 21CFR part 161.173 for shrimp. The crab meat was packed without liners to 6 1/2 ounce fill as recommended by the NFPA. The cans were processed according to Bulletin 26-L of the National Food Processors Association (NCA-1976).

The pouched products were packed in the spirit of the canning regulations with exceptions being in the fill of container sections. Initial experiments with pouched products were a series of 3 or 4 levels of duplicate pouches at varying brining levels. The chosen ratio of product to brine levels was used for subsequent heat penetration experiments. Three trials of 4 pouches for each product were used to estimate heat penetration characteristics of a product concept. Various levels of processing aids were intermixed with heat penetration runs. This interfacing of experiments was based on the assumption that low levels of additives would not affect heat penetration characteristics.

The pouch retort was an experimental model constructed specifically for the processing of institutional-sized retort pouches under controlled and measurable flow conditions of pressurized water or steam-air over the racking system. A Digistrip II (Kaye Instruments) data logger recorded entrance and exit rack temperatures, internal temperatures of pouches, and the differential pressure outputs of the two orifice flowmeters.

Nine inch (5 lb. pouches) and six inch (2 1/2 lb. pouches) 1/8" O.D. needle thermocouples (O.F. Ecklund Custom Thermocouples, Cape Coral, FL) were supported at the geometric center of pouches by two thick rubber disks. The thermocouples were inserted into the thickest part of shrimp segments. Because of problems with movement of thermocouples while processing of shrimp, these pouches had 2 thermocouples per pouch. All pouches were processed at 250°F with pressurized water (25 psig) at a flow rate of 110 gpm (Reynolds number ≈ 3300 between racks). The pouch heating profile

of 1 inch was maintained in racks having 3/8" holes on 9/16" staggered centers (40% open area). Eight pouches were processed simultaneously. Retort come-up times during heating ranged from 4 to 7 minutes, because the retort was not pre-heated. This extended come-up was reasonably close to commercial operations. Canned products were processed in a No. 3 Dixie still retort using steam at 250°F. Data analysis was completed by using standard graphic techniques and statistical programs using an Amdahl 470 computer at the Northeast Regional Computer Center located on the U.F. Campus. Approximate process times (B values) were calculated by methods and from tables in Stumbo (1973) using an initial temperature of 50°F, retort temperature=250°F, Z=18°F and F_o=6 minutes. Table 1 lists the pouched seafoods and associated water used for process time determinations. Post processing drained weights were measured on cans and pouches.

Single pouches of each processing aid trial were stored at room temperature for up to 6 months to evaluate effectiveness of additives in color and texture retention. Subjective evaluations (flavor, texture, and color) of the products were conducted to rapidly optimize ingredients and processing conditions used to produce a superior product. Evaluations were limited to a five member panel using a simple hedonic scale. Approved processing aids were used to correct problems found during evaluation sessions. Samples stored under ambient temperatures were not tasted, but none of the 40 pouches stored at ambient exhibited signs of bacterial spoilage.

RESULTS AND DISCUSSION

Flake Tuna

Fresh deheaded and eviscerated yellow fin tuna were steamed for one half to one hour at 215-220°F to firm the flesh and facilitate deboning. The cooled flesh was scraped clean of dark meat and flaked. The flakes (2270g) were processed in 12 x 16 x 1" pouches with 1, 30, and 100g of brine containing 0.5% salt (% of flake weight). Duplicate pouches were processed at 250°F. Because of insignificant differences between pouches having different brine levels, the additional heat penetration trials of 12 pouches were conducted with the arbitrarily chosen 30g brine level. The drained weights for tuna averaged 83% (s=7.8, n=18). The calculated B values would suggest a process time on the order of 44 minutes. This time was less than the recommended process times of 55 minutes for 307 x 113 cans (6 1/2 oz) and of 190 minutes for 603 x 408 cans (4 lbs, 2 1/2 oz). The can sample of tuna consisted of 155g of tuna, 65g of water and 0.5% salt. The cans were processed 55 minutes at 250°F.

The flake tuna was evaluated 2 weeks and 2 months after processing. Both products had excellent color, although the color of the pouched product was rated as slightly lighter than the canned product. The addition of citric acid seemed to lighten the color slightly. The odor of both pouched and canned tuna was also rated excellent. This odor was mild and reminiscent of a typical tuna product. The texture of the pouched product was slightly better than the canned product, which seemed a bit tougher. The flavor of the pouched product was that of a typical clean, distinctive canned tuna. The canned product seemed less distinctive and slightly oxidized. In general, the pouched tuna product was slightly better than the canned product and would seem to have excellent potential for commercialization.

TABLE 1. POUCHED PRODUCT SUMMARY

SEAFOOD	FILL WEIGHT (g)	WATER ADDED (g)	ADDITIVE LEVEL (% of Seafood)	APPROXIMATE PROCESS TIME (min @ 250°F)
SHRIMP				
120-130 Count	1135	70	0.5 Salt 0.2 Citric Acid	40
30-40 Count	1135	70	0.5 Salt 0.2 Citric Acid	34
Lean Fish (RED SNAPPER)	2270	100	0.5 Salt 0.2 Citric Acid	44
High Fat Fish (SPANISH MACKEREL)	2270	100	0.5 Salt 0.2 Citric Acid	46
FLAKE TUNA	2270	30	0.5 Salt 0.2 Citric Acid	44
BLUE CRAB				
Body Meat	1135	200	0.5 Salt 0.2 Citric Acid 275ppm EDTA	39
Claw Meat	1135	200	0.5 Salt 0.2 Citric Acid 275ppm EDTA	40

Shrimp

Fresh shelled and deveined 80-count shrimp were blanched in two batches in boiling 5.0% salt brine for two minutes. Weight losses for these treatments were 39 and 43%. Blanched shrimp (1135g) were placed in 9 1/2 x 13 x 1" pouches and 28, 70 or 140g of 3.0% salt brine was added. The heat penetration data obtained suggested some decrease in process time (B value) with increasing brine levels, but this consideration was not as important as the excessive fluiditity of 140g brine level pouch and the severe clumping of shrimp in the 28g brine level pouch. Thus, for further experiments the 70g brine level was chosen as a compromise.

Additional fresh peeled and deveined shrimp of 20-30 and 60-80 count were pouched. The blanching process of 11 to 15.5 kg/batch resulted in weight losses of 39, 39 and 33% for the 60-80 count shrimp. The 20-30 count shrimp had weight losses of 30 to 26% for batches of 16.5 and 19 kg/batch. After blanching the effective canning count was 120-130 shrimp/lb and 30-40 shrimp/lb, respectively.

The small shrimp were processed with citric acid levels of 0.1, 0.2, 0.5, and 1.0% of shrimp weight. The citric acid was not observed to have any beneficial effect above the 0.2% level and the increased acid taste was evident at the 0.5 and 1.0% level. Therefore, subsequent trials were conducted at 0.2% levels. After processing the small shrimp had average drained weights 86% (s=6.9, n=17) of their blanched fill weight (976 out of 1135g), while the large shrimp had average drained weights 78% (s=7.1, n=11) of their blanched fill weight (885 out of 1135g). Thus, the total weight yield of shrimp from fresh to canned drain weight was 54% and 56% for the small and large shrimp, respectively. The calculated B values for the small and large shrimp would suggest process times on the order of 40 to 35 minutes, respectively. The reasons for this difference in process times between shrimp sizes was not apparent. Those process times are longer than the 19 minute process for 502 x 510 wet pack shrimp (2 1/2 lbs) but shorter than the 55 minute process for 307 x 400 dry pack shrimp (10.5 oz). In essence, the pouched shrimp formulations are mid-way between the canned wet-pack and dry-pack shrimp.

The canned shrimp (307 x 113) consisted of a 130g shrimp fill with 90g brine of 0.5% salt and 0.27% citric acid. The cans were processed at 250°F for 14 minutes. The products were evaluated within 5 days of processing and after 2 months of ambient storage. The pouched shrimp products were compared to experimental canned and commercial canned shrimp. The pouched shrimp were basically equivalent in quality to those canned in these experiments and superior to the commercial canned product. This difference between the commercial and experimental canned was probably negligible since the experimental product was not exposed to the environment history of the commercial pack. The evaluation of the pouched shrimp products revealed very little difference between small and large shrimp, so they are discussed subsequently without any distinction between the two sizes.

The color of pouched shrimp was judged outstanding and slightly superior to the canned products. The pouched products had white flesh

with distinctive red skins. The canned products were slightly greyer in the flesh. The addition of citric acid definitely decreased the greying of the flesh, while the added EDTA had no observable effect. A defect of the pouched shrimp was a sulfury or hydrogen sulfide odor present when the pouch was first opened. This odor dissipated rapidly if the product was soaked in water (a practice recommended on commercial canned shrimp). The addition of citric acid noticeably reduced this odor problem. The canned shrimp were noticeable sulfury in odor, but they also possessed a metalic or briny odor which the pouches did not have. The texture of the pouched shrimp was not as firm or dry as the canned product which may be due to not using optional blanching conditioning. The flavor of pouched shrimp was superior to the canned products because they possessed a definite shrimp-like characteristic. The canned products were bland and lacked any distinctive shrimp flavor.

Blue Crab Body and Claw Meat

Picked blue crab body meat (1135g) was processed in 9 1/2 x 13 x 1" pouches with 30 and 100g brine, and in 10 x 13 x 1" pouches with 200 and 300g brine. All pouches contained 0.5% salt based on meat weight. This product produced a dilemma in whether to use a brine level based on organoleptic or abuse resistance judgements. The two high brine levels produced pouches sufficiently fluid in nature to question their resistance to the rigors of transportation without additional carton cushioning. Additionally, at low brine levels, the meat maintained its lump character. The two pouches with low brine levels were greyer in color, lacked distinct "crab" flavor, and had higher perceived levels of cooked flavor. Because the observed B values did not demonstrate any decreasing trend with increasing brine levels, there appeared to be no advantage to the high brine levels from a heat transfer viewpoint. However, the loss in the apparently delicate crab flavor was considered sufficiently important to justify the 200g brine level for future work.

Additional crab body meat was processed with varying levels of citric acid (0.0, 0.2, 0.5, 1.0% of meat weight) and 275ppm EDTA added to the pouches. The average drained weight for these pouches was 102% (s=6.0%, n=16). The calculated B values for blue crab body meat suggested a process time on the order of 39 minutes. This process time is comparable to a 307 x 113 can of blue crab which requires 37 minutes at 250°F.

Blue crab claw meat was processed with 200g of water and 0.5% salt based on meat weight added to each pouch. Citric acid (0.1 or 0.2%) and 275ppm EDTA was added to some pouches. The drained weights for claw meat averaged 103% (s=2.2%, n=12). The calculated B values for claw meat would suggest a process time on the order of 40 minutes.

The pouched blue crab products were evaluated the day after processing and after 5 and 6 months (body meat) and 4 months (claw meat) of ambient storage. The color of the pouched crab body and claw meat was very dependent on additive levels used. Without the addition of citric acid and/or EDTA the crab meat would turn blue in spots and grey overall. By increasing the citric acid level from 0.0 to 1.0% the meat changed from a grey background to a yellow off-white. EDTA aided in preventing blue coloration, but did

not seem to effect the overall greying. Stored pouches containing EDTA alone, 0.2% citric acid alone, and EDTA plus 0.1% citric acid demonstrated objectionable blue spots and greying formation. However, the combination of EDTA and 0.2% citric acid had no blue spot formation and little background greying. Thus, there seemed to be a synergistic effect with the combination of EDTA and citric acid. But, the pouch containing body meat and salt only, and stored 6 months did not have any bluing problems. This pouch was rated the best pouch for color overall. Therefore, as some literature has stated, this bluing phenomenon is a transient problem which must be constantly monitored. The color of the crab claw meat was darker than the body meat due to the blue skin layers on claw meat.

The odor of the pouches was rated mild to moderate in strength depending on the brine and additive levels. The pouches with the two lower brine levels had stronger cooked seafood odors. The 200g level had only slightly perceptable levels of this cooked odor. The sulfury seafood odor was evident with all of the crab products but citric acid definitely reduced this sulfury odor. A level of 0.2% citric acid produced a product where this odor was not objectionable. Citric acid levels above 0.5% produced an acidic odor which was objectionable. The canned products were similiar to the pouched ones except that all but one canned product had an additional ammonia odor which was objectionable. The can with citric acid and EDTA was not objectionable. The pouches and cans were processed on the same day, so that the age of the product would not explain this difference.

The texture of the body meat was superior to the canned products. The lump character of body meat was maintained to a greater degree with decreasing brine levels. Citric acid at the 0.2 and 0.5% levels seemed to make the body meat firmer and juicy. The 1.0% citric acid level made the body meat stringy and chewy. The body meat in cans was shredded leaving little or no lump character. The claw meat, because of its characteristic short meat fibers, did not maintain its lump character in either pouches or cans.

The flavor of the pouched products was superior to the canned products if sufficient brine was added to pouches. The pouched products retained some of their fresh crab-like flavor which was totally absent in the canned products. With the 30 and 100g brine levels the crab possessed an unpleasant cooked flavor which the cans did not have. However, with the 200g brine level this flavor was not present. The addition of 0.2% citric acid was sufficient to minimize the sulfury flavor present in pouches and cans.

Lean Fish (Red Snapper)

Fresh red snapper fillets (375g/ave. fillet) were laid 2 deep in 12 x 16 x 1" pouches with a single thermocouple positioned between fillets at the center of the pouch. Five grams of salt were sprinkled on top of fillets and 0, 30, 100 or 200g of water was added. Duplicate pouches were processed at 250°F. The 200g brine level produced a pouch with excessive fluidity, while the 0 and 30g levels seemed to compress the fillets and increase their adherrance to each other. Therefore, the 100g brine level was chosen for further experiments. Additional fillets (275g/ave. fillet) were processed in 12 x 16 x 1" pouches with 100g brine (0.5% salt

of fillet weight). Also, some pouches had 0.2% citric acid added. The drained weights for red snapper averaged 74% (s=3.4%, n=20). The B values calculated from heat penetration data suggest a process time on the order of 44 minutes. The only comparison of this pouch process with existing can operations is with canned flakes made from cod or haddock. A typical process for a 300 x 407 can (425g) of fish flakes is 75 minutes at 250°F.

The products were evaluated the day following processing and after 3 and 4 1/2 months of ambient storage. The color of the fillet flesh was off-white and not perceptibly different from non-processed flesh. The odor of the fillets was bland and mild in strength with a slight cooked fish odor. Citric acid in the brine seemed to "clean-up" the odor, but because the odor level was so low, its addition was considered optional. The texture of the fillets varied from a dry flakiness to a softer, sticky cohesiveness with increasing brine levels. The addition of citric acid produced a firmer fillet. When the fillets were packed with skin sides against each other, they were easily separated after processing. The processed fillets possessed a bland and clean cooked fish flavor which was free of fishy or oily notes. It was an excellent fully-cooked fish product.

High Fat Fish (Spanish Mackerel)

Frozen spanish mackerel fillets were thawed and layered 3 high in a 12 x 16 x 1" pouch with a thermocouple positioned above or below the middle fillet. Two percent salt of fillet weight was spread over the surface of the fillets and 0, 28, 140, or 280g of water was added to a pouch. Duplicate pouches were processed at 250°F. After processing, pouches containing 200g of brine demonstrated excessive fluidity and the 0 and 28g brine levels produced fillets considered too firm and dry. Therefore, 100g of brine was chosen for future work.

Additional frozen fillets (approx. 150 grams each) were processed with 100g of water and salt (0.5% of fillet weight) added to all pouches, citric acid (0.2% of fillet weight) was added to some pouches. The drained weights for mackerel average 102% (s=19.3, n=8). The B values calculated from heat penetration data would suggest a process time on the order of 46 minutes. This projected process time is close to the recommended process of 50 minutes at 250°F for mackerel fillets in a 1 pound oval can.

For the can samples, mackerel cubes (155g, 1/2") were placed in a 307 x 113 can, filled with 45g water, 0.5% salt and 0.2% citric acid (% of cube weight), exhausted and sealed. The cans were processed for 47 minutes at 250°F.

The products were evaluated after processing and 3 months ambient storage. The color of the fillet flesh for the pouched products was white to a yellow white, while the canned mackerel had considerable surface browning and some internal browning of the flesh. The pouched mackerel fillets was an attractive product. The odor of the pouched fillets was clean but strong and typical of a canned fish product. The canned mackerel cubes had a stronger odor than the pouched product and possessed a fishy and oily off-note.

The texture of the pouched fillets was firmer and more cohesive than the red snapper fillets. The fillets exhibited an excellent flake character. The canned cubes were firmer than the fillets, lacked flake character and tended to give a grainy mouth-feel.

The flavor of the pouched fillets was considered to be a mild cooked fish one which possessed a clean and distinctive character. The addition of citric acid seemed to aid in providing the clean distinctive flavor. For consumers who enjoy mackerel, this product was considered to have a very good flavor. The canned cubes were oily and had a fishy flavor which lacked any character.

CONCLUSIONS

All of the products processed in pouches were as good as or superior to canned products. The most obvious advantage to pouching these products was the greater flavor retention obtained compared to cans. All of the products had similiar or lesser process times than comparable canned products. It was judged that the tuna, shrimp and blue crab products would have few obstacles to their commercialization. Some further work would be necessary with the shrimp products, however, to improve their firmness. The fish fillet products did present problems with fillet integrity and adherance to each other. Because all of these products demonstrated good flavor retention, it seems quite plausible that their formulation with butter, lemon oils and other flavorings and sauces would demonstrate similar flavor retention. A couple of preliminary pouches processed with added butter and lemon oil produced excellent products.

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A COMPARISON OF THE EDIBILITY CHARACTERISTICS AND CHEMICAL COMPOSITION OF SIXTEEN SPECIES OF SOUTHEASTERN FINFISH

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INTRODUCTION

Because of high and rising food and fuel prices, there is a critical need to use nontraditional fish species to the greatest advantage. Many species that surely have desirable edibility characteristics, if not discarded at sea, are left in the market or on the dock, simply because of the consumer's confusion and lack of knowledge about them. These species should be classified into groups depending on their potential usefulness and similarities in edibility characteristics (and possibly chemical composition), so that processors can consider product options and consumers can choose according to their taste preferences and nutritional values, rather than buying only what is well-known and often more expensive.

Recognizing the need for an improved market nomenclature system, in 1977 the National Marine Fisheries Service (NMFS) contracted the Brand Group, Inc., a Chicago-based marketing firm, to develop a Model Identification Plan, using consumers' attitudes toward seafood. This plan (3) identified eight attributes as important in grouping fish species: odor, flakiness, coarseness, flavor, moisture, fat, firmness and color. The Natick Laboratory (U. S. Army) expanded on this study in a subsequent NMFS contract. They identified 13 sensory factors they felt provided maximum discrimination and also investigated instrumental methods to correlate with sensory measurements of edibility characteristics (8).

Anticipating the findings of the Natick study, Chapman et al. (4) used a taste panel trained in flavor, texture and appearance evaluation principles to describe the edibility characteristics of 23 underutilized southeast species, based on the approach of the Brand Group. This panel found coarseness to be rather vague in relating to finfish; fat content, which can be measured by chemical analysis, was not clearly perceived as an edibility characteristic. Therefore, these two attributes were omitted and the other factors were evaluated on a 5-point rating scale (1=least intensity, 5=greatest intensity). The Chapman preliminary study showed that the southeast foodfish species evaluated lend themselves to such a classification procedure.

The purposes of the present study were to extend the observations of our Laboratory to other southeastern species, refine sensory techniques and, more specifically, broaden the study to include several chemical tests in the profiling, using two statistical tests to aid in the categorization (grouping) process. In this study, the methodology was modified in order to construct a simpler yet relevant experimental procedure. Samples were cooked in

a microwave oven, rather than using boiling bags in boiling water, because the microwave method consumed less materials and time. In addition, samples could be cooked immediately when a panelist arrived, thereby eliminating changes caused by holding the cooked samples at warm temperatures. Odor was deleted as a characteristic because taste panel ratings of odor were shown to be highly variable in our previous study (4) and presence of odor of seafoods is, generally, a negative factor. Analysis of the earlier data required the use of non-parametric statistics, due to the use of discrete numbers in the scale. For this study a new continuous line rating scale was implemented that allows the use of standard statistical techniques, since a normal distribution of ratings can be assumed.

Each species was evaluated by the taste panel on at least three separate occasions to achieve greater statistical reliability. Sixteen species, representing four ecological groups (Fig. 1), were evaluated and classified using cluster analysis and multidimensional scaling. The species were also grouped by cluster analysis on the basis of (a) proximate chemical composition and (b) fatty acid profiles, and the results were compared with edibility characteristics groupings.

MATERIALS AND METHODS

Fresh fish of designated species were purchased from local seafood markets, usually on the day of taste panel tests. Fresh fish were also obtained on occasion from survey cruises of the South Carolina Wildlife and Marine Resources Department. Fish were judged fresh by redness of gills, clearness of eyes and firmness of flesh. After purchase, fish were measured and weighed, then headed, gutted and filleted. Samples of approximately 100 grams were taken from the fillets for proximate and fatty acid analyses. Remaining fillets were cut into 3 cm x 5 cm portions, each weighing approximately 20 grams. Care was taken to obtain samples from a similar part of the fish, i.e., the mid-section, with no tail portion included. Samples were placed in glass Petri dishes, marked with randomized 3-digit codes, covered and stored at 5°C until the taste panel began. Three or more taste panel evaluations of each species were performed over a one year period.

The proximate analyses, performed on at least one sample of each species, included protein, moisture and ash determinations according to AOAC procedures (2). Fat analysis, also performed on one or more samples of each species, was by the method of Smith et al.(6). Fatty acids were analyzed using a Hewlett Packard 5830A gas chromatograph equipped with packed columns (DEGS) and flame ionization detector, and fatty acid peak identifications were based on the method of Ackman and Burgher (1).

The taste panel consisted of 11 laboratory employees who had been trained in sensory evaluation of seafood (4). Panel members were briefed as to the purpose and nature of the study and the same panel was used throughout the testing.

On arrival of the taste panel, samples were taken from the refrigerator and the lids removed; the samples were placed in a Sharp[1/] model R-9600 microwave oven and covered with paper toweling to prevent splattering. Samples 1 cm and less were cooked 40 seconds on full power; samples thicker than

1/ The use of trade names does not imply endorsement by National Marine Fisheries Service, NOAA.

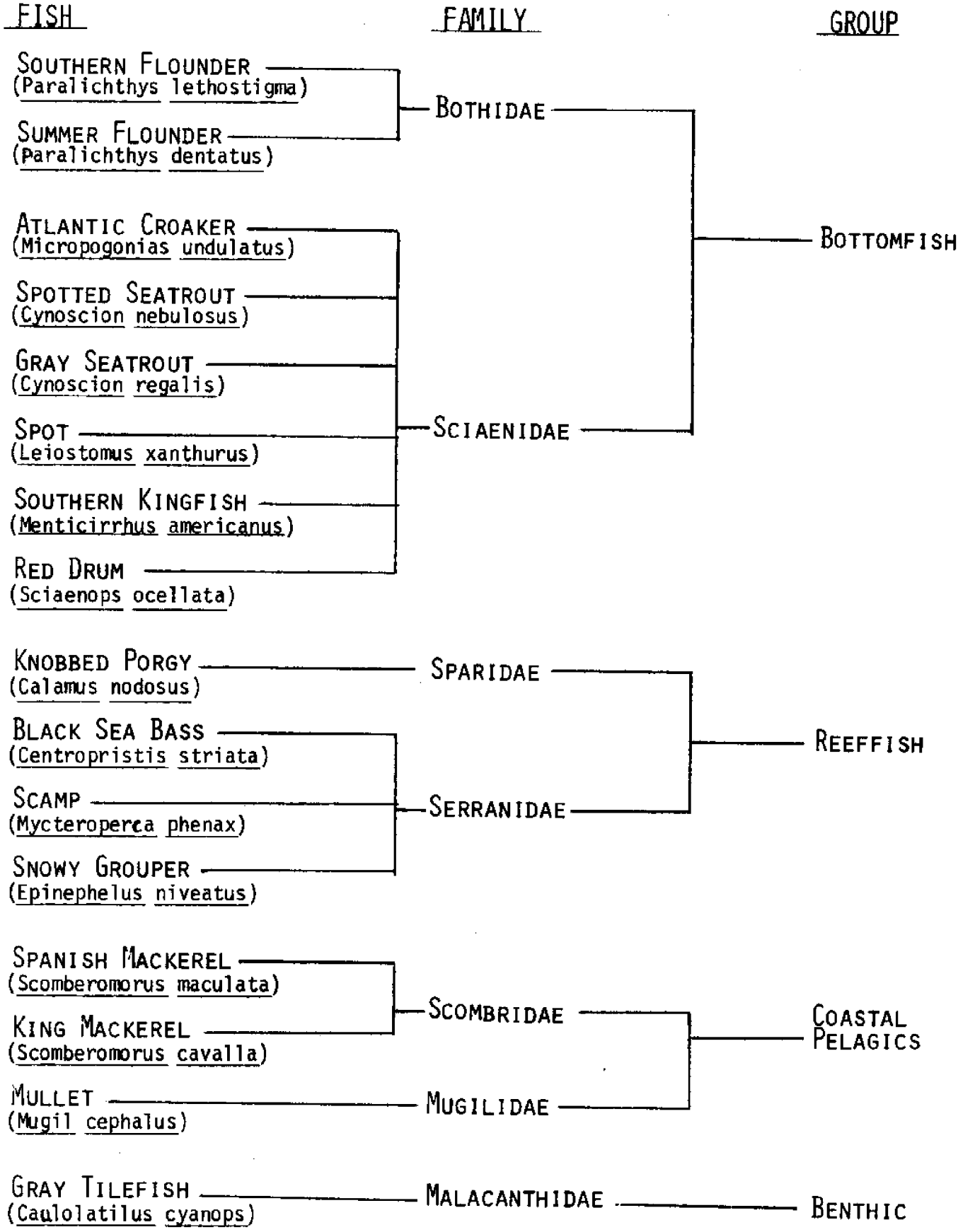

Figure 1. Family and ecological grouping of 16 southern species.

1 cm were cooked 50 seconds. Final internal temperature was approximately 70°C. Lids were replaced and samples passed to panelists in taste panel booths for evaluation.

The rating scale is illustrated in Figure 2, with characteristics listed in the order of evaluation. Panelists were requested to place a vertical slash across each line in a position reflecting the intensity of each characteristic.

Both the BMDP2M computer program, for cluster analysis of cases and the KYST-2A program, for multidimensional scaling, were employed to analyze the taste panel data and to group the species according to similarities in edibility characteristics (5). While an analysis of variance would be useful to compare different species by individual edibility characteristics, the complexity of five characteristics and 16 species is better represented and more readily understood through the grouping methods employed here. Before grouping was done, the average characteristics for the 16 species were standardized; then the dissimilarities between species were computed by taking the Euclidean distance between the two species, using the standardized average characteristics as the coordinates. A criteria for two species to belong to the same group is that the dissimilarity measure between the species is small.

RESULTS AND DISCUSSION

Edibility Characteristics

A continuous line rating scale was felt to be more statistically reliable for descriptive analysis tests of this kind (Fig. 2). Panelists may feel apprehensive about using numbers at either extreme of a rating scale. It also has been theorized that panelists may have a preference for some integers over others (7). The continuous line scale eliminates this problem by presenting a 200 mm line with lowest intensity on the left and highest intensity on the right, with no numbers listed. Panelists seem to be more willing to make judgements on the entire scale when only a vertical mark is required. In addition, there is a better justification for assuming a normal distribution of the data. Many statistical analytical techniques are only valid for data that can be assumed to be normally distributed.

From the results of the cluster analysis (Fig. 3) and the multidimensional scaling (Fig. 4), judgement was employed to define the four logical groupings. Group A consisted of black sea bass, snowy grouper, summer flounder and scamp. Group A was light in color, not flakey, ranged from scamp (very soft) to snowy grouper (firm) in texture, had weak flavor intensity and was medium moist. Group B consisted of southern kingfish, red drum, Atlantic croaker, gray seatrout (weakfish) and spotted seatrout. This group had medium color, was moderately flakey to not flakey, medium to soft in texture, moderate in flavor intensity and moist. Group C consisted of knobbed porgy, gray tilefish and southern flounder. This group was medium to light in color, medium to very flakey, firm, ranged from weak flavor (southern flounder) to strong (knobbed porgy) and was medium moist to dry. Group D consisted of Spanish mackerel, king mackerel, striped mullet and spot. This group was dark in color, moderate to very flakey (Spanish mackerel), medium to very firm, very strong flavor intensity and dry.

DATE _____

INITIALS _____

SAMPLE _____

SENSORY PROFILE OF FISH FLESH AFTER COOKING

Place a vertical slash on the line to represent the intensity to which the sample corresponds to the listed characteristics.

COLOR

LIGHT ——————————————————————————————— DARK

FLAKINESS

HARD TO SEPARATE ——————————————————————————————— EASY TO SEPARATE

FIRMNESS

SOFT ——————————————————————————————— FIRM

FLAVOR

BLAND ——————————————————————————————— STRONG

MOISTURE

DRY ——————————————————————————————— MOIST

Figure 2. Rating scale.

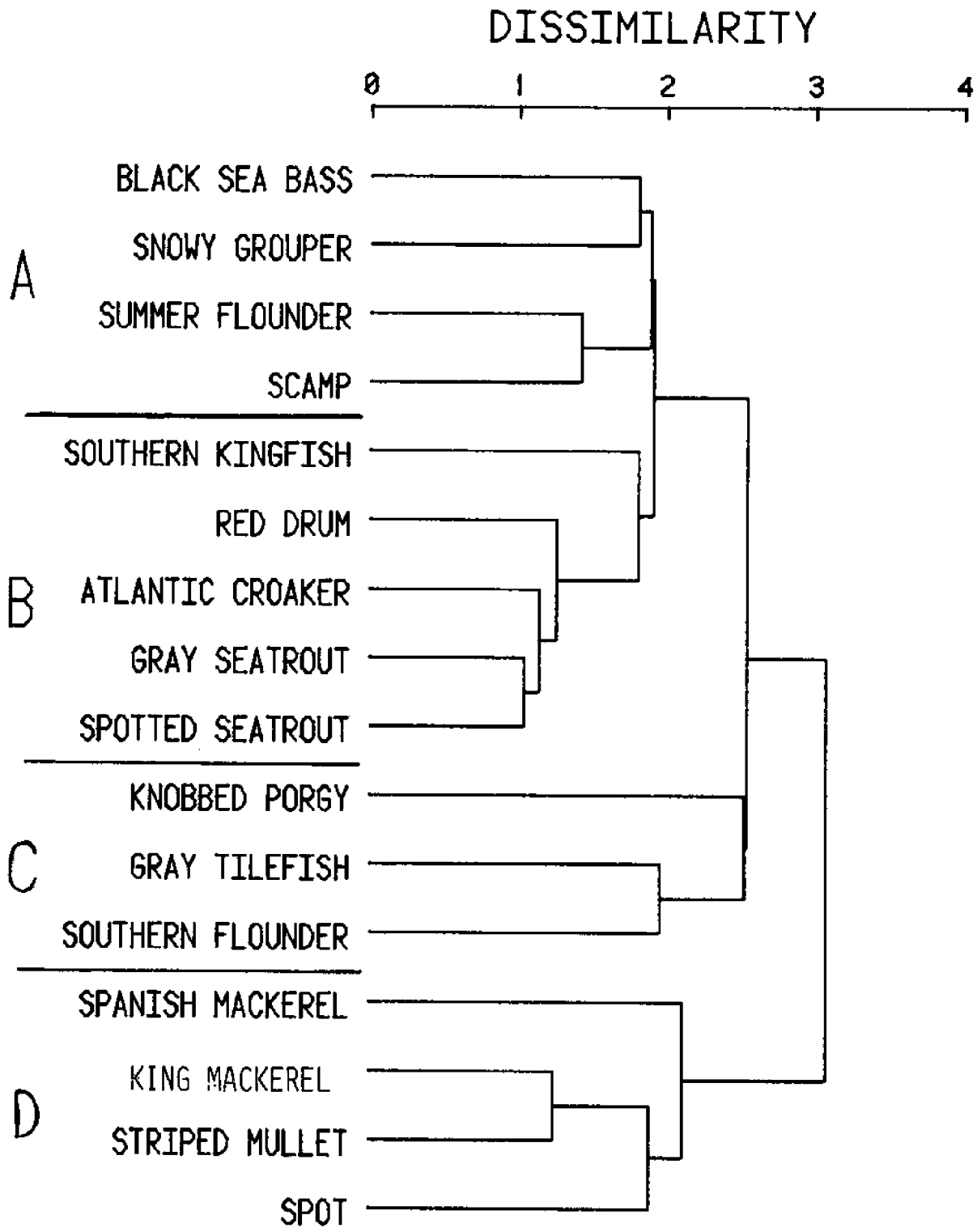

Figure 3. Cluster analysis of edibility characteristics.

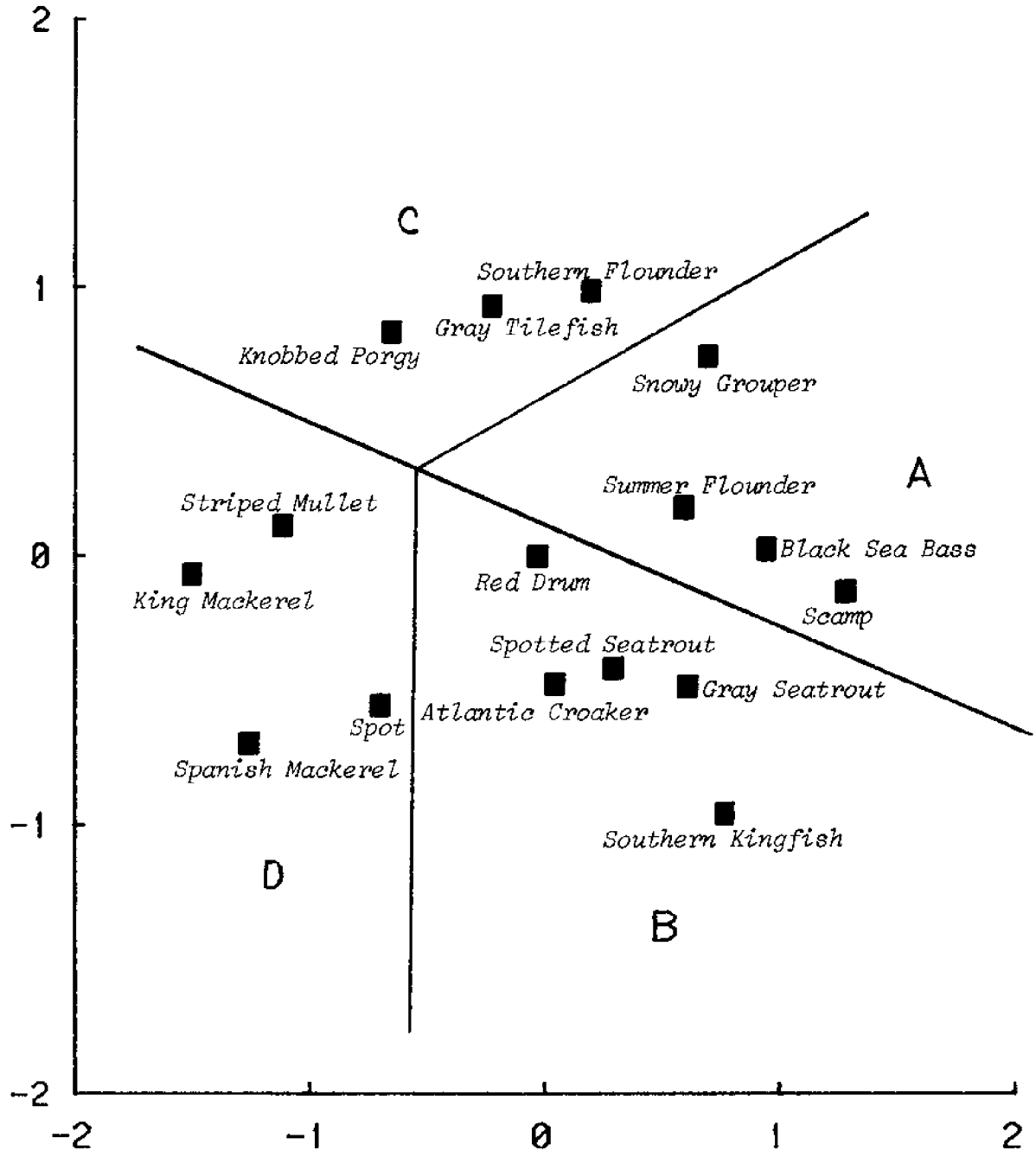

Figure 4. Multidimensional scaling of edibility characteristics.

Proximate Analyses

Proximate data were evaluated using cluster analysis of cases. This analysis divided the 16 species into four groups (Fig. 5). A summary of proximate data is listed in Table 1. Group A consisted of spot and mullet, which were very similar in having relatively low moisture and ash, higher fat and moderate protein content. Group B consisted of knobbed porgy, scamp, spotted seatrout, red drum, king mackerel and southern flounder, which were similar in having high protein and ash, moderate moisture and fat contents. The third group (C), consisting of gray seatrout, croaker, Spanish mackerel, gray tilefish, black sea bass, snowy grouper and summer flounder, was characterized by relatively low protein and fat and high moisture and moderate ash. The Spanish mackerel had a low fat content because the fish that were available fresh (locally) were small, averaging only about 0.6 lb each. The fourth group (D) was composed solely of southern kingfish, although it was similar to group A, having low protein, moisture and ash, with the highest fat content of all species.

Fatty Acid Profile

Seven of the more biochemically important fatty acids were picked for evaluation by cluster analysis. These were 14:0, 16:0, 18:0, 16:1, 18.1, 20:5 and 22:6. (See Table 2 for complete fatty acid profiles.) The resulting cluster tree consisting of six groups is illustrated in Figure 6. In describing these groups, it must be emphasized that the terms "high," "moderate" and "low" are used to compare species, not the total percentages; i.e., "high" could refer to 9%, if other species contained 2%. Illustrated are Group A, consisting of spot, red drum, croaker, tilefish, snowy grouper and scamp, showed similarity in low percentages of 18:0 and high percentages of 18:1, while other fatty acids were uniformly moderate to low. Group B was composed of gray seatrout, black sea bass, king mackerel, southern flounder and summer flounder. This group had low concentrations of 14:0, 16:1 and 18:1, with high levels of 22:6; other fatty acids were present in moderate amounts. The next two fish on the cluster tree, knobbed porgy and Spanish mackerel (C & D), are alike in their high level of 18:0 and fairly low level of 16:1, 18:1 and 20:5, but differ in the other acids, so are not a clear group. Southern kingfish and spotted seatrout (E) are extremely similar in having high concentrations of 16:1 and 18:1, with low levels of 18:0 and 22:6. Mullet, the last fish in the cluster tree (F), is significantly different from all other species studied in having relatively high levels of 14:0 and 20:5, with moderate to low levels of other fatty acids. All species had high levels of 16:0, a characteristic fatty acid in fish of the southeast region.

The ratios of total saturated fatty acids to total unsaturates (monoenes plus polyenes) were calculated for each species. The species could be divided into four groups on the basis of the ratios, but the correlations with groupings based on edibility characteristics were fair to poor.

Comparison of Groups

As can be seen from the previous discussion, few fish are grouped together in two of the three analyses, while none fall together in all three. The clearest overlap occurs between edibility and ecological groupings. All

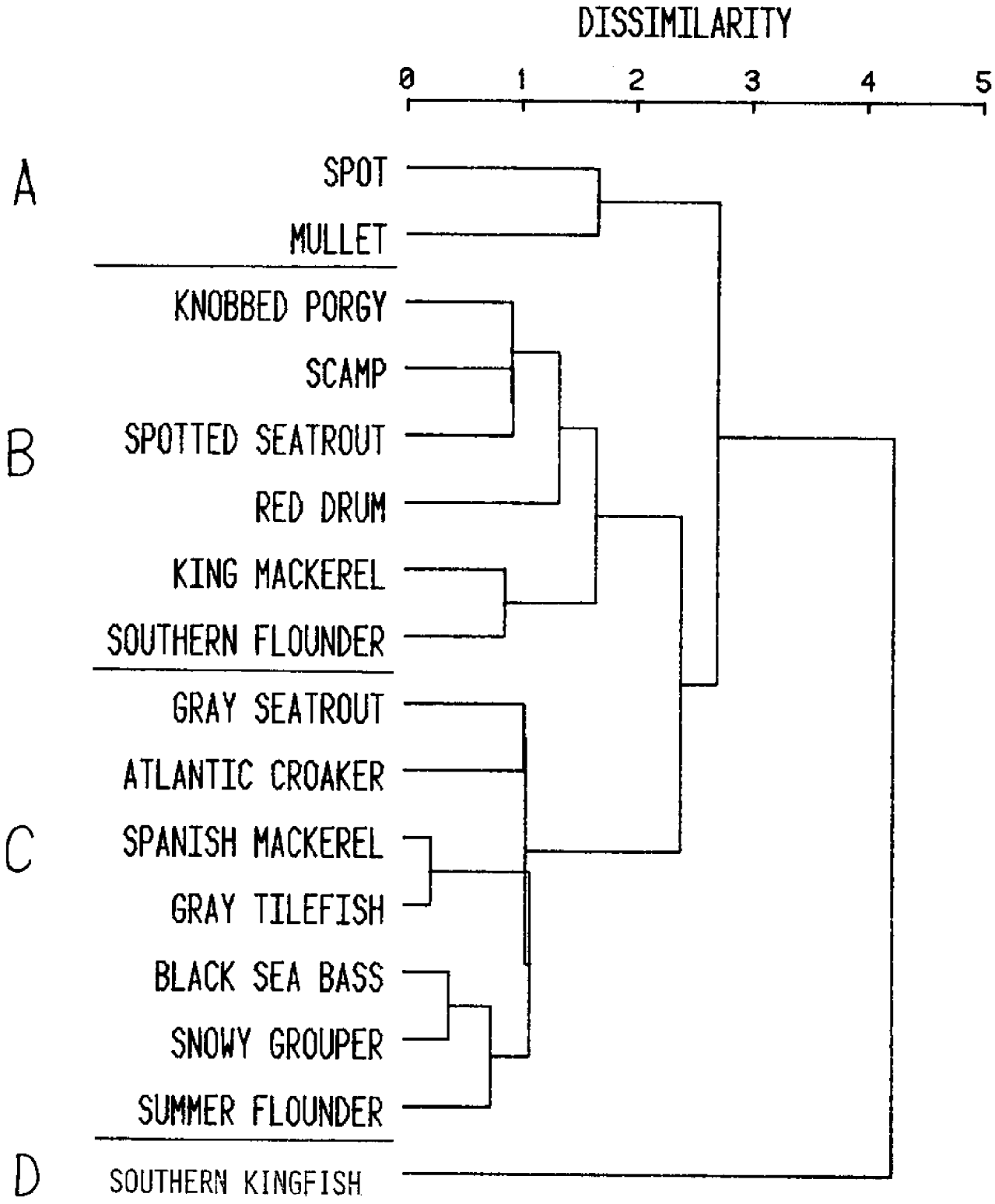

Figure 5. Cluster analysis of proximate composition.

SPECIES	COMPOSITION (%) PROTEIN	MOISTURE	SAK FAT	ASH
Spot	19.2	76.2	4.50	.993
Mullet	20.8	75.0	4.09	1.07
Knobbed Porgy	21.1	77.1	1.86	1.23
Scamp	20.7	76.2	3.02	1.26
Spotted Seatrout	19.9	77.0	2.54	1.20
Red Drum	20.7	78.3	0.68	1.20
King Mackerel	20.9	77.1	1.54	1.41
Southern Flounder	20.8	78.2	0.54	1.45
Gray Seatrout	18.5	80.7	0.85	1.16
Atlantic Croaker	18.1	79.6	1.87	1.09
Spanish Mackerel	19.5	79.9	0.54	1.04
Gray Tilefish	19.3	79.9	0.86	1.03
Black Sea Bass	19.3	79.8	0.63	1.17
Snowy Grouper	19.1	79.7	1.14	1.21
Summer Flounder	19.8	79.0	1.24	1.15
Southern Kingfish	17.2	75.9	6.74	0.957

Table 1. Proximate analysis data

SPECIES	FATTY ACID (%)													
	14:0	16:0	16:1	18:0	18:1	20:1	22:1	18:2	18:3	18:4	20:3 +20:4	20:5	22:5	22:6
Spot	1.7	19.4	8.45	7.0	16.4	2.8	.4	1.4	1.8	.1	3.8	4.9	3.1	7.6
Red Drum	1.1	20.9	7.55	7.6	16.4	.458	.9	2.0	1.0	2.4	8.2	5.4	5.2	9.8
Atlantic Croaker	1.7	22.5	10.07	7.66	15.8	1.8	.6	1.1	.8	3.1	3.3	4.9	3.8	10.4
Gray Tilefish	2.6	19.9	4.8	6.1	14.8	4.1	0.0	1.7	1.3	.9	3.5	3.3	3.2	18.8
Snowy Grouper	2.5	20.4	5.1	6.5	19.4	4.8	-	1.3	.4	.1	3.6	2.9	4.0	17.2
Scamp	3.0	24.9	10.6	6.8	18.0	.6	.3	1.6	.7	1.1	1.9	3.5	3.8	16.6
Gray Seatrout	1.4	20.6	3.7	8.1	9.5	1.3	-	1.1	.4	.6	5.9	5.5	5.8	21.1
Black Sea Bass	2.0	19.5	6.2	7.4	15.5	.1	.1	1.3	.3	.9	4.8	5.0	4.8	24.6
King Mackerel	.6	21.6	2.4	9.2	11.4	.4	.2	1.5	.5	.9	5.1	4.9	4.0	30.4
Southern Flounder	1.9	16.1	4.2	8.9	9.9	-	-	1.6	.5	1.0	3.0	4.0	2.6	21.9
Summer Flounder	1.0	22.5	3.1	7.5	7.6	.6	-	1.1	.4	.8	6.5	2.8	5.9	21.1
Knobbed Porgy	2.7	17.8	5.0	11.8	13.8	-	-	.8	.5	.2	4.6	4.2	5.7	9.7
Spanish Mackerel	.9	23.3	3.3	13.7	13.0	.3	.2	1.1	.8	1.0	3.2	3.6	3.3	22.6
Southern Kingfish	3.0	27.6	20.5	4.4	20.9	.3	.1	.6	.2	1.0	1.9	4.5	2.4	4.7
Spotted Seatrout	2.2	24.0	18.4	5.1	20.1	.7	.1	1.3	.7	.8	2.9	4.5	2.8	7.4
Mullet	4.7	20.6	13.0	4.8	8.0	2.2	-	1.3	1.6	1.0	2.8	9.4	4.5	5.8

Table 2. Fatty acid profile data.

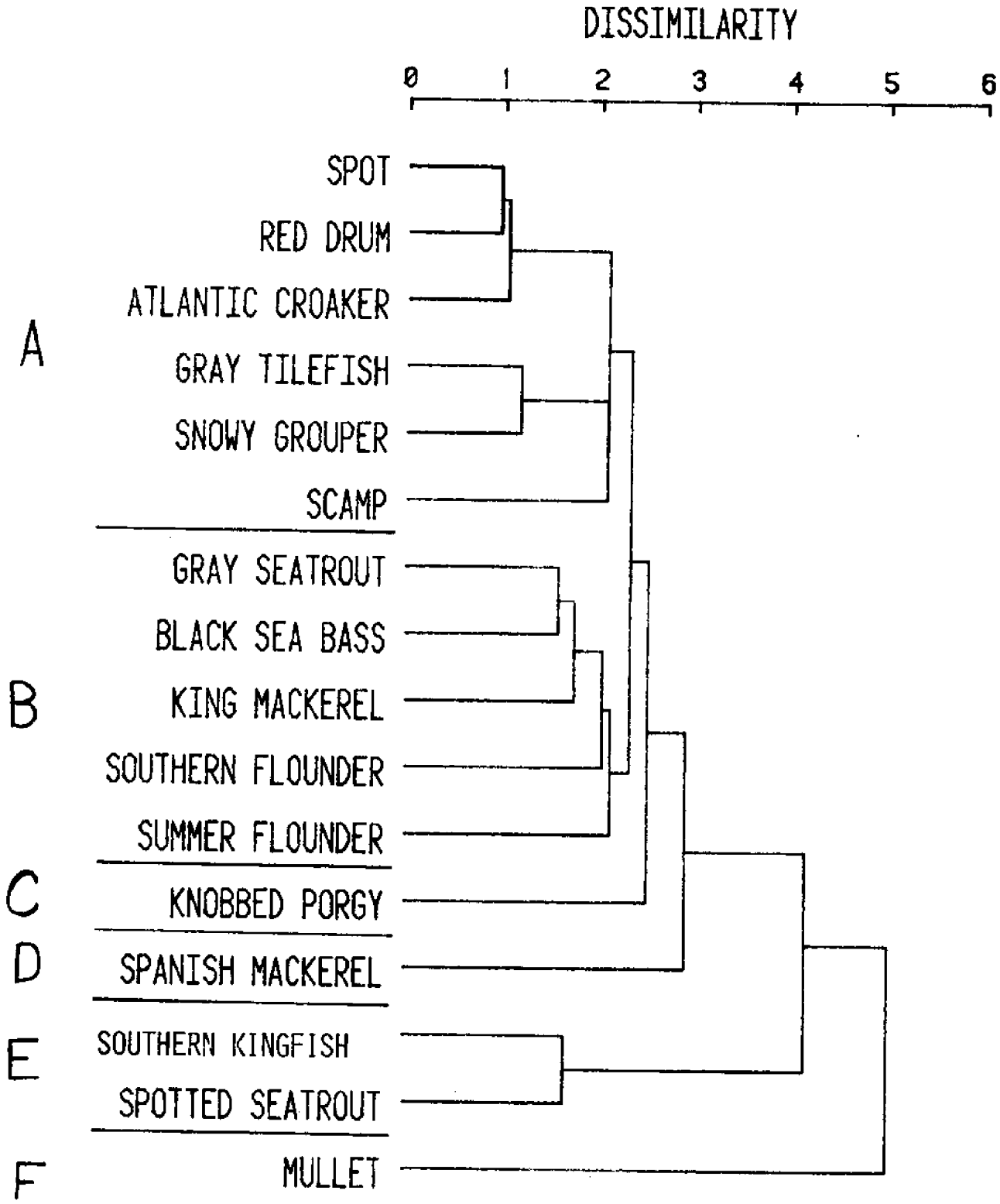

Figure 6. Cluster analysis of fatty acid profiles.

of edibility group B is contained in the bottomfish, while Group A contains all the reeffish except knobbed porgy. The coastal pelagics constitute a majority of Group D.

In edibility characteristics and proximate composition, spot and mullet are grouped together, as are Atlantic croaker and gray seatrout and knobbed porgy and southern flounder, representing three separate groups. Snowy grouper and scamp fall in the same group for edibility characteristics and fatty acid profile, while black sea bass and summer flounder are grouped together for these attributes. Scamp and red drum fall in the same groups for proximate and fatty acids, as do gray seatrout, black sea bass and summer flounder.

CONCLUSIONS

A simplified method for the evaluation of edibility characteristics, based on the rating of five characteristics of microwave-cooked samples on a continuous line scale, was successfully applied to the description of 16 southeastern finfish species. Treatment of the resulting data by cluster analysis and multidimensional scaling identified four separate groups of species with similar edibility characteristics within each group. Most of the southeastern species in our previous study (4) were reeffish and only 4 species were included in both studies. The red drum sample of the earlier study was atypical, as noted in that publication. The other species, scamp, black seabass and knobbed porgy, were placed together in one large group in the previous study. A review of the cluster analysis and multidimensional scaling diagrams, however, indicates that a strong case can be made for splitting the large group into two, placing knobbed porgy into a separate group as was done in the present study.

Data on proximate chemical composition and major fatty acids were also analyzed by cluster analysis, but the resulting groups were less distinct and did not match the groupings determined on the basis of edibility characteristics. Relationships of chemical composition to edibility characteristics deserve further study, however, and we plan to carry out additional studies on selected species with variables such as sex, size, habitat and season, closely controlled. The use of a capillary column gas chromatograph in future work in place of the packed column may result in more accurate differentiation of important highly unsaturated fatty acids that may correlate with edibility characteristics.

The most striking correlation in this study is that of edibility groups to ecological groups. Although it appears that family or habitat of each species can serve as a guide to its edibility grouping, there will be obvious exceptions and actual sensory testing will be required to establish the proper edibility classification.

ACKNOWLEDGMENTS

The technical assistance of Tom Brown and Jim Bonnet, statistical analyses of Lysander Ng, and technical and editorial guidance of Jeanne Joseph, Lloyd Regier and Harry Seagran are greatly appreciated.

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GLYCOGEN AND CHOLESTEROL CONTENT OF THE VENEZUELAN SCALLOP PECTEN PAPYRACEAU (MOLLUSCA, BIVALVA), AT THE CONSUMER LEVEL

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INTRODUCTION

The cholesterol content of scallops reported in the literature varies to such a great extent that it is difficult to make an assessment of this parameter in the nutritional tables. The cholesterol values ranged from 29 mg/100 g to 180 mg/100 g of tissue.

Almost all the work done in the determination of the cholesterol content of the scallop, as well as other molluscs, was done by chemical determinations such as a colorimetric reaction or by titration. Kritchevsky et al. (5), Pearson, (7) and Grodner et al. (3) were the first to use gas-liquid chromatography. Idler et al. (4) combined gas-liquid chromatography with mass-spectrometry to determine cholesterol in seafoods. Other factors not taken into consideration in this type of determination are variation within species and the time of year samples are taken.

The glycogen content of scallop is another area in which little information is to be found.

In the investigation glycogen and cholesterol analyses of the Venezuelan scallop Pectin papyraceau (Mollusca-Bivalva) were done monthly. Also, a monthly proximate analysis was made to characterize the organism.

According to Salaya (10), the scallop analyzed in this study constitutes about 98 percent of the capture in Venezuela.

MATERIALS AND METHODS

Sampling. Scallop samples were collected by the personnel of the Universidad Simón Bolivár (U.S.B.). The samples were brought to the distributor at the wholesale market in Caracas, Venezuela. They were taken into the laboratory in a small icebox.

The samples were shucked, packed unwashed in 500-ml plastic containers, and iced as soon as they arrived at the laboratory. Samples used for proximate analyses were analysed at their arrival. Samples for glycogen and cholesterol analysis were frozen and stored at 25°C. Six samples were taken for each examination. Proximate analyses were run in duplicate and glycogen and cholesterol analyses in quadruplicate.

Glycogen Determination. The determination of glycogen was done according to Grodner et al. (3) from approximate 15 g of tissue. Samples were treated with trichlorocetic acid, and the starch was precipitated with 95 percent ethanol. The precipitated glycogen was determined according to Carrol et al. (2). The optical density of the glucose produced was determined spectrotometrically in Bausch and Lomb Spectronic Colorimeter at 620 nm, being established the amount of glycogen in mg/100 g of tissue.

Cholesterol Determination. Scallop samples were removed from the freezer, thawed and weighed. Due to variations in the size of individual scallops (12.35-24.8 g), the size of the sample varied from 20.0400 to 25.6667 g. The lipid extration was done according to Lanc (6) with methanol for 24 h and chloroform for another 24 h. The extracts were combined, and the lipids were collected and stored in a suitable flask and under nitrogen at 4°C overnight, to facilitate the precipitation of suspended tissue particles in the extracts.

Upon removal from the refrigerator the 2:1 chloroform/methanol mixture was allowed to come to room temperature and was filtered through a coarse grade Buchner filter. Several washings with KCl (0.88%) were done. The upper phase was removed by aspiration and the lower phase was washed with a 1:1 methanol/water several times. The lower phase, containing the purified lipids, was refrigerated overnight.

The solvent in the purified lipids was removed with a rotary evaporator under vacuum at 42°C. Then the lipid residue was saponified in KOH in 95% ethanol and refluxed for 3 h. The sterols were extracted with 40 ml of petroleum ether and mixed. The sterols found in the upper layer were dried in a volumetric flask with Na_2SO_4 under nitrogen

In preparation for the chromatographic analysis, the sterol extract was warmed to room temperature and 1 ml was pipetted into a Teflon-capped vial for derivatization (silylation) by the addition of 1 ml of Tri-Sil reagent (9). The reaction is complete in one hour. Microliter samples of the derivatized extract were injected into a Hewlett Packard 5830A Gas Chromatograph (GC). The operating conditions for GC analysis of cholesterol is shown in Table 1.

Cholesterol standards were derivatized the same way and injected both alone and with the sterol extract. The cholesterol content was determined by subtracting the internal standard.

Proximate Analysis. Samples were analyzed for moisture, ash, crude protein and crude fat according to AOAC. (1).

Statistical Analysis. To determine if the difference found in the results of each analysis was the product of the treatment or an error in the methodology, an analysis of variance was performed. Differences between the monthly averages were established by using a Least Square Difference Test for the proximate analysis and a Duncan's Multiple Range Test for cholesterol and glycogen, after Sokal & Rohlf (11) and Ott (7).

ITEM	CONDITION
Column	
Liquid phase	10% OV-30
Support	Chromasorb BW-AW-DMC S
Mesh	80/100
Length O.D.	4 feet 1/8 inch
Carrier gas	Helium
Flow rate	26 ml/min
Chromatograph	
Operating temperature	260C
Detector	Flame ionization
Injection temperature	300C
Detector temperature	300C
Integrator	
Attenuation	8-16
Chart speed	0.5 min/in

Table 1 Conditions for Gas Chromatograph Analysis for Cholesterol with a Hewlett Packard 5830A Chromatograph.

RESULTS AND DISCUSSION

Glycogen. The values for glycogen for April 1978-March 1979 are shown in Table 2. The data are represented in Figure 1.

As can be observed in Figure 1, the glycogen content shows three different rises. January, February, May and September values were higher ($p<.01$) than the rest. May and September values were statistically equal and both were smaller than in January and February. The glycogen values agreed with the reproductive period reported by Salaya, (10), who stated that this organism has three reproductive periods. Reproduction can occur year-round but reproductive activity peaks during December and January, again in May and again, at varying times. Glycogen, a reserve energy material, is liberated together with the sexual material. Other factors, such as salinity and water temperature, can affect scallop gonad formation, according to Salaya (10). These two variables are important for glycogen formation.

Cholesterol. Values for cholesterol for April 1978-March 1979 are shown in Table 3. These data are represented in Figure 2.

Cholesterol content has a marked rise in August ($p<.01$). July and August levels were greater than others and they both were statistically equal. Cholesterol content of scallops reported in the literature varies considerably, and there is variation in cholesterol content throughout the year.

Pearson (8) and Kritchersky et al. (5) reported punctual values of 29 and 175 mg/100 g of tissue, respectively, for the cholesterol content of scallops. These papers did not mention the time of year, or species.

Proximate Analysis. Moisture content (Table 4) was highest ($p<.01$) during June and July. Ash content (Table 4) was highest ($p<.01$) in March and April. Crude protein did not vary significantly during the year (Table 4). Crude fat was highest ($p<.05$) in May and June (Table 4).

CONCLUSION

Glycogen content varied monthly from 146.79 mg/100 g in June to a high of 616.69 mg/100 g in January.

The cholesterol content also varied monthly from 13.22 mg/100 g in the month of November to 219.39 mg/100 g in July.

Month	Year	mg/100g of scallop tissue [a]*	s
APRIL	1978	180.00	18.19
MAY	1978	223.89	26.62
JUNE	1978	146.79	4.58
JULY	1978	152.13	27.58
AUGUST	1978	159.21	18.01
SEPTEMBER	1978	247.29	30.28
OCTOBER	1978	210.64	27.74
NOVEMBER	1978	220.01	16.57
DECEMBER	1978	206.68	44.68
JANUARY	1979	616.69	15.44
FEBRUARY	1979	267.96	33.87
MARCH	1979	203.92	28.83

[a] Based on wet weight
* The number of samples: 6

Table 2 Monthly Mean Concentration of Glycogen in the Venezuelan Scallop (*Pecten papyraceau*).

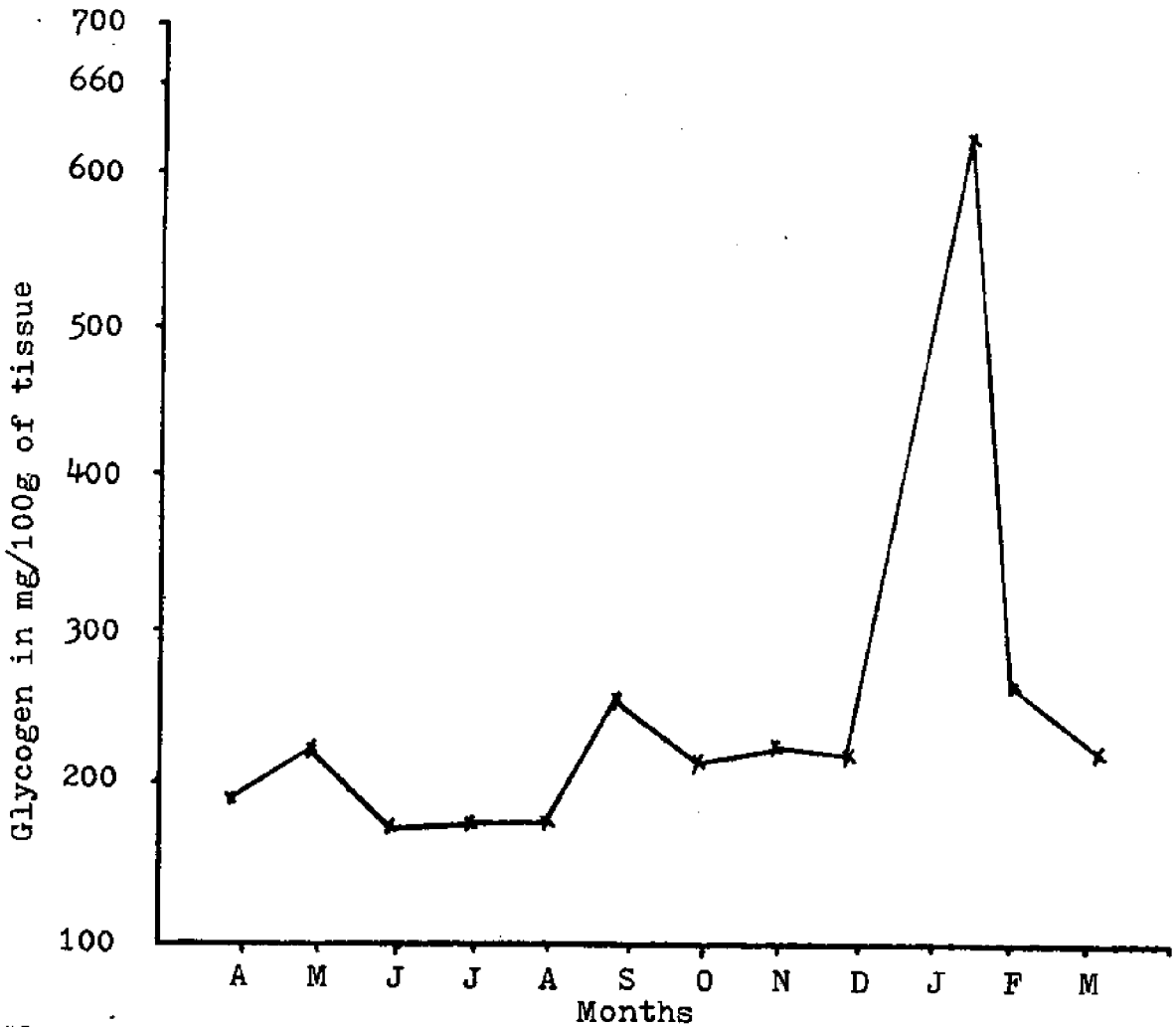

Figure 1 Glycogen content of the scallop Pecten papyraceau from April 1978- March 1979

Month	Year	mg/100g of scallop tissue[a] *	s
APRIL	1978	37.72	7.35
MAY	1978	132.83	22.98
JUNE	1978	139.84	7.14
JULY	1978	219.39	11.50
AUGUST	1978	193.90	28.10
SEPTEMBER	1978	18.20	1.34
OCTOBER	1978	14.55	0.35
NOVEMBER	1978	13.22	1.98
DECEMBER	1978	25.40	8.23
JANUARY	1979	30.29	2.53
FEBRUARY	1979	26.48	4.83
MARCH	1979	68.60	6.83

[a]Based on wet weight
* The number of samples: 6

Table 3 Monthly Mean Concentration of Cholesterol in the Venezuelan Scallop (*Pecten papyraceau*).

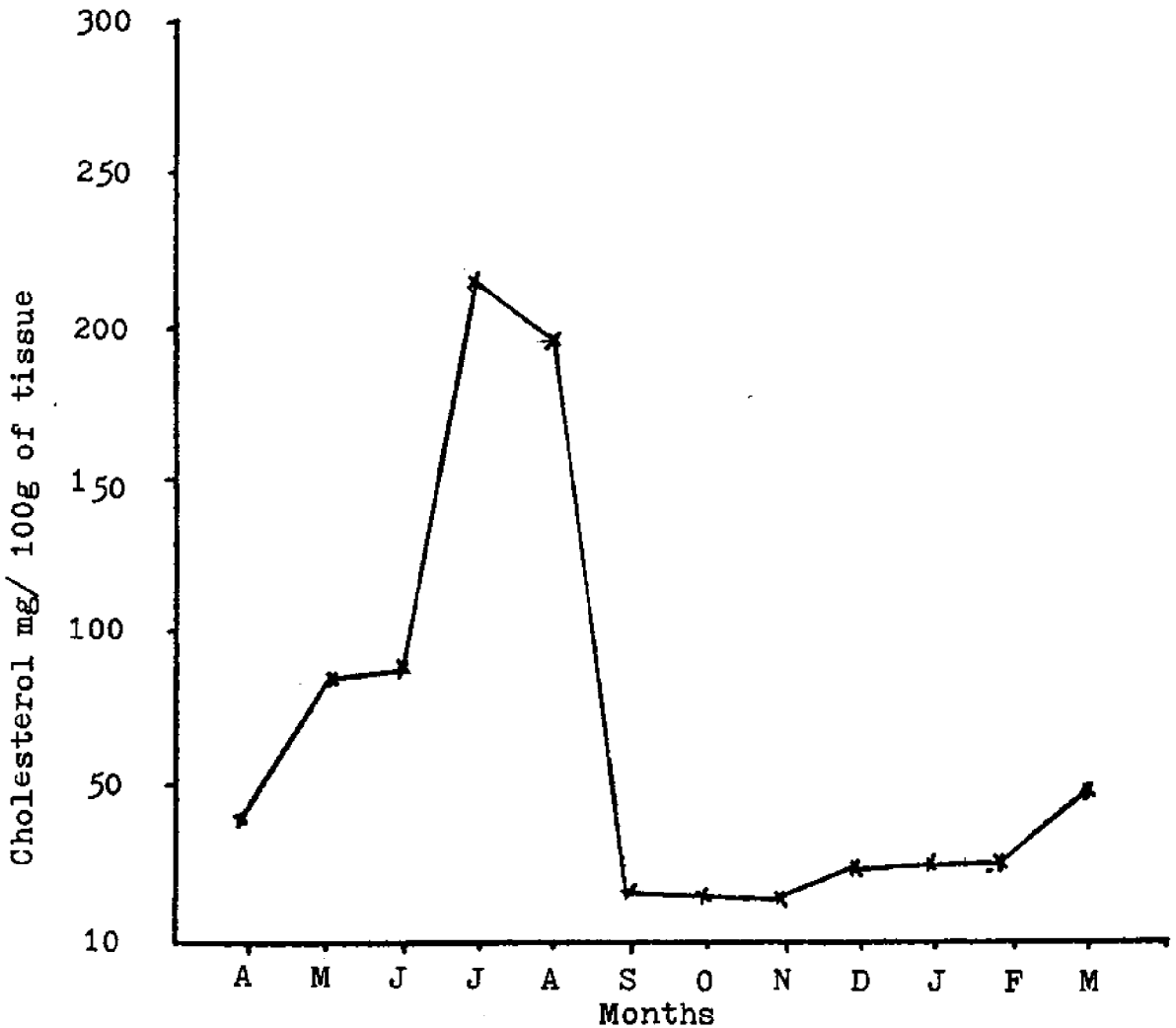

Figure 2 Cholesterol content of the scallop Pecten papyraceau from April 1978- March 1979

	Moisture %		Ash %		Protein %		Fat %	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
JANUARY	79.57	0.65	2.71	0.18	13.31	1.22	0.48	0.17
FEBRUARY	77.94	0.62	4.10	0.29	13.78	0.88	0.54	0.21
MARCH	78.99	2.02	6.60	1.81	11.93	1.30	0.56	0.20
APRIL	79.60	1.82	6.59	2.05	12.82	1.65	0.69	0.30
MAY	81.98	1.19	2.38	1.17	13.99	1.10	2.44	0.27
JUNE	85.80	1.61	2.40	0.97	11.91	1.06	2.98	0.16
JULY	86.03	0.38	1.34	0.14	14.69	0.97	1.61	0.32
AUGUST	83.58	0.47	2.14	0.48	12.37	0.76	0.53	0.13
SEPTEMBER	81.78	1.28	2.79	0.24	14.37	0.76	0.53	0.13
OCTOBER	80.73	0.51	2.01	0.09	14.31	1.94	0.41	0.06
NOVEMBER	80.70	0.81	2.04	0.18	13.53	0.37	0.56	0.18
DECEMBER	-	-	-	-	-	-	-	-

* The number of samples: 6

Table 4 Monthly mean and standard deviation of the proximate analysis of the Venezuelan scallop (Pecten papyraceau) during 1978

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TRIMETHYLAMINE FORMATION IN DRIED FISH

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INTRODUCTION

The change of trimethylamine (TMA) content during the storage of fresh fish is often associated with the microbiological spoilage of fish (3,4, 9). The formation of TMA may be from emzymatic hydrolysis of trimethylamine oxide (TMAO) through either microbiological or autolytic actions. (3,7,10). It may also be formed through non-enzymatic action such as chemical reduction in the presence of metal ions (1,8,11). In most cases, the presence of TMAO is the prerequisite for TMA accumulation during the storage. Fish muscle generally contains a large amount of TMAO, even in tissues where the concentration of TMAO splitting enzyme is not significant. High concentrations of this type of enzyme could be found primarily in kidney tissue, blood, spleen, and pyloric caeca of the fish (3,10). Where endogenous TMAO splitting enzyme is not present, TMA formation primarily occurs as a result of microbiological proliferation. Thus, the TMA formation in microbiologically stable food items, such as dry fish is of interest.

In the processing of dried fish where microbiological proliferation no longer occurs after dehydration to the shelf stable moisture content, accumulation of TMA and other amino compounds has been reported to occur during storage (2,8,9). Although lipid changes and browning reactions are more noticable problems associated with dried foodstuffs during the storage (1), establishing the fact that TMA formation occurs only in conjunction with microbiological proliferation would justify the use of TMA levels in dried fish as an index of the quality of the fish prior to drying and the extent of microbiological activity in the product as the fish is being dried. Knowing the rate of TMA formation under varying temperatures and water activity can also be useful in optimizing drying conditions such that minimal TMA formation occurs.

MATERIALS AND METHODS

Materials. Fresh mullet was purchased from a local seafood market, dressed, filleted, and sliced in two ways for either air drying or freeze drying. For the air drying, the mullet was filleted with skin on, and trimmed to 1 x 3 x 1/4 inches. For freeze drying, mullet fillets with skin on were cross-sectioned to one-half-inch thickness and frozen before freeze dried. An antibiotic solution for sanitizing samples was made from

50 ppm chlorotetracycline and 25 ppm nystatin (United States Biochemical Corporation, Cleveland, Ohio).

Drying and storing conditions. Air drying took place in a forced-air dryer originally constructed to be a demonstration fish smoker with a chamber of 20 x 22 x 17 inches. Trimmed mullet fillets were dipped with the antibiotic solution for one hour before drying. Drying was conducted at 45°C, 55% relative humidity, and 52 ft/min average air velocity. It took 24, 20.5, and 6.5 hours to dry each 3-lb batch of mullet fillets to moisture contents of 26, 43, 55%, respectively. The dried samples were shredded by a Waring blender into fibers aseptically and stored at an ambient temperature of 5°C in plastic buckets containing salt slurries. According to the desorption isotherm as shown in Figure 1, salt slushes of NaCl, KCl, and K_2SO_4 were selected to maintain samples with 26, 43, and 55% moisture contents under water activities of 0.75, 0.95, and 0.98, respectively. In this study, the water activity was determined by the jar equilibrium method using a hygrometer with wide range humidity sensors (American Instrument Co., Silver Springs, MD).

Freeze drying was carried out in a freeze dryer for two days until the fish was bone dry. At the termination of the drying cycle, the chamber was purged with formaldehyde gas to reduce the microbiological content. The dried sample was then shredded using the same method as the air dried sample. The shredded samples were divided into three batches and mixed with calculated amounts of water to bring the moisture contents to 39, 31, and 18%. These samples were kept with salt slushes of K_2SO_4, KNO_3, and NaCl. Their water activities measured at the end of two weeks were 0.94, 0.93, and 0.85, respectively. As expected, the water activities of the rehydrated freeze dried samples were higher than those where moisture content was adjusted through desorption as was the case with the air dried samples.

Trimethylamine determination. TMA was determined using a TMA-specific electrode according to Chang, et al. (5), except that the amount of formaldehyde was doubled to decrease the interference from ammonia (4). When standard solutions of TMA with concentrations ranging from 0.1 to 1 mM were analyzed using the electrode, a standard curve was obtained showing the relationship between the readings (X) and concentrations (Y mM) as:

$$Y = (X + 63.76) / - 57.53$$

The concentration of TMA-N in mg/100 g dry tissue became:

$$TMA\text{-}N = 14 * Y * U / Z * V * (1 - M)$$

where Y (mM) was the concentration in mM found from the previous equation, U (ml) the volume of water used for sample extraction, Z (ml) the amount of extract used in the 10-ml solution into which the electrode was immersed, Z (g) the sample weight used for extraction, and M (%) the moisture content of the sample. A strip chart recorder connected to the output of the pH meter recorded the electrode potential so that correct steady readings could be obtained, usually in 10 to 30 minutes. The TMA contents were calculated on a dry basis in order that comparison could be made among samples having different moisure contents.

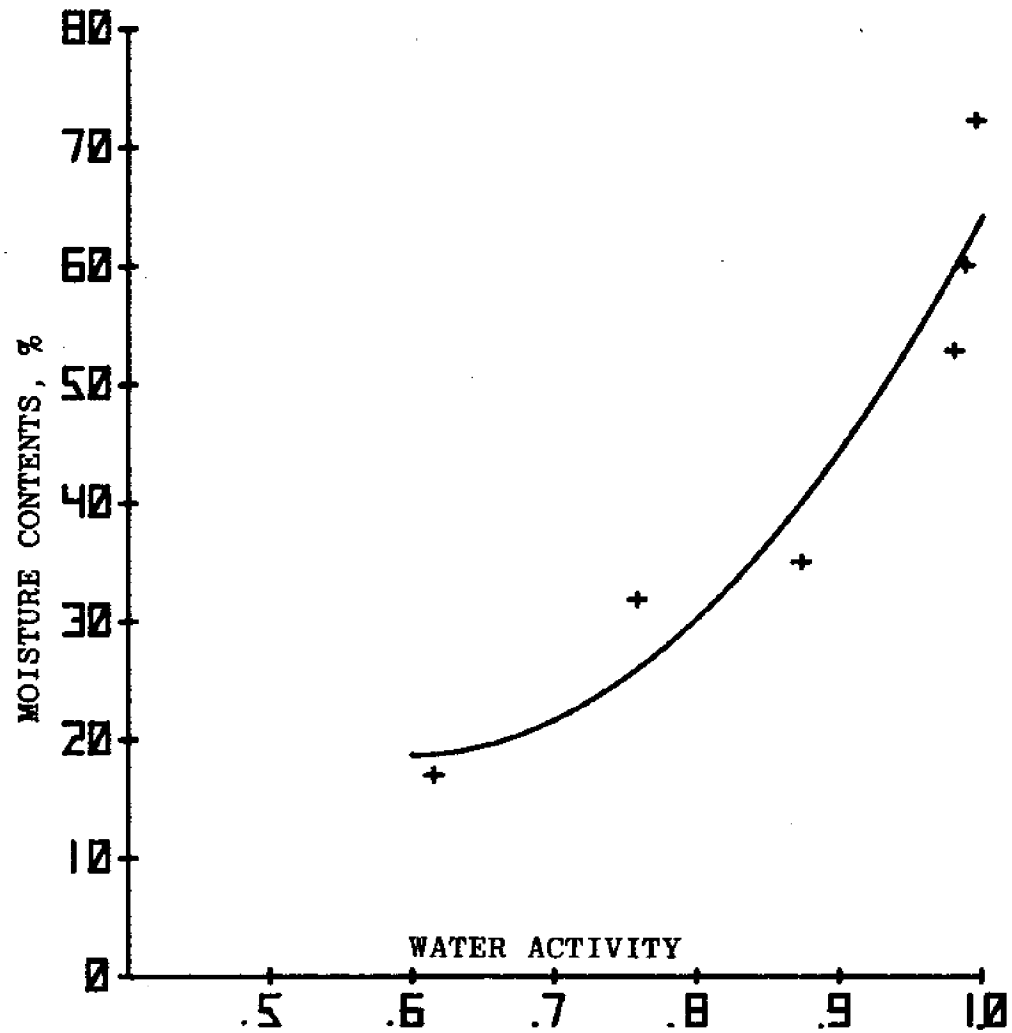

Figure 1. Water activity of air dried samples of different % moisture.

RESULTS AND DISCUSSION

Air-dried samples. After the drying process, the TMA contents of three batches with three moisture contents were around 6.2 mg N/100 g dry tissue. The difference caused by the range of 6 to 24 hours of drying time was not significant. Minute differences exist in TMA levels among samples which might be due to differences in TMA contents of fresh samples used in the various batches before processing. As shown in Figure 2, the TMA content remained at this initial level when the three samples with three different a_w values were stored at 5°C for seven days. During this period, the total aerobic plate count (TPC) of the samples remained stable at less the 10^8 per gram (Table 1). After seven days, TMA content of the sample with 0.75 a_w remained constant for another two weeks. However, mold started to grow in samples with 0.95 and 0.98 a_w and a trend toward increasing TMA content in these samples became evident. The reason for the fluctuation of TMA content in the sample with 0.98 a_w was undetermined, but maybe due to the nonhomogeneity of the sample. Samples with 0.75 a_w were observed for three weeks and no increase in either TPC or TMA content occurred.

When stored at ambient temperature, samples with 0.75 a_w remained microbiologically stable for three weeks. A slow decrease in TMA content was observed. Whether this decrease is due to further degradation of TMA or some other cause was undetermined. Bacteria and mold started to grow on the other two samples with higher a_w after one day storage (Table 1) and TMA content increased rapidly after three days storage to 10 and 16 mg N/100 g dry tissue (Figure 2) for samples with 0.95 and 0.98 a_w, respectively.

Freeze dried samples. Shortly after freeze drying, the TMA content was about 0.7 mg N/100 g dry tissue. After equilibration with three different salt slushes for seven days at ambient temperature, the moisture content of the samples stayed at less than 20% and their TMA contents remained unchanged. The same situation occurred with samples stored at 5°C for 10 days. Because of the slow uptake of water from the salt slushes, an estimated amount of water was added to each sample to raise the a_w. After water addition and equilibration, the final a_w values were 0.85, 0.93, and 0.94. The zero day used on Figure 3 was the day after water addition and was actually the seventh day and tenth day following drying for room temperature and 5°C stored samples, respectively. In any event, the TMA content of freeze dried mullet samples was much lower than the air dried samples right after drying. The freeze drying process under subzero temperatures slowed down the action of microorganisms (Table 2) and reduced autolytic activity resulting in lower TMA content than the air drying process at 45°C.

After rehydration to the designated moisture contents, all three batches stored at 5°C maintained a stable TMA level at less than 1 mg N/100 g dry tissue for 11 days. Their TPC remained at less than 10^3 and even decreased gradually. Mold growth was observed on samples with 0.93 and 0.94 a_w on the 11th day and the TMA contents of these two samples increased to 2.4 and 4.4 mg N/100 g dry tissue after 18 days, respectively.

TEMPERATURE:	AMBIENT			5°C		
% MOISTURE:	26	43	55	26	43	55
A_w :	0.75	0.95	0.98	0.75	0.95	0.98
Days						
-1	3.02×10^5	----	----	3.02×10^5	----	----
-1/2	----	2.54×10^5	----	----	2.54×10^5	----
-1/4	----	----	9.20×10^5	----	----	9.20×10^5
0	1.31×10^6	1.48×10^6	8.38×10^5	1.31×10^6	1.48×10^6	8.38×10^5
1	6.20×10^5	MOLDY	MOLDY	3.90×10^5	---	1.25×10^5
3	1.36×10^7	---	---	8.50×10^5	1.35×10^6	1.36×10^5
7	1.32×10^8	---	---	1.30×10^8	8.90×10^5	1.28×10^6
14	4.6×10^7	---	---	2.4×10^8	MOLDY	MOLDY
21	2.8×10^7	---	---	8.1×10^5	---	---

Table 1. Total plate counts for air-dried mullet fillets stored at 5°C and ambient temperatures with the zero day as being the time when the air drying was finished.

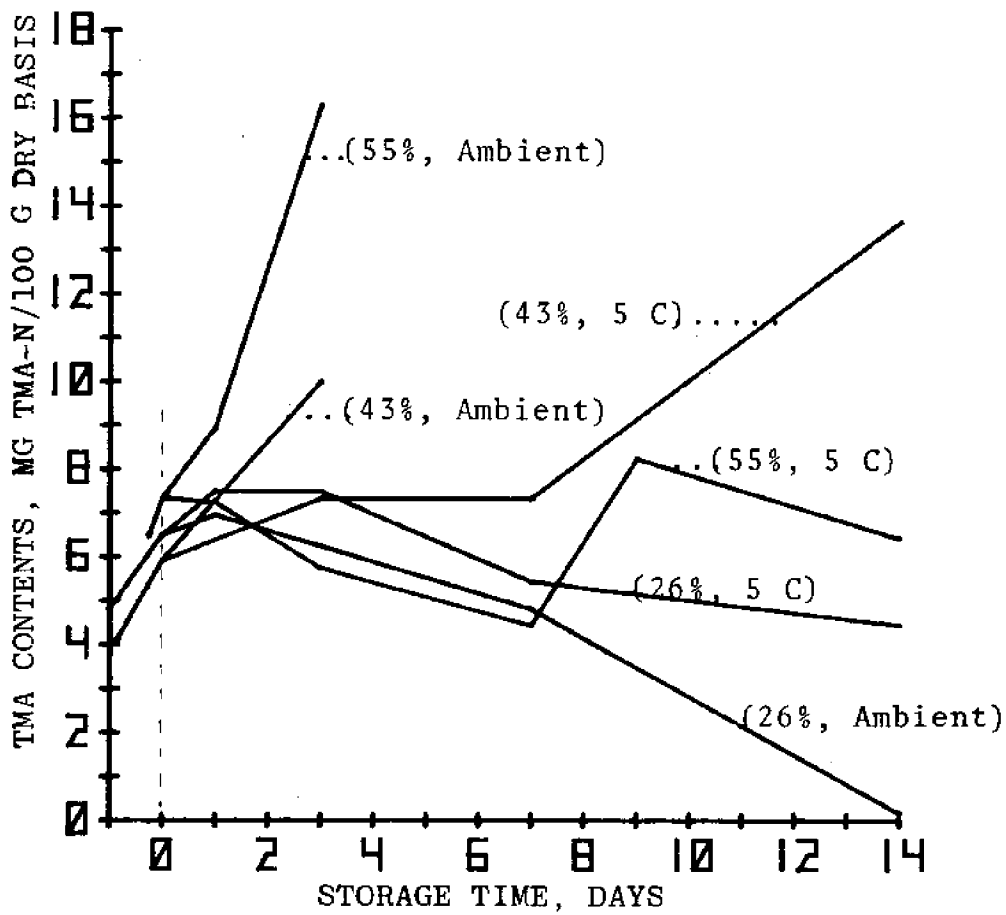

Figure 2. TMA formation of air dried samples with different % moisture contents. stored under 5 C and ambient temperature.

TEMPERATURE:	AMBIENT			5°C		
A_w:	0.85	0.93	0.94	0.85	0.93	0.94
% MOISTURE:	18.2%	31.1%	38.5%	18.3%	32.2%	38.5%
Days						
0	5.40×10^4	5.10×10^4	1.85×10^5	6.10×10^2	5.30×10^2	2.10×10^2
2	8.60×10^8	1.03×10^4	8.80×10^6	4.30×10^2	3.30×10^2	2.10×10^2
4	----	1.04×10^7	MOLDY	3.90×10^2	2.90×10^2	2.4×10^2
5	5.30×10^7	MOLDY	-----	TLC	TLC	TLC
7	9.30×10^4	-----	-----	TLC	TLC	TLC
11	MOLDY	-----	-----	TLC	MOLDY	MOLDY

Table 2. Total aerobic plate counts of freeze dried mullet flake samples containing different moisture contents, being stored at 5°C and ambient temperatures; TLC = too little to count.

The sample with 0.85 a_w maintained the same TPC and TMA contents at 5°C throughout a three month observation period.

When stored at ambient temperature, samples with 0.93 and 0.94 a_w showed a rapid increase in TMA content as bacteria and mold growth progressed (Figure 3 and Table 2). The TMA content reached 9 mg N/100 g dry tissue in two days for the sample with 0.94 a_w and 8 mg N/100 g dry tissue in six days for the sample with 0.93 a_w following a two day lag period. The amount of TMA also increased in the 0.85 a_w sample, although the rate of increase was slower than those with higher a_w.

In conclusion, TMA increases with increasing TPC can be observed in samples with water activities ranging from 0.75 to 0.98 and stored at either 5°C or ambient temperature. When no microbiological proliferation occurred, no increase was observed. When conditions allowed growth of microorganisms, TMA increased depending upon the a_w of the sample and the amount of microbiological growth. Although the heat denaturation of endogenous TMAO splitting enzyme system in the fish was minimized by freeze drying, the TMA accumulation in these samples in the absence of microbiological action was not evident and not different than those in the air dried samples. The study proves that TMA formation in dried mullet fillets was primarily caused by microbiological proliferation.

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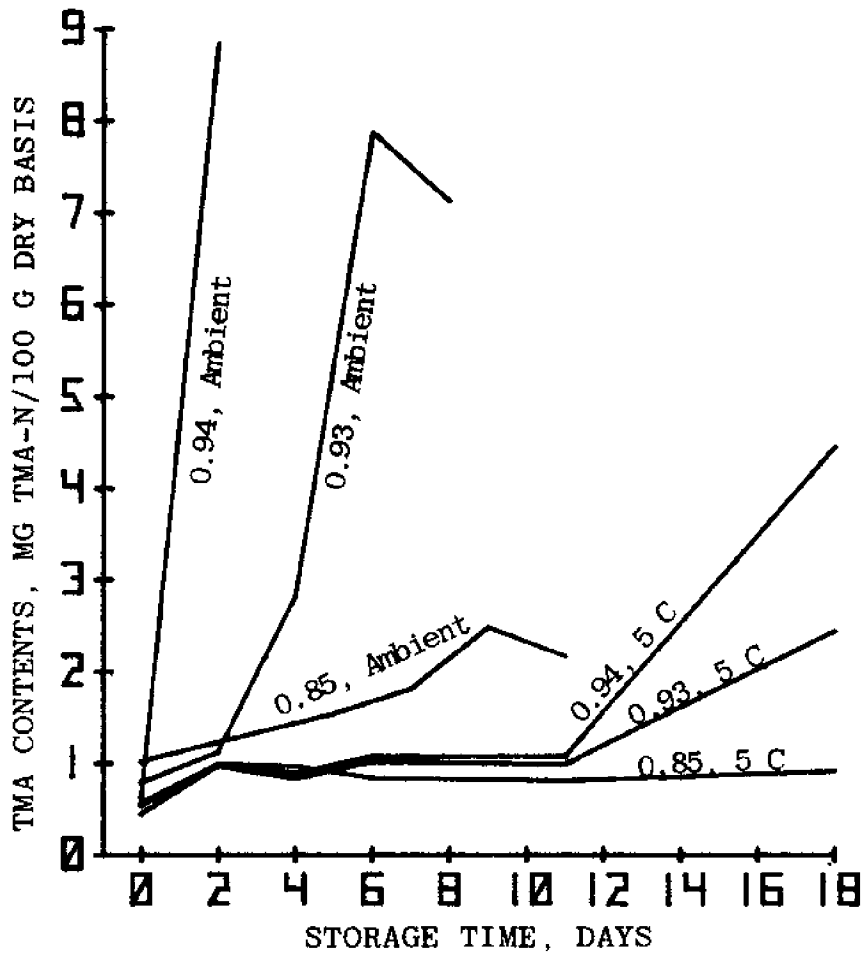

Figure 3. TMA formation of freeze dried samples with different a_w.

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FABRICATION OF STRUCTURED FOODS FROM UNDERUTILIZED FISH

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INTRODUCTION

In many coastal regions of the world, lack of refrigeration facilities results in the loss of a large proportion of the fish catch. In most of these regions rice is a staple food. The coextrusion of rice and fish offers the possibility of producing shelf-stable foods with variable properties which can be nutritionally balanced fairly easily. In other regions of the world coextrusion of fish and high carbohydrate materials may also find acceptance as finger (snack) foods or as foods requiring relatively little additional preparation before eating. If they do, the economic value of the fish catch will be greatly increased.

Extrusion cooking, a means of preserving and enriching foods, offers several advantages. Among them are: longer shelf life; absence of thermolabile growth inhibitors and microorganisms; ready-to-eat property, as the product is already cooked; and the possibility of mixing and enriching with nutrients from several sources (Figure 1).

Quantitative measurement of product quality. It is necessary to specify the product quality in terms of some measurable parameters. If the product is to be eaten as a ready-to-eat snack food, it must possess a quality called "crispiness". Fracturability and "impact shear strength" are the parameters which are close to the crispiness of the product and these two values are required to be measured.

If the product is to be consumed in a curry, it must have the "chewiness" characteristics of fish. This parameter should be measured after the product has been rehydrated in boiling water for about 10-15 minutes.

Fracturability and chewiness of a product may be determined from the textural profile analysis of the product (Bourne, 1978). An Instron Universal Testing Machine can be used for the purpose and the product should be tested under compression. For the chewability test, the product must first be rehydrated in boiling water and then tested under compression. No rehydration is necessary for either the crispiness or the fracturability test.

Impact shear strength is a measure of crispiness and may be determined by means of an impact shear tester. Impact shear strength is the reduction in the amount of kinetic energy of a heavy weight when it is allowed to fall on, and break, the sample. In this machine, the product is very quickly deformed; the rate of deformation produced is too high to be obtained by means of an Instron Universal Testing Machine. Zuilichem et al (1975) studied the strength of extruded corn grits by this machine and specified a value of 0.6 kg/cm^2 as an acceptable upper limit of the strength of extrudate corn grits.

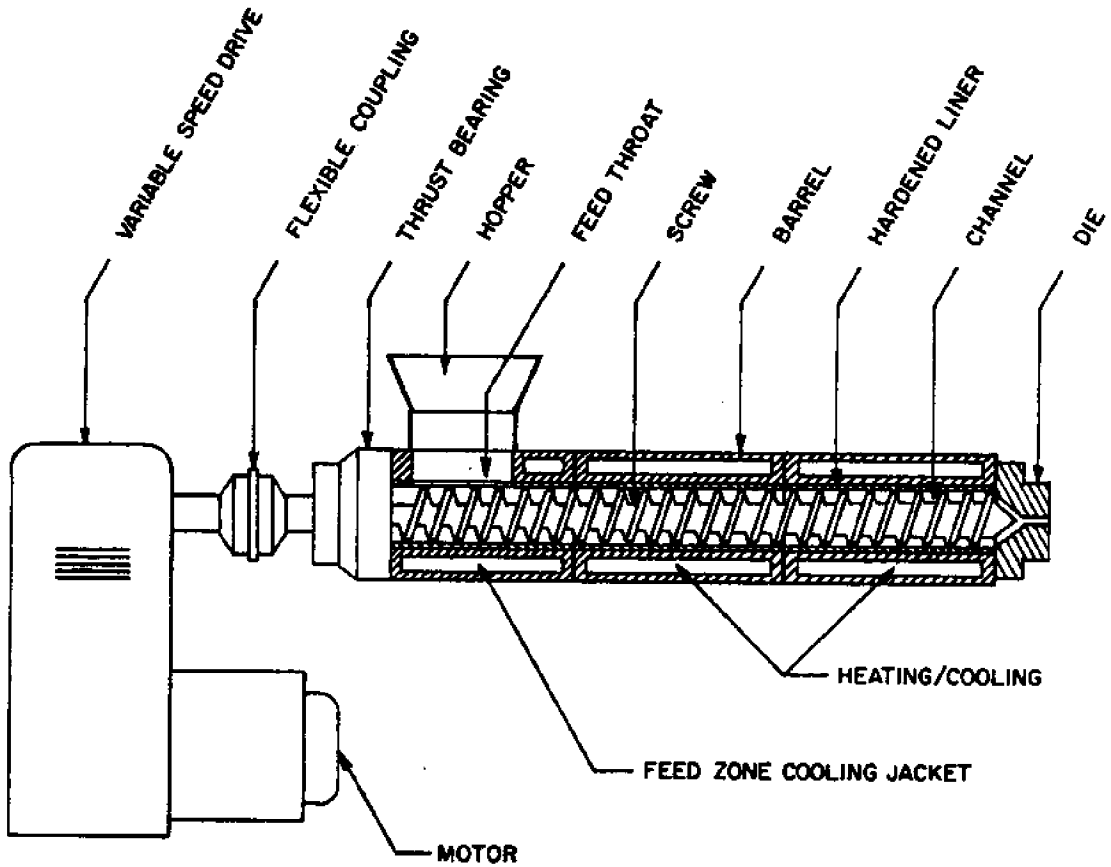

Figure 1. Elements of a food extruder. The food material is fed from a hopper through the feed throat into the channel of the screw. The screw rotates in a barrel which has a hardened liner. A motor drives the screw through a gear reducer, and the backward thrust of the screw is absorbed by a thrust bearing.
As the food material is conveyed along the screw channel, it is simultaneously subjected to mixing, heating, and shear. When it is close to the discharge end, i.e., near the die, it has been transformed into a thermoplastic, viscoelastic material at high pressure. Finally, the material flows through the die.

Physico-chemical changes in product. Several physico-chemical changes occur in the ingredients of the feed when it passes through an extruder. Due to the rotation of the screw, a considerable amount of mixing of the ingredients takes place. The temperature of the product rises due to the viscous shearing taking place within the material. The temperature of the material within the flights of the screw may thus be several degrees higher than the temperature of the barrel where it is measured. Pressure at the end of the screw increases when the material is forced through a small die opening. A considerable amount of restructuring of the material takes place when it flows through the die. A pressure of about 100 atmospheres and a temperature of about 250°C are not uncommon operating parameters. As the product comes out of the die, the superheated water vapor quickly escapes, giving a porous and expanded structure to the extrudate.

Gelatinization of starch, denaturation of protein and the texturization of protein are the major changes that occur inside the extruder. Feed moisture, barrel temperature and screw speed are the factors responsible for bringing about these changes.

Starch gelatinization. Gelatinization of starch occurs due to the swelling of starch granules. Amylose (a straight chain polysaccharide with D glucose as a repeating unit, linked together by α (1→4) linkage) and amylopectin (a branched chain polysaccharide with D glucose as a repeating unit, linked together by α (1→4) linkage) are the two major constituents in a starch granule. Due to the hydrogen bonding between them, amylose molecules form a crystalline region within the starch granule. Because of this crystalline structure, water cannot readily penetrate between the amylose molecules. On the other hand, the amorphous regions formed mainly by branched chain amylopectin molecules form easily accessible places for water penetration. At higher temperatures, however, as in food extruders, the hydrogen bonds between the amylose molecules break and water penetration occurs readily.

Under atmospheric pressure and temperature conditions no appreciable swelling or gelatinization of starch occurs unless the moisture content of the starch exceed 30%. However, under extrusion conditions gelatinization occurs at a much lower moisture content.

As a result of gelatinization, several changes in the properties of the material take place, included are an increase in cold paste viscosity, loss of birefringence, higher water solubility and greater swelling. For the extruded product, the lower the difference in cold and hot paste viscosities, the higher will be the degree of gelatinization of starch.

Expansion of the extruded product is partly due to the gelatinization of starch inside the extruder and partly due to the escape of superheated steam entrapped within the extrudate. Water absorption is usually positively correlated to the expansion of the product. Breaking strength of the extrudate is also affected by the gelatinization of starch.

Effect of extruder variables on starch gelatinization. Our objective was to get an extruded product made of rice and fish which would have the desired quality. Gelatinization of the starch of a rice-fish mixture

affects the quality of the extrudate. Extruder variables like barrel and die temperatures, compression ratio, moisture content of the feed, amylose content of the starch, and screw speed affect the process of gelatinization of starch which in turn affects the viscosity, breaking strength, expansion, water solubility and water absorption of the product. None of the published literature discusses these properties relative to rice and fish mixtures. Most of the published literature deals with the extrusion of corn and rice separately.

The following conclusions were drawn from a number of sources (Anstaett, et al (1969); Conway (1970); Lawton et al (1972); Mercier and Feillet (1973); Charbonniere et al (1973); Zuilichem et al (1975)):

1. Extruder barrel temperature and the feed moisture content are the two most important factors in the gelatinization process of the starch in extrusion processing.
2. Some of the characteristic changes in starch probably occur when the barrel temperature lies between 130°C and 170°C. This has been observed in the extrusion processing of corn starch.
3. Water absorption index is a measure of gelatinization.
4. High barrel temperature and low feed moisture or low barrel temperature and high moisture improve gelatinization.
5. An increase in breaking strength is related to the increase in cooking paste viscosity.
6. An increase in feed moisture content reduces expansion but improves water absorption.

Effect of food additives on gelatinization. Incorporation of various food additives into the feed modify the process of gelatinization of starch. The effects of the following food additives are of interest:

1. Sugar competes with starch for the water present and retards the swelling of the starch granules.
2. Strong alkalinity causes increased swelling of starch granules.
3. Salt increases the firmness and gel strength of the hydrated products.
4. Natural fat does not have any effect on the viscosity of the gelatinized starch, but the temperature at which the maximum viscosity is reached gradually lowers as more fat is added. Conway (1970).
5. Surfactants have the opposite effect on gelatinization as that of natural fat. They increase the temperature at which maximum viscosity is reached.

Denaturation and texturization of protein. While passing through an extruder proteins experience high temperatures and denaturation. This is a part of the cooking process and makes proteins more digestible. Effects of protein denaturation are: (1) unfolding of the peptide chain of the protein molecule, resulting in an increase in intrinsic viscosity; (2) decrease in solubility; and (3) availability of peptide chains for hydrolysis by proteolytic enzymes.

Heat is an important denaturing agent. Through heat, the native secondary, tertiary and quatenary structure of protein are disrupted and

the peptide chains of the protein molecules form a shearable solution of random coils. In addition to heat, alkaline pH, detergents, urea, quanidine hydrochloride and certain amides enhance the denaturation process. If urea and quanidine hydrochloride are used as denaturing agents, they must be removed after the extrusion process.

Texturization of protein is achieved when the random coils of peptide chains are sheared, stretched and aligned as they pass through a die. Rapid cooling of the product as it comes out of the die sets the fibers before they return to the random coil arrangement. Burgess et al (1976), Stanley et al (1978) and Shen et al (1979) have shown that the formation of the intermolecular amide bond is responsible for the final texture of the protein. They also have shown that the formation of the disulphide bond does not play a role in the texturization process.

As a result of texturization of protein, shear strength of the product is increased and the chewability is improved.

Effect of extruder variables. Barrel temperature, protein and moisture content in the feed, and screw speed are the important factors responsible for the texturization process. Adequate feed moisture improves the flowability of the product. Screw speed determines the residence time of the product inside the extruder. Adequate residence time is necessary for the completion of denaturation of the protein.

No research has been reported on the coextrusion of rice and fish. Most of the reported work has been conducted on defatted soy flour, which usually contains about 50 percent protein, and has been reported by: Cumming et al (1972); Maurice et al (1976); Aquilera et al (1976); among others. From these reports, the following conclusions have been drawn:

1. Texturization of protein consists of two main steps: (a) the unfolding of the peptide chain from the native structure of protein by the action of heat, and (b) reorientation of these peptide chains by the action of shear.
2. One of the measurable properties of the texturized protein is its breaking strength, which increases with the increase in the degree of linear orientation of the peptide chains.
3. Breaking strength of defatted soy flour increases with the increase in barrel temperature, starting from about 130°C and reaches its maximum at about 170°C.
4. Decrease in screw speed increases residence time and thus increases the denaturation and the texturization of protein.
5. Moisture effects on the texturization process remain inconclusive. Most researchers have worked with feed moisture contents varying between 24% and 35%.
6. Increase in protein content increases the "degree" of texturization.

Starch-protein interactions. Gelatinization of starch and the texturization of protein are the two major processes that occur within an extruder. Both of these processes affect the shear strength and the water absorption capacity of the extruded product. Barrel temperature, feed moisture content, screw speed and the relative proportion of starch

and protein in the feed affect these two product qualities.

With an increase in the barrel temperature, starch gelatinization and protein texturization processes are enhanced. Moisture content plays a positive role in starch gelatinization. However, its effect on protein denaturation is not very well defined. Reducing the screw speed increases the time required for the gelatinization of starch and for the texturization of protein.

Coextrusion with fish. Stanley and deMain (1978) coextruded a mixture of soybean and minced cod muscle which contained 90% protein. A combination of 80% soy meal and 20% fish was used leading to a final protein content of 58%. The moisture content of the feed was 36% and the product was extruded at 170°C barrel temperature. The screw speed was 100 rpm. Micrographs of the extruded product resembled that of soy alone. No other reports have been found on the coextrusion of fish.

Composition and characteristics of fish muscle. Fishes vary widely in the proportions of protein, fat and moisture in their tissues. Compositions for five categories of fish are given in Table 1. From 40 to 60 percent of the weight of a whole fish is edible. The analysis given in Table 1 is for "light" meat only. As seen in Table 1, there is apparently an inverse relationship between the fat and moisture content of fish, such that the sum of the fat and moisture percentage is approximately 80%.

The range in isoelectric points of fish protein is from about 4.0 to 5.5. At the isoelectric point the protein is electrically neutral, is least soluble and is easily denatured. By addition of a neutral salt, like sodium chloride, the isoelectric point is displaced towards a lower pH.

Under alkaline pH, fish protein shows increased ability to hydrate and swell. Calcium ions reduce swelling. Under alkaline conditions salt like sodium chloride increases solubility and swelling of fish proteins. However, under acidic pH, salt has the opposite effect. It reduces swelling and solubility of proteins.

Fish muscle contains free amino acids which supply nutrients to invading microorganisms causing the fish to spoil quickly after death. Another source of free amino acids is the strong enzyme-like cathepsins which reside in the fish muscle and attack the muscle to produce amino acids.

Lipids in fish are susceptible to hydrolysis by various enzymes producing free fatty acids. One of these enzymes, phospholypase is remarkably heat stable, even at a temperature of 100°C. However, the severe thermal treatment given inside an extruder inactivates these enzymes.

Composition of rice. On a dry basis, milled rice has an approximate composition of: starch, 90%; protein, 8%; fat, 0.8%; and ash, 1.2%. Assuming an average moisture content of 10%, wet basis, the composition of rice would be: moisture, 10%; starch, 81%; protein, 7.3%; fat, 0.7%; and ash, 1%. The amylose content of nonwaxy milled rice may constitute 7 to 33% of its dry weight, or 8 to 37% of its starch content

(Holston, 1972). Waxy (glutinous) rice has an apparent amylose content of about 0.8 to 1.3%. Gelatinization temperatures are different for these two kinds of rice.

TABLE 1. Composition of Fish (Heen and Kreuzer, 1962)

CATE-GORY	TYPE OF FISH	COMPOSITION				
		Example	Moisture	Protein	Fat	Ash
A	Low oil, high protein (under 5%)(15-20%)	Pacific Cod	81.5	17.9	0.6	1.6
B	Medium oil, high protein (5-15%) (15-20%)	Mackerel	67.5	18.0	13.0	1.5
C	High oil, low protein (over 15%) (under 15%)	Siscowet Lake Trout	52.5	11.3	36.0	0.53
D	Low oil, very high protein (under 5%) (over 20%)	Skipjack Tuna	72.4	26.2	0.7	1.5
E	Low oil, low protein (under 5%) (under 15%)	Butter Clams	83.0	13.3	1.3	1.9

MATERIALS AND METHODS

Composition of rice and fish mixture. From previous work it appeared that the rice-fish mixtures should have moisture contents varying between 25 and 40%. Using Mackerel fish having a moisture content of 67.5% (Table 1) and assuming that rice has a moisture content of about 10%, wet basis, the mixtures of rice and fish shown in Table 2 were considered. Table 3 shows the estimated composition of the extruded product after drying to 8% moisture content.

TABLE 2. Composition of Rice and Fish Mixture Before Extrusion

Mixture	Rice	Fish	COMPOSITION*			
			Moisture	Carbohydrate	Protein	Fat
I	100	100	38.8	40.5	12.7	6.9
II	100	75	34.6	46.3	11.9	6.0
III	100	50	29.2	54.0	10.9	4.8
IV	100	25	21.5	64.8	8.2	3.2

*When the above mixtures are dried to about 8 percent moisture content, wet basis, the percentage composition of the dried product is as shown in Table 3.

Carolina rice and fresh Mackerel fish were purchased locally. The rice was ground in a laboratory grinder. Following grinding, the rice was sieved through 20 and 30 mesh sieves. The portion of the ground rice

which passed through the 20 mesh screen and was retained on the 30 mesh screen was used in the experiments. Deboned fish was cut into pieces and was ground in a laboratory meat grinder.

TABLE 3. Estimated Composition of Extruded Product After Drying

Mixture	Proportion of Rice to Fish Before Extrusion		Percentage Composition			
	Rice	Fish	Moisture	Carbohydrate	Protein	Fat
I	100	100	8	60.8	19.0	10.3
II	100	75	8	65.2	16.7	8.4
III	100	50	8	70.1	14.1	6.2
IV	100	25	8	75.9	11.1	3.7

Rice and fish in the proportion of 2:1 were mixed in a laboratory mixer and then processed through the extruder. The temperatures in the first two extruder zones were maintained at 50°C and 70°C throughout the experiments. The temperature of the third zone ("the barrel temperature", including the die) was operated at temperatures of 125°C, 150°C, and 175°C. A 1/8-inch (0.3175 cm) diameter die was used in all trials. Screw speed was fixed at 50 rpm.

Brief description of the extruder and performance equations. A 3/4" extruder made by Haake, Inc., Saddle Brook, New Jersey, was used to carry out the experiments. From the available knowledge of the modelling of extruders, the capacity, power requirements and the residence time of the extruder were determined. These quantities depended upon the specification of the geometry of the screw, operating parameters of the extruder, and the properties of the rice-fish mixture. Details of these quantities were required before the characteristic equations for the capacity, power requirements and residence time of the screw could be determined.

In the absence of any data on the viscosity of fish-rice mixtures, it was decided to use corn flour dough as a model and its viscosity was determined by the equation proposed by Harper (1979) at a temperature of 150°C, a moisture content of 32.5% and a screw speed of 50 rpm. As the viscosity changes with the temperature and moisture content of the product and with the speed of the screw, the use of the equations below has many limitations. It was also found that equations (1) and (4) predict values for power required and pressure differential across the die that are considerably smaller than those normally encountered.

All of the following performance equations* required assumption of an average value of viscosity:

*Derivations of performance equations are not included in this paper. They may be secured by request to the authors.

1. Capacity: Q in cm^3/min,

$$Q = 0.643\ N$$

where N is the speed of the screw in rpm

2. Power required, E, in watts to rotate the screw

$$E = 5.053 \times 10^{-6} N$$

3. Residence time of material, $\bar{t}$ in min inside the extruder

$$\bar{t} = \frac{25.09}{N}$$

4. Pressure, $(\Delta P)_d$ in N/M^2 at the die of the extruder

$$(\Delta P)_d = 117.2\ N$$

Choice of operating variables. Our objective was to achieve two characteristic properties of the extruded rice-fish mixture for its two different uses. The product should have "crispiness" for one of its uses and "chewiness" for the other. It is possible to vary the starch-protein ratio in the feed by varying the proportions of fish and rice to achieve the desired qualities of the extruded product. High initial moisture content of the fish, however, might place limitations on the achievable starch to protein ratios.

Initially the following ranges of operating variables were selected:

Barrel temperature:	120°C to 180°C
Feed moisture:	25% to 40%
Screw speed:	50 rpm to 125 rpm
Ratio of rice to fish:	1 to 4

Choice of these ranges was arbitrary. The judgment was based on experiments carried out on other products by other researchers.

Wet basis moisture content determinations for the ingredients were: ground rice, 10.05%; ground fish, 77.6%. Moisture content of 2:1 mixture was

$$\frac{(0.1005)\ (2) + 0.776}{3} = 32.6\%$$

RESULTS

Extruder output at various barrel temperatures was as follows:

Sample	Barrel Temp. (°C)	Pressure at Die (psi)	Output (g/min)
1.	125°C	500	18.47
2.	150°C	500	16.64
3.	175°C	500	20.21

It was difficult to come to a definite value for the specific volume of the extruded product. Material processed at a barrel temperature of 125°C was not puffed; material processed at 150°C was slightly puffed; and the material processed at 175°C was puffed and curled.

The "fishy odor" of the Mackerel was almost wholly removed during processing.

Processed material kept at room temperature for several weeks did not show visible signs of degradation.

When rehydrated, the material exhibited the "chewiness" of fish.

CONCLUSIONS

Coextrusion of ground fish and high carbohydrate materials such as rice can yield a product that is shelf stable. Textural properties of the product can be made quite variable by varying mixtures and extruder operating parameters. The process also has potential for improving the utilization of so-called "trash fish" and fish "pieces" presently used for animal feeds, fertilizers, and other low-grade purposes.

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A MORPHOLOGICAL STUDY OF MUSCLE PROTEOLYSIS IN THE TAILS OF MACROBRACHIUM ROSENBERGII

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INTRODUCTION

As the cost of harvesting seafood from the oceans increases, alternative means of production, such as aquaculture, are being investigated more intensively. Freshwater shrimp have become popular in the last decade and, of the many species tested, Macrobrachium rosenbergii has emerged as an animal suitable for intensive culture.

There have been many studies on the nutrition, biology and mechanics of growing Macrobrachium, but few concerning the consumer acceptance of the product. Ultimately, the success of the product will be determined by whether or not it can compete in consumer markets. Macrobrachium could be an ideal alternative to marine shrimp, but it has a postmortem texture problem. The texture of the cooked product is often described as mushy, soft or chalky if it is held on ice two days or more before being cooked. A statement from the National Aquaculture Plan of 1980 sums up the problem:

> "Additional work is required on processing and packaging whole shrimp; the "mushiness" problem also needs attention. Mushiness, coupled with variations of quality and packaging of imported fresh shrimp, gives many U.S. wholesalers a negative opinion of shrimp. Lower prices offered for imported shrimp compared to those of marine shrimp of equal size reflect this".

Macrobrachium have an appearance similar to large crawfish. Like crawfish, the edible meat is taken from the tail. They are generally split down the middle of the underside and broiled in a butter sauce with the heads still attached. They are served several to a platter in the same manner as small lobsters.

A large majority of Macrobrachium consumed are grown in tropical waters. They are harvested and frozen in large blocks, then shipped to consumer markets. Often, they are thawed and held on ice several days before being consumed, but this practice results in a much deteriorated product.

Nip and Moy (1979) investigated the effects of post-thawing, ice chilled storage on the quality of Macrobrachium at the University of Hawaii. They determined that storage on ice for 48 hours significantly decreased the firmness of the product when measured with a PEP texture tester and by sensory evaluation methods. They also found that relatively little difference existed in the quality of fresh and frozen-thawed shrimp held on ice for 48 hours. This indicated that when proper freezing techniques are used, the quality of the product is not significantly affected.

Preliminary investigations at Texas A&M showed that the texture of 48 hour old fresh Machrobrachium stored on ice with the heads left on was significantly less firm than the texture of those stored with the heads removed.

The implications were that leaving the heads on the shrimp during storage was in some way affecting the textural quality.

The head portion of Macrobrachium really includes both the head and the carapace. All the major digestive organs are located in this region. The vein, which is also involved in digestion, extends the length of the tail on the dorsal surface. It was suspected that digestive enzymes from the hepatopancreas and the vein were migrating into the muscle tissue during postmortem storage and causing proteolysis of the tissues and subsequent textural deterioration. When the head was removed, along with the digestive tissues, less deterioration of textural quality was noticed.

The objective of this study was to examine histogically the muscle tissues of the tail for evidence of proteolysis.

PROCEDURE

Live Machrobrachium were obtained from experimental ponds at Texas A&M. They were killed, treated and held in ice for 48 hours, and then they were placed in fixative and processed for observation with light and electron microscopy.

One treatment consisted of leaving the heads on the animals and rupturing the hepatopancreas with a knife. A second treatment consisted of deheading the animals and removing the veins. The samples were washed well and injected with 0.1 ml of a trypsin solution (5 mg/ml). A third treatment consisted of deheading the animals and rinsing them well but leaving the veins intact. All the animals were held in ice for 48 hours.

Two tissue samples were taken from a tail muscle that was near the head in the six ruptured, trypsin treated and washed Macrobrachium tails. Then two additional samples were removed from a muscle adjacent to the vein of only the six washed deheaded Macrobrachium tails.

All the samples were fixed in a solution of 3% gluteraldehyde/2% paraformaldehyde in 0.1 M phosphate buffer held at 4°C. They were washed and placed in 4°C 1% aqueous osmium textroxide, washed again and placed in 0.5% aqueous embedded in an epon 12 epoxy resin. They were sectioned for light microscopy and transmission electron microscopy on an MT-2-B ultramicrotome.

The thick sections for brightfield microscopy were stained with 1% methylene blue. The thin sections for TEM were stained with lead citrate and uranyl acetate.

RESULTS AND DISCUSSION

Proteolysis in postmortem muscle has long been considered to be one of the possible mechanisms by which tenderization occurs. This subject has been investigated extensively in red meats. Summarily, it was found that proteolysis, as measured by classical methods, such as increased free amino acids and non-protein nitrogen, was not related to early changes in tenderness. Evidence did indicate, though, that specific changes in the myofibrillar proteins may have been occurring.

Research by Goll et al. (1971) indicated that trypsin acted on muscle tissue to simulate changes which occurred in postmortem muscle during postmortem tenderness increase. Several enzyme systems with the ability to degrade specific muscle proteins have been identified in muscle tissue. The major enzyme thought to be responsible for proteolytic breakdown in muscle at neutral pH is the calcium activated protease (CAF), which was isolated and purified by Daton et al. (1975). This enzyme causes partial degradation of myofibrils by removing a-actinin from the z-line and hydrolyzing tropomyosin, troponin-T, troponin I, C protein, connectin, and desmin. Unpublished data has shown that CAF is localized only at the z-line and at the sarcolema.

Though CAF may be responsible for some of the proteolysis that occurs in meats, it is by no means the only proteolytic system responsible for tenderization. Postmortem tenderization is probably due to several enzyme systems working together, each with a catalytic effect on the other.

Proteolytic activity can be seen in light micrographs of muscle tissue that was held on ice for 48 hours. Tissue taken from washed deheaded samples is illustrated in Figure 1. The structural integrity of the myofibers and z-lines is intact. Z-lines appear as white cross bars all throughout the tissue. The myofibers are not greatly separated, indicating that the sarcoplasmic proteins are fairly intact. Tissues taken from the vein area (Figure 2) show evidence of slightly increased proteolytic activity. The z-lines were illustrated by white lines or cross bars. There is more evidence of gapping between myofibers; however, the tissue still appears fairly cohesive. Muscle tissue from samples that were taken from Macrobrachium with the head left on and the hepatopancreas ruptured is illustrated in Figure 3. The dark bands throughout the tissue are the location of z-lines. They are dark, presumably because they are no longer intact and the space left in the tissue tends to trap stain. Gapping in the sarcoplasm is evident, and the tissue looks more disorganized than that in Figures 1 and 2. Tissue taken from deheaded washed samples treated with trypsin is illustrated in Figure 4. The integrity of the tissue is very disrupted. Sections of myofibers several sarcomeres in length are evident throughout the tissue, indicating complete breakdown of z-lines. There is also wide gapping between myofibers, indicating breakdown of proteins in the sarcoplasm.

Transmission electron micrographs of the samples showed more detail of proteolytic activity. Z-lines in tissue from washed deheaded samples are illustrated in Figure 5. The same material is illustrated in this micrograph as in Figure 1, but at a higher magnification. The z-lines are still intact and myofibrils are organized in a normal manner. Tissue in Figure 6 corresponds to that illustrated in Figure 2. Z-lines are still evident, but there is some relaxation apparent in the structure of the myofibrils. Complete absence of z-lines is illustrated in Figure 7, a micrograph of tissue from samples with the hepatopancreas ruptured. Myofibers are disorganized and much of the protein between myofibrils is no longer structurally intact. Tissue from trypsin treated samples, illustrated in Figure 8, is similar to that in Figure 7. The z-lines are absent and the sarcoplasm is widened. The integrity of the tissue has disintegrated substantially.

Tissue that was not exposed to enzymes associated with the digestive tract did not have evidence of proteolytic activity associated with tenderization in other meats. The ruptured and trypsin treated samples had gapping

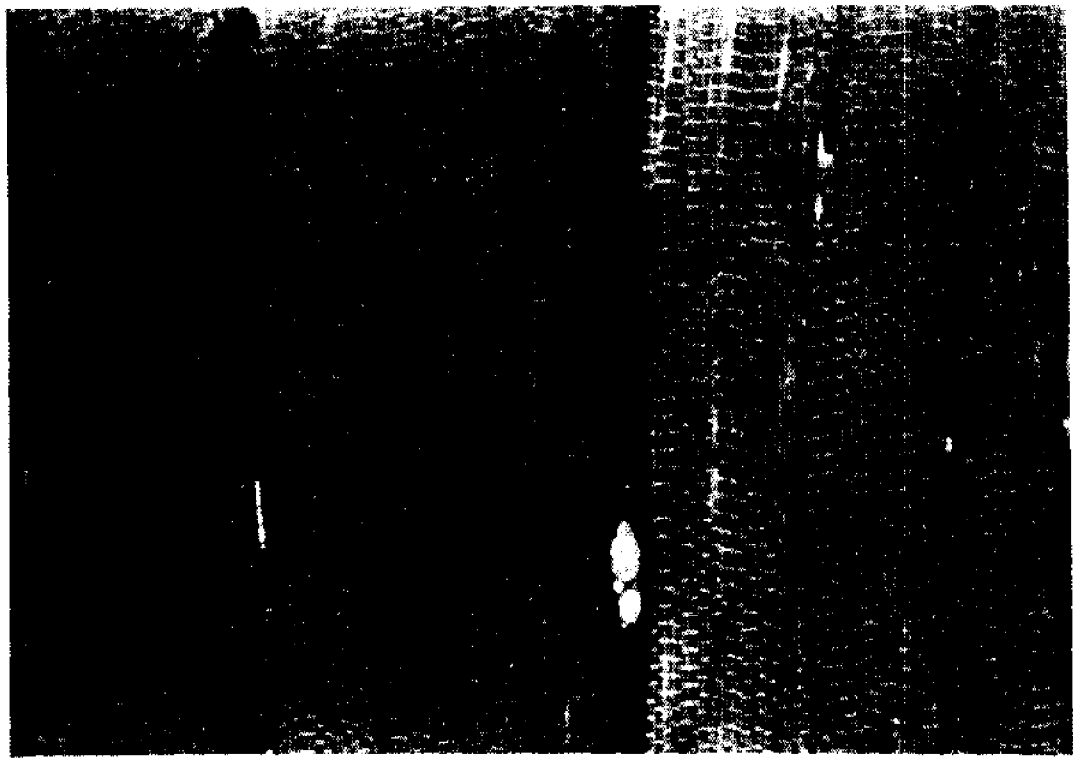

Figure 1. Light micrograph of normal muscle tissue after 48 hours on ice. Magnification = 668X.

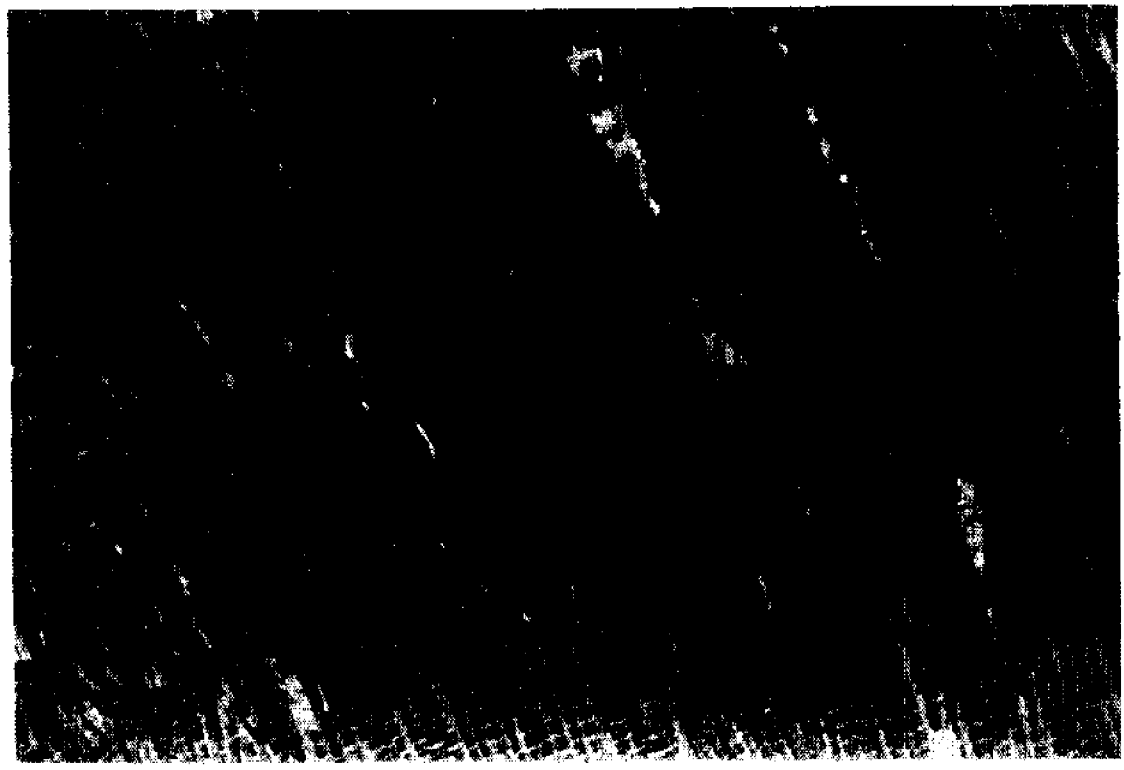

Figure 2. Light micrograph of muscle tissue from the vein area of Macrobrachium tails after 48 hours on ice. Magnification = 663X.

Figure 3. Light micrograph of muscle tissue from the tail of a Macrobrachium that had the hepatopancreas ruptured. Note the dark z-lines compared to those in Figures 1 and 2. Magnification = 666X.

Figure 4. Light micrograph of muscle tissue from the tail of a Macrobrachium that was injected with a trypsin solution. Note the dark z-lines and general degeneration of the tissue. Magnification = 670X.

Figure 5. Electron micrograph of normal muscle fibers in Macrobrachium tails stored for 48 hours on ice. Magnification = 66,000X.

Figure 6. Electron micrograph of muscle fibers taken from the vein area of Macrobrachium tails. Magnification = 66,000X.

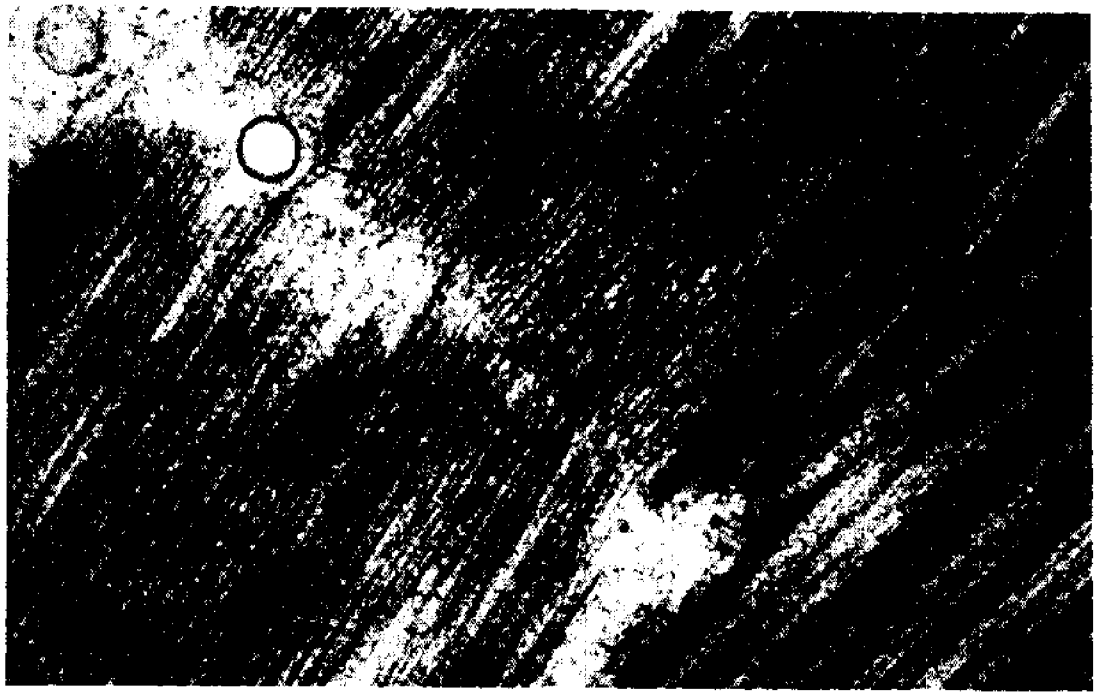

Figure 7. Electron micrograph of muscle fibers taken from a Macrobrachium that had the hepatopancreas ruptured. Note that the z membrane is not present and the myofibrils are disorganized. Magnification = 66,000X.

Figure 8. Electron micrograph of muscle fibers treated with a trypsin solution and stored on ice for 48 hours. Note that the z-line is absent. Magnification = 66,000X.

between myofibers, breakdown of structure at the z-lines and general disorganization of tissues. Also, the histological evidence corrolates with the preliminary taste panel results and suggests that proteolysis does occur due to migration of enzymes from the digestive tracts of animals held on ice with the heads left on.

The problem of mushiness that occurs in Macrobrachium is still not completely understood. Evidence from this study shows that enzymatic digestion of tail muscle tissue is occurring when the animals are stored on ice with the heads left on. How to resolve this problem is still a matter that needs attention. The enzyme systems involved and possible ways to inhibit them will be the next questions addressed.

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FORECASTING EXVESSEL SHRIMP PRICES FOR THE NORTHERN GULF

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INTRODUCTION

The United States is the largest single market for shrimp. In spite of being the second largest world producer of shrimp, domestic production meets about half of the country's demand. Imports from major producers such as Mexico, Ecuador, and India supplement domestic landings to provide the supply needed to meet market demand. Final demand is met throughout the year by release of foreign shrimp from inventory and to a lesser extent fresh supplies. The sale of shrimp through institutional and restaurant outlets is well known to account for over 60 percent of consumption. This consumption pattern, the seasonality of landings, and the importation of shrimp combine to make it essential that supplies be secured by market intermediaries well in advance of ultimate purchase by consumers. Buyers and brokers in the market chain must take positions in the market long before a sale to the final consumer occurs. Insight to supply and demand factors in determining shrimp prices within a twelve month period can be a valuable aid in taking a position in the market.

Three developments in 1981 signaled additional importance to the acquisition of a better understanding of exvessel shrimp pricing. Short-term interest rates in the high teens and low twenties during 1981 were certainly a new factor in the shrimp marketing system. A system which is unable to meet demand without sizable inventories of frozen shrimp. The cost of financing inventories in a period of high interest rates could depress exvessel prices. The extent to which this development influences prices needs to be identified over time.

Pond shrimp culture in Ecuador yielded the success in 1981 that aquaculture proponents in general have long suggested were possible. Between 50 and 65 percent of Ecuador's 20.2 million pounds of shrimp exported to the United States in 1980 was pond grown (Fish Boat 1981). The 1981 pond production was 30 percent higher than 1980. Shipments to the United States in 1981 will approach 24 million pounds (NMFS 1981). Expansion in acreage and increased yield will continue in the near future. There are implications for the United States shrimp market of major advances in aquaculture. The fact that the shrimp can be grown to size, harvested, and sold in a manner conducive to counter undesirable price fluctuations will introduce a new element in shrimp price determination. The problem in the future for culturists will be one of shrimp price forecasting to facilitate maximum generation of revenue for culture businesses (Collins and Stowe 1981).

The third element of the 1981 shrimp situation was the use of a 50 day closed season off Texas in an effort to increase the catch and average size of shrimp. This effort under the auspices of the Gulf of Mexico Fishery Management Council signals a major perturbation in the historical process of price determination. Benefits of a lengthy closed season will depend in large part on the impacts the closure has on shrimp prices. As quantity and sizes are altered during and after the closure, a price response is certain. The extent to which all other determinants of the exvessel shrimp price interact with the physical quantity of shrimp produced by the closure will eventually reveal whether the benefits and their incidence are worth the costs.

The research outlined in the paper was prompted by an absence of academic work on forecasting and three 1981 developments which impact shrimp prices. It is an opportune time for an attempt to fill the void. The nature of shrimp price movements, forecasting using historical patterns, and multiequation models are used in the paper in an initial attempt to gain insight.

THE NATURE OF SHRIMP PRICE MOVEMENTS OVER TIME

Time series in business and economic applications typically exhibited three types of movements through time: seasonal, trend, and cyclical. Seasonal movements describe the pattern of change recurring within a one year period. Trend refers to the long term movement of a series. Cyclical movements describe cycles of expansion and contraction that require more than one year to complete. The commonly referenced "business cycle" is an example of a cyclical movement in business activity over time. The monthly exvessel price of 26-30 count shrimp (heads-off) was analyzed for seasonal, trend, and cyclical movements. The results of this analysis are briefly summarized as follows.[1]

The price series begins with a price of $0.57 per pound in January 1961 and ends in June 1981 with a price of $3.86 per pound. Price ranged from a low of $0.57 to a high of $5.15 per pound in June 1979. A data base of twenty years was selected so that recurring cyclical patterns could be identified.

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[1]The three components of variation in the shrimp price series were derived using a procedure called multiplicative decomposition (Bowerman and O'Connell 1979, Granger 1980). The mathematical model behind this procedure can be written:

$$P_t = SN_t \times TR_t \times CL_t \times IR_t$$

where:

P_t = price in period t.
SN_t = seasonal component in period t.
TR_t = trend component in period t.
CL_t = cyclical component in period t.
IR_t = irregular component in period t.

The multiplicative model is more appropriate than an additive model when the amplitude of the cyclical component is increasing over time.

A graph of seasonal price indexes is shown in Figure 1. Each monthly index shows the relationship between prices in that month and the average for a typical year. The nature of seasonal variations in exvessel shrimp prices is such that beginning in January prices are about at the yearly average. The price rises in the first part of the season to a high in May where prices are approximately five percent above the yearly average. From May prices start to decline until September when they turn back up towards the annual average and continue upward throughout the remainder of the year.

The seasonal indexes can be used to make short-run predictions of price. Say, for example, we want an estimate of the price in November, 1981. Assuming June is the latest month for which actual prices are available the predicted price in November is:

$$\frac{P_J}{SI_J} = \frac{\hat{P}_n}{SI_n}$$

or

$$\frac{3.89}{102.9} = \frac{\hat{P}_n}{97.7} \qquad \hat{P}_n = 3.69$$

where:

P_J = the actual price of June, 1981 ($3.89)

SI_J = the seasonal index for June.

SI_n = the seasonal index for November.

$\hat{P}_n$ = the predicted price for November.

In this case the price move from June to November is estimated to result in a decrease of 20 cents per pound. We are, of course, only considering the seasonal component of price variation in making this prediction. A concise discussion of the calculation, interpretation, and application of seasonal indices can be found in Purcell (1979, pp. 154-159).

The nature of the trend component in the deseasonalized shrimp price series was estimated with the use of ordinary least squares regression. Deseasonalized prices were regressed on time in months to estimate the linear relationship between prices and time.[2] A curvilinear relationship was also estimated by regressing deseasonalized prices on time and time squared. The linear trend component is such that price is estimated to increase about one and a half cents each month throughout the data series. The trend in price per month estimated by the quadratic line depends on where in the data series the change in time takes place. For example, using the quadratic trend line price is estimated to increase about one half cent between January and February, 1965. The estimated price increase between the same two months in 1980 is four cents per pound.

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[2]Deseasonalized prices are obtained by dividing the original prices by the seasonal index corresponding to the month the price was observed. Using deseasonalized prices decreases the possibility that the estimate of the trend line will be effected by seasonal variation.

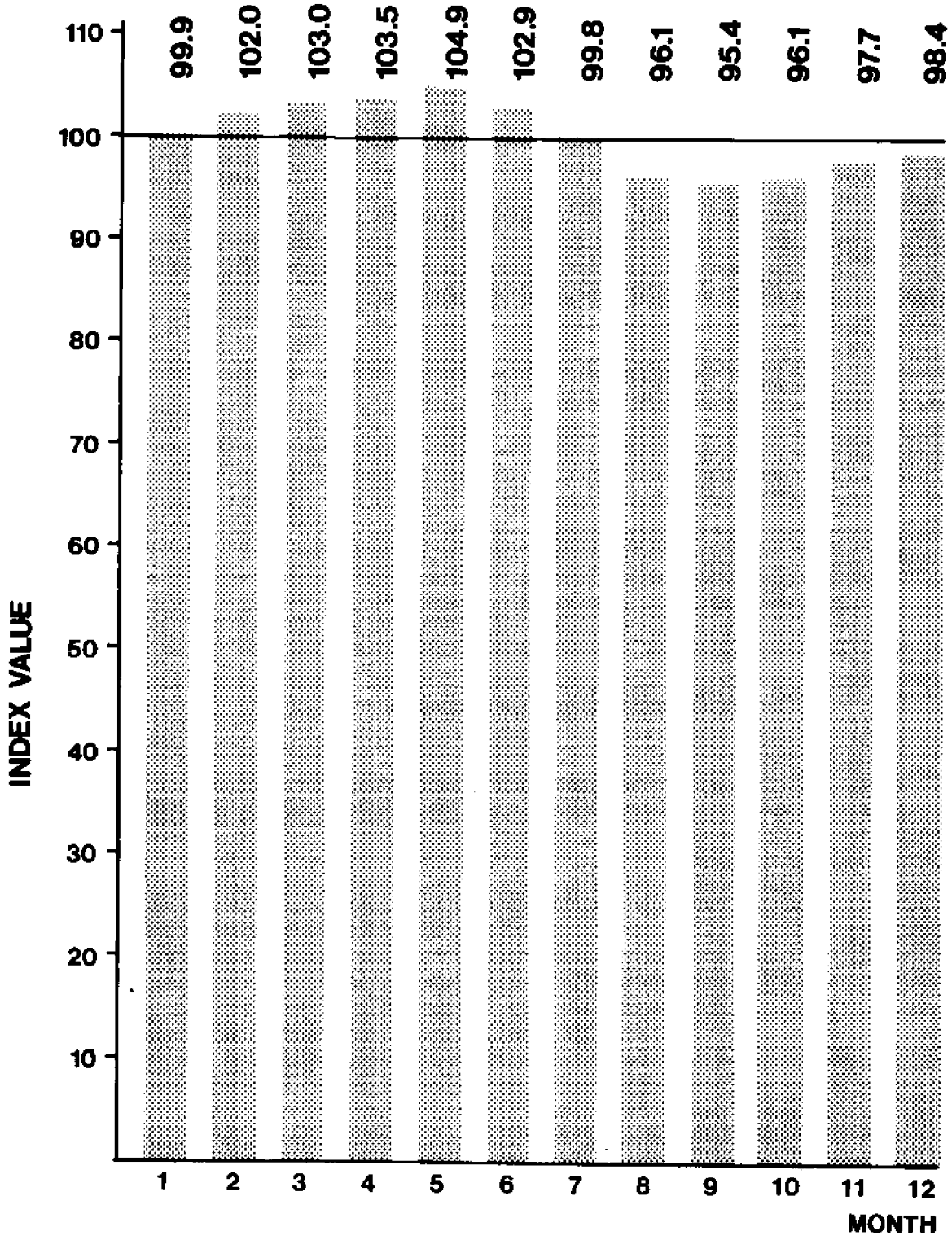

FIGURE 1. Seasonal price indices of 26-30 count shrimp at the exvessel level, 1961-1981.

Because of the superior fit and the greater intuitive appeal, the quadratic trend line was used to estimate the trend component in the price series. Both equations are plotted in Figure 2 against the deseasonalized data. A visual inspection of Figure 2 also leads to the choice of the quadratic equation as the best estimate of trend.

The calculation of the cyclical factors of price variation is possible after trend and seasonal movements have been isolated. The cyclical components of the price series are shown in Figure 3. It is obvious from visual inspection of Figure 3 that shrimp prices do not follow a consistent cyclical pattern. However, shrimp prices have in the past exhibited periods of steady growth followed by steady decline. For example, in the latter part of 1977 prices turned around from a cyclical fall which had lasted about two years and began a cyclical climb. Mid-1979 marked a cyclical turning point where the cycle reached a maximum and once again started a downward phase. This phase persisted through 1981. Although actual prices in some cases increased between mid-1979 and the present, prices were in a downward trending phase of a cycle. This becomes apparent after seasonality and long term trend are removed from the series. The irregular nature of previous cycles makes prediction of the next turning point in shrimp prices extremely difficult.

FORECASTING USING HISTORIC PRICE PATTERNS

The cyclical movement in shrimp prices has been observed to be inconsistent from cycle to cycle. It is not possible to forecast future cyclical movements based on a "typical" cycle since one could not be identified. Seasonality and long-run trend have followed a relatively consistent course making these components easier to identify and project.

Price predictions made using only seasonal and long-run trend factors are likely to be reasonably accurate during periods when the cyclical component is near one.[3] However, such periods are quick in passing as the cycle is usually moving from a high to a low and visa versa (see Figure 3). A more complicated econometric model would be desirable if predictions of price are made in periods where the cyclical component cannot be disregarded. Such a model would incorporate variables that are thought to cause or influence cyclical movements in shrimp prices.

Average monthly exvessel shrimp prices are available for 7 count sizes (Shrimp Statistics). One may want to know if the results from the analysis of the price of 26-30 count shrimp can be applied to the other count sizes. To answer this question a technique called principle component analysis was applied to the 7 series of prices (Doll and Chin 1970; Anderson 1958; Morrison 1967). Principle component analysis was used in this study to determine if the 7 price series have historically exhibited similar movements through time. The results of the analysis strongly suggest that the 7 series of prices display the same type of variation.

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[3]A cyclical component near one implies that the price cycle has little influence on shrimp prices. Notice from the model presented in footnote 1 that a cyclical component of 1.0 results in no impact on price from this component.

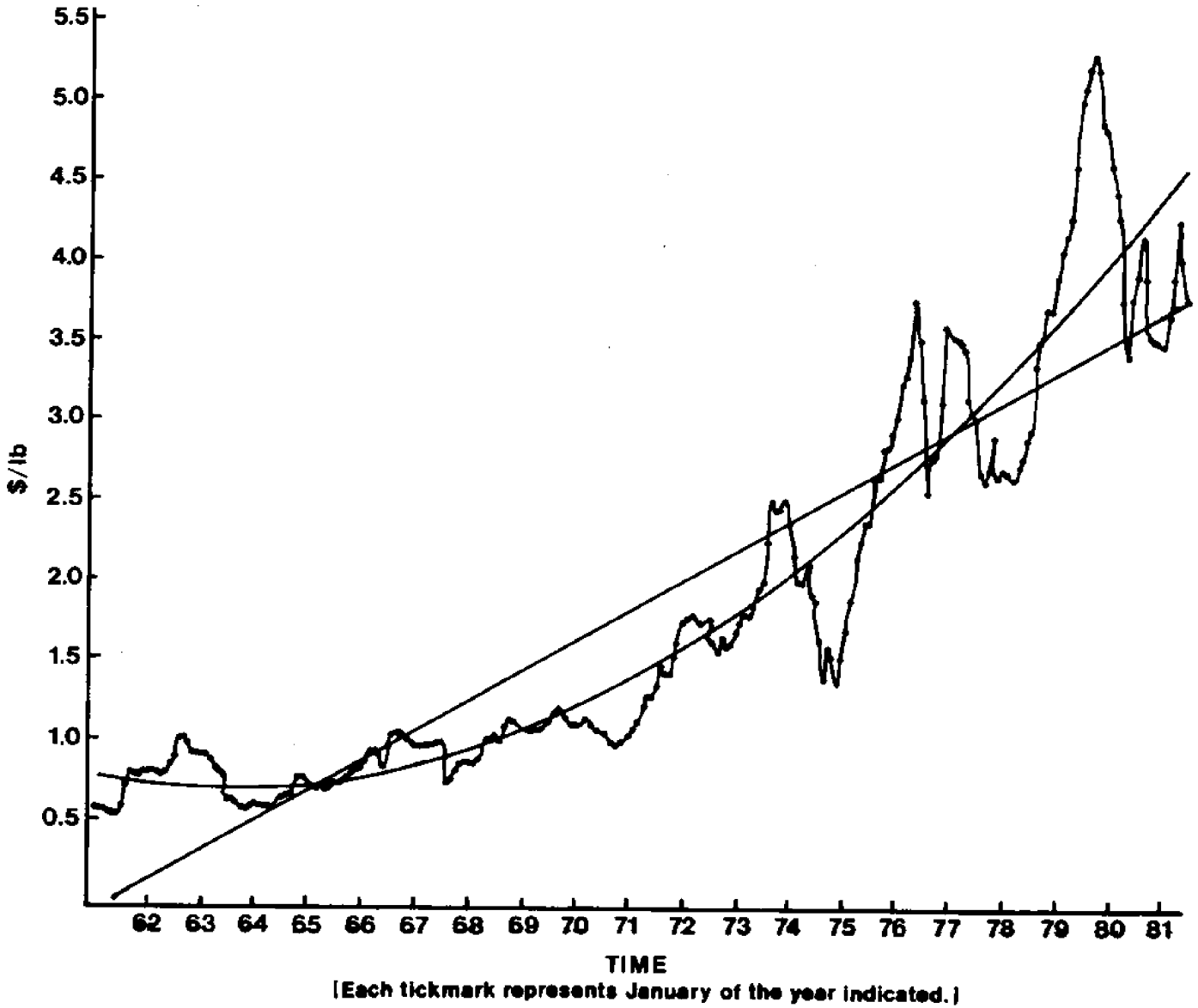


FIGURE 2. Plot of monthly deseasonalized prices, quadratic and linear trend lines.

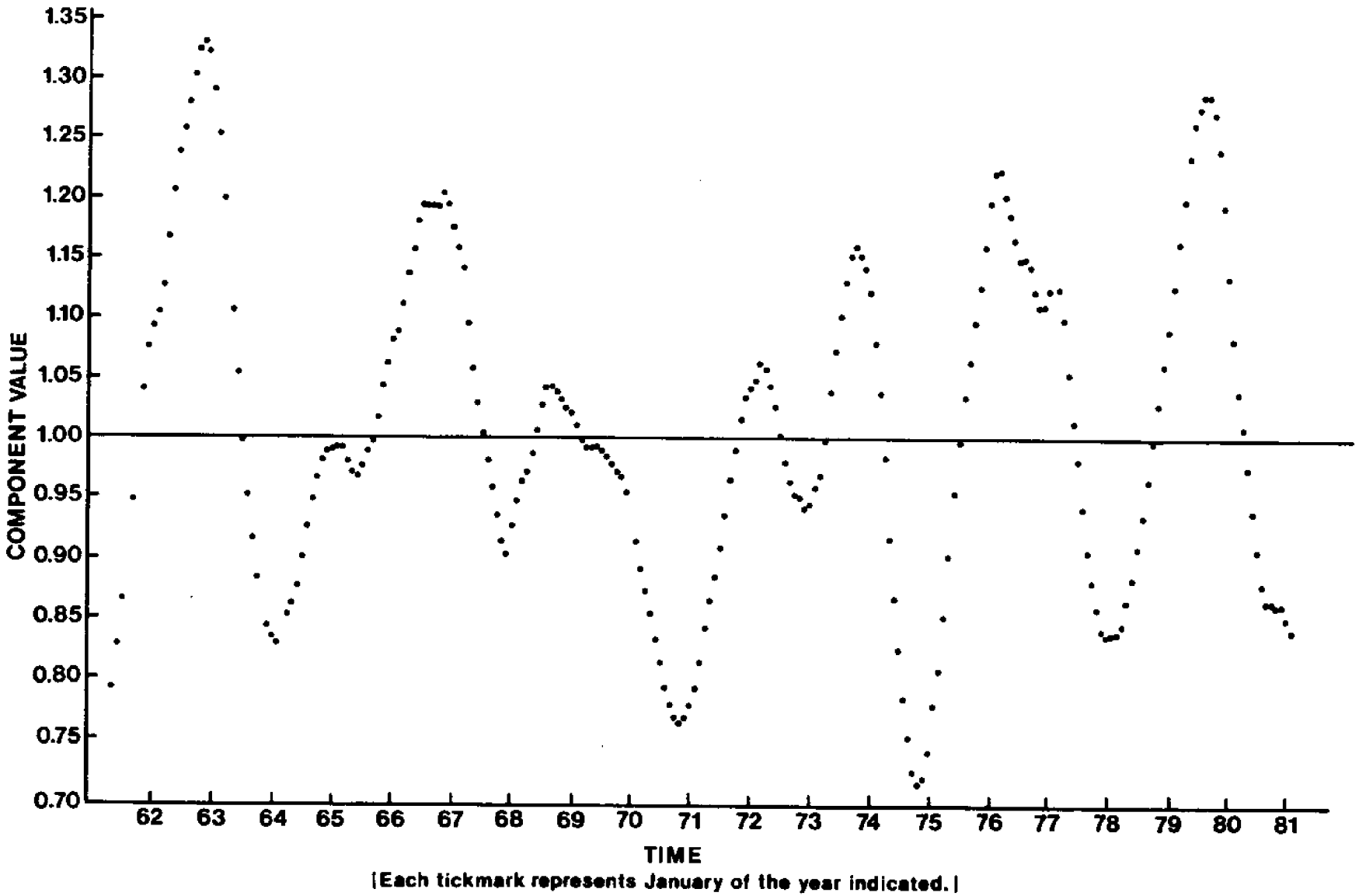


FIGURE 3. Plot of monthly cyclical components of exvessel prices, 26-30 count shrimp, 1961-1981.

It is, therefore, concluded that the seasonal indexes and long-term trend components exhibited by the price of 26-30 count shrimp are applicable to the other six count sizes.

THE FORECASTING MODEL

Single equation models typically used in forecasting economic variables can be classified as either time series equations or equations that imply causality. The decomposition of price variation over time into seasonal, trend, and cyclical movements from the previous section is a type of time series model. A causal model has variables in the equation which are theoretically expected to influence the value of the variable for which a forecast is desired. Other than the initial analysis of seasonal, trend, and cyclical variation our research has primarily dealt with models which imply causality.

An example of a single equation model based on hypothesized causal effects is price expressed as a function of the supply of shrimp and disposable income. Assuming a linear relationship the equation can be written as:

$$P_t = b_0 + b_1 Q_t + b_2 Y_t \tag{1}$$

where:

P_t = the exvessel price of shrimp in period t.
Q_t = the quantity of shrimp supplied in period t.
Y_t = the level of disposal personal income in period t.
and b_0, b_1, and b_2 are coefficients to be estimated.

It is, of course, possible to include other independent variables which have an impact on price in equation 1. One advantage of the causal type model is that price predictions will react to predicted changes in economic prosperity, as measured by Y. A price change in direct response to changes in income would not be possible with a time series model.

For a model such as equation 1 to be applicable it must be assumed that the values of the independent variable (Q and Y) are not influenced by values of the dependent variable P. That is, it must be assumed that P is dependent on Q and Y but Q and Y are determined independently of P. When there is an independent variable in the equation whose value in a given period is affected by the value of P (the dependent variable) the two are said to be jointly-dependent (also called jointly-determined). If P is jointly-dependent with Q, for example, the coefficients estimated by the ordinary least squares procedure will be unreliable. In addition to unreliable coefficient estimates it will probably be very difficult to find predictions of Q on which to base the forecast of P if the two are jointly-dependent. The single equation model is inadequate when one or more variables in the equation are jointly-determined with price.

AN INTRODUCTION TO MULTIEQUATION MODELS

A multiequation model is simply a system of equations where each equation describes the behavior of one of the jointly-dependent variables

in the model.[4] The number of equations in the system will equal the number of jointly-dependent variables. Consider the following model:

(2) $$P_t = b_0 + b_1 Q_t + b_2 Y_t$$

(3) $$Q_t = \alpha_0 + \alpha_1 P_t + \alpha_2 B_t + \alpha_3 F_t$$

where:

P, Q, and Y are defined as before.
B = biological factors affecting shrimp population levels.
F = costs of factors of production (e.g., fuel, groceries, nets, etc.).
and b_0, b_1, b_2, α_0, α_1, α_2, α_3 are coefficients of the system to be estimated (also called structural coefficients)

The model contains two jointly dependent variables (P and Q) and three independent variables (Y, B, and F). Equations 2 and 3 are called structural or behavioral equations since they describe the relationship between the variables in the model. The price equation 2 describes the behavior of the demand sector of the system while the response of the supply sector is depicted with equation 3. Equations 2 and 3 are referred to as a system of simultaneous equations.

The coefficients in the two equation system must be estimated using a technique that accounts for the simultaneity between P and Q. That is, the two equations are estimated together, instead of separately without regard to the dual dependency between P and Q (as per equation 1). There are several methods available for estimating simultaneous systems of equations. For a discussion of these methods see Theil (1971), Johnston (1963), and Goldbenger (1964). For the purposes of this paper it is more important to understand how the coefficients of the system are used to make forecasts.

The jointly-dependent variables in a system of simultaneous equations can be expressed as a function of only the independent variables. This expression, accomplished through simple algebraic manipulation of the structural equations (e.g., equations 2 and 3) is referred to as the reduced form equations. The reduced form equations are particularly useful for forecasting because the equations no longer contain jointly-dependent variables to the right of the equality sign. Consider again the system of equations defined by equations 2 and 3. To forecast price, for example, using equation 2 would require an estimate of Q. Since Q is determined simultaneously with P an estimate of Q on which to base a prediction of P could not be obtained. The reduced form equations eliminate this problem since Q is not an argument in the equations. Assume that the coefficients in equations 2 and 3 have been estimated by one of the available methods for estimating simultaneous equations. The reduced form

[4]The reader interested in a more detailed introduction to forecasting with multiequation models is referred to Suits (1962). An indepth introduction to the theory and mechanics of multiequation models can be found in Theil (1971), Goldberger (1964), and Johnston (1963).

equations are then calculated by solving the structural equations for P and Q in terms of Y, B, and F and are:

$$(4) \qquad P_t = b_0' + b_1' Y_t + b_2' B_t + b_3' F_t$$

$$(5) \qquad Q_t = \alpha_0' + \alpha_1' Y_t + \alpha_2' B_t + \alpha_3' F_t$$

The primes above the coefficients are used to indicate that they are different from the coefficients of the structural equations.

A SYSTEM OF SIMULTANEOUS EQUATIONS FOR FORECASTING SHRIMP PRICES

The model presented in this paper is one which has evolved from several previous specifications. The development of this forecasting model is a continuing process and the model to be discussed is certainly not the final product. The authors feel, however, that the research is at a point where some interesting characteristics of the model and forecasts can be conveyed. Some characteristics are discussed after a brief introduction to the forecasting model. Changes in the model that are presently being considered are also discussed. In its' current form the shrimp price forecasting model is one in which the exvessel price of shrimp, landings from the Gulf, fishing effort, imports, cold storage holdings, and consumption are determined simultaneously within the framework specified by six structural equations. All equations are linear and are presented in functional form below:

$$(6) \qquad P' = f_6 (S_{t-1}, L', I', C', r, P_{t-1}, P_{t-2}, Q_2, Q_3, Q_4)$$

$$(7) \qquad I' = f_7 (P_{t-2}, X_{t-2}, Q_2, Q_3, Q_4)$$

$$(8) \qquad L' = f_8 (PR_{t-2}, T_{t-2}, TR', M, M^2, M^3, M^4)$$

$$(9) \qquad TR' = f_9 (P', F, L', Q_2, Q_3, Q_4)$$

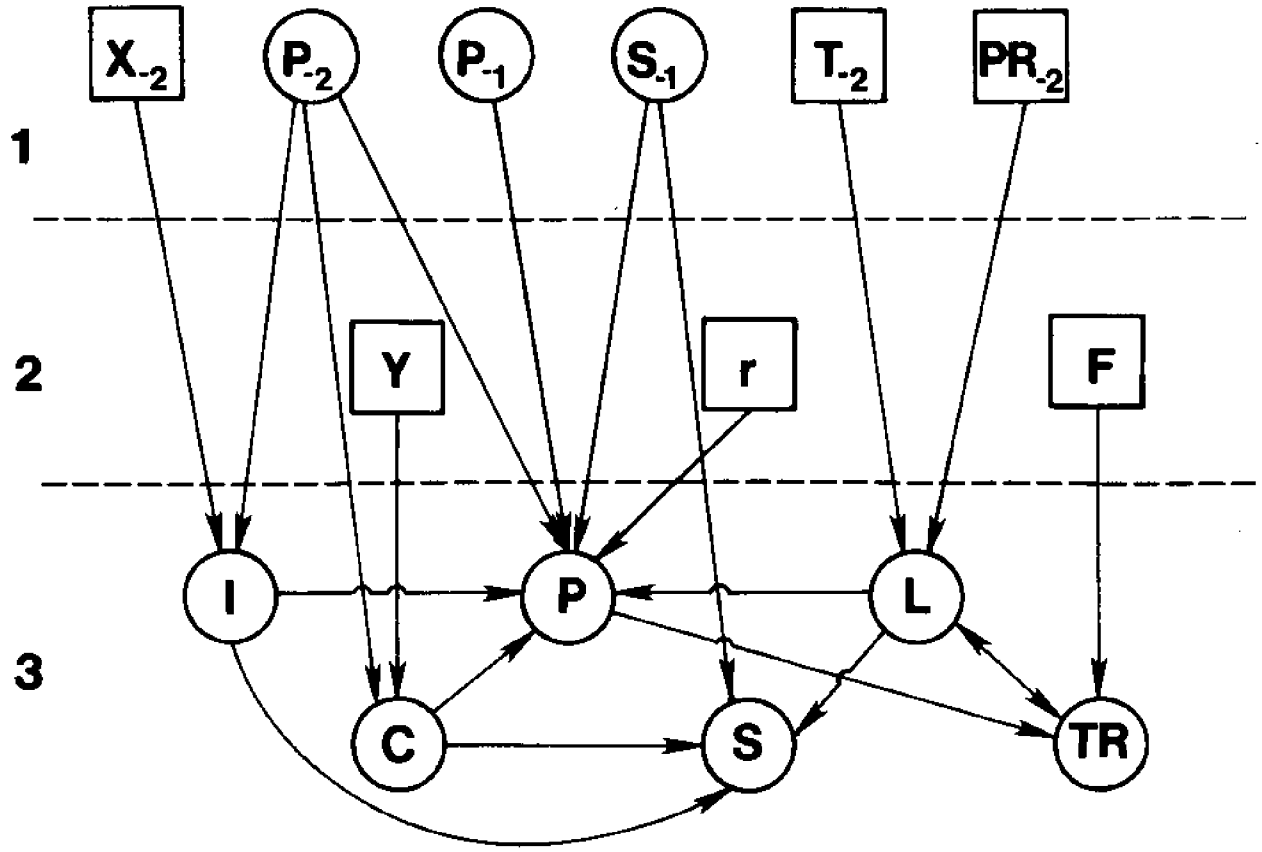
$$(10) \qquad S' = f_{10} (S_{t-1}, L', I', C')$$

$$(11) \qquad C' = f_{11} (P_{t-2}, Y, Q_2, Q_3, Q_4)$$

The primes indicate the jointly-dependent variables in the model. Lagged variables are identified by the subscript $t-\alpha$ where α equals the order of the lag. Monthly data beginning in September 1974 was used to estimate the coefficients in equations 6-11. Variable symbols and a brief description of each variable are listed in Table 1. In addition each variable is identified in Table 1 as being either jointly-dependent or exogeneous (where independent and exogeneous are used interchangeably). The relationships expressed by the equations in the system are presented in flow chart in Figure 4. It is possible to trace the interactions of the variables in the system by referring to Figure 4. For example, one can see how diesel fuel costs effect the price of shrimp by following the arrows. Fuel prices (F) will affect the number of fishing trips (TR) which will have an impact on landings (L) which will cause shrimp price (P) to be changed. Each equation will be discussed below.

TABLE 1. Glossary of Variables

SYMBOL	BRIEF DEFINITION	TYPE
P	Exvessel price of 26-30 count shrimp, northern Gulf (head off, $/lb.)	jointly-dependent
I	Imports, thousands of pounds	jointly-dependent
L	Landings from the U.S. Gulf, thousands of lbs.	jointly-dependent
TR	Number of shrimping trips by Gulf shrimpers	jointly-dependent
S	Cold storage holdings, thousands of lbs.	jointly-dependent
C	Consumption, thousands of lbs.	jointly-dependent
X	Currency exchange rate between U.S. and Japan (yen/$)	exogenous
Y	Disposable personal income (1972 dollars)	exogenous
r	Prime rate of interest on short-term business loans	exogenous
F	Fuel price (diesel, $/gal.)	exogenous
PR	Average precipitation coastal Louisiana	exogenous
T	Average atmospheric temperature, coastal Louisiana (°F)	exogenous
Q_2	Dummy variable indicating the second quarter	exogenous
Q_3	Dummy variable indicating third quarter	exogenous
Q_4	Dummy variable indicating fourth quarter	exogenous
M	Month (Jan=1, Feb=2, etc.)	exogenous



1 Lagged Exogenous and Jointly-Dependent Variables

2 Current Exogenous Variables

3 Current Jointly-Dependent Variables

FIGURE 4. Arrow scheme of model of the shrimp market. Exogenous variables are in boxes and jointly-dependent variables are in circles.

THE PRICE EQUATION

The exvessel price of shrimp was expressed as a linear function of beginning stocks, landings, imports, consumption, the interest rate, exvessel price lagged one and two months, and three quarterly dummy variables. The supply of shrimp in any given month is made up of three sources: beginning stocks, landings, and imports. Consumption was included directly in equation 6 instead of demand shifters such as disposable income and population. Increasing interest rates may have a negative impact on exvessel prices. A highly serially correlated error term made inclusion of the price lags necessary and appropriate.[5] The dummy variables were included to reflect seasonal patterns in shrimp prices.

THE IMPORTS EQUATION

Equation 7 expresses imports as a linear function of the exvessel price of shrimp lagged two months, the rate of exchange between Japanese and American currency, and dummy variables to accommodate seasonal fluctuations. The quantity of imports supplied in the current month is likely to have been based on market conditions from previous months. A lag on price and the exchange rate of 2 months was chosen after considering lags of zero to six months. The United States and Japan are the major competitors for world supplies of shrimp. To reflect the competition for total world supplies the currency exchange rate between these two countries is included in equation 7. As the dollar becomes more valuable relative to the yen (i.e., X increases) suppliers of imported shrimp prefer to sell more of their product in the United States to obtain the now more valuable dollar. Conversely, imports may decrease when the dollar slips in value relative to the yen. Seasonal indicator variables were also included in the import equation.

THE LANDINGS EQUATION

The quantity landed is dependent on the amount of shrimp available for harvest and the extent of fishing effort exerted by the industry. Three species of shrimp (brown, white, and pink) account for 98 percent of annual Gulf landings. Brown shrimp make up 56 percent of total Gulf of Mexico catch and are highest along the Texas, Louisiana, and Mississippi coasts. White shrimp, also primarily found in northern and western Gulf areas, account for 30 percent of annual Gulf landings. Pink shrimp are the only species found primarily in the eastern Gulf of Mexico. Twelve percent of Gulf of Mexico catch comes from pink shrimp.

Brown, white, and pink shrimp are estuary dependent during the early stages of their life cycle (Shrimp Fishery Management Plan, 1979, pp. 4-1 to 4-19). Growth rates during this time will be adversely affected by low salinity levels and water temperatures. There is a well documented correlation between salinity levels in the estuaries and amounts of precipitation

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[5]The Durbin-Watson statistic estimated from application of OLS to equation 6 without the price lags was 0.46. After inclusion of the price lags the D-W statistic was 1.94.

along the coastal areas (Shrimp Fishery Management Plan, pp. 4-18). Average precipitation in coastal Louisiana is used to reflect salinity levels in Louisiana wetlands. Data on precipitation was used instead of salinity levels because the latter was not consistently available on a monthly basis.[6] Average atmospheric temperature was used instead of water temperature in the estuaries as the latter was not available in a consistent and timely fashion.

Shrimp landings follow a consistent seasonal pattern from year to year. Landings are typically low from January through April and high in May, June, and July reflecting the spring season for brown shrimp. The gradual decline in landings after July is interrupted by the fall season for white shrimp which reaches a peak in October. To reflect the two-peaked seasonal production of domestic shrimp a fourth order polynomial in months was included in equation 8.

THE EFFORT EQUATION

Equation 9 describes the response of the shrimp harvesting industry in terms of the number of fishing trips completed in a given month, to changes in the economic and biological conditions facing the industry.[7] The price of shrimp, the price of diesel fuel, the quantity landed, and seasonal dummy variables are all arguments in the effort equation. Depending on the size of the vessel, fuel costs account for 50 to 70 percent of the variable costs associated with making a fishing trip. The rapidly escalating fuel prices of recent years and the large percentage of variable costs attributable to fuel make diesel price a logical choice for inclusion in an effort response function.

THE STOCKS EQUATION

Equation 10 expresses cold storage holdings at the end of the month as a linear function of beginning stocks, landings, imports, and consumption. If landings included Atlantic and Pacific coasts landings and there was no measurement error associated with the other variables, it would be appropriate to express equation 10 as an identity (i.e., all coefficients would

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[6]Previous research has found landings to be negatively correlated with discharge of the Mississippi River (Barrett and Gillespie 1973, Griffin et al. 1976). Mississippi River discharge was included in place of precipitation in some of the trial runs of the landings equation. Precipitation gave somewhat better results and the data is easier to obtain.

[7]The number of fishing trips (TR) could be improved upon as a measure of fishing effort by application of a fishing power index. Such an index would reflect the relative increase in fishing efficiency of individual boat and vessel trips made in 1975 and 1980, for example. A trip made in 1975 probably did not exert the same amount of effort on the resource as a trip made in 1980 because of increases in fishing efficiency. A fishing power index was not constructed because of the time lag involved in acquiring the relevant data.

equal plus or minus 1). The coefficients were not forced to those values because measurement error is likely to be a problem with S and C.[8]

THE CONSUMPTION EQUATION

Current month consumption of shrimp is dependent on exvessel price lagged two months, the current level of disposable personal income, and seasonal dummy variables. Shrimp prices are lagged two months to reflect the lag between exvessel and retail prices. One would also want to include prices of substitute and complementary products to satisfy the dictates of economic theory. Previous studies have not found the price of such products to have a significant impact on shrimp consumption and were, therefore, not considered (Batie 1974, p. 81; Doll 1972, p. 443; Timmer 1968, p. 247; Gillespie, et al. 1969, p. 34).

Before proceeding with a discussion of the results it is useful to discuss the price variable. The price of 26-30 count shrimp (heads off) as reported by the National Marine Fishery Service (NMFS) for the northern Gulf (Louisiana, Mississippi, and Alabama) was selected for use as the exvessel price variable. However, several other possible formulatons of the price variable were available. The NMFS Market News reports exvessel prices for 7 count sizes at three major regions in the Gulf. The authors decided to confine the initial selection to the northern Gulf to make the forecasting model most relevant to applications to the Louisiana shrimp fishery.

As was mentioned earlier a principle component analysis indicated that there is not a significant degree of difference in the variation between the seven price series for the northern Gulf. Simple and weighted average prices were also considered but neither improved the overall significance of the model. Other count sizes were tried in the model as well. However, the results are similar as those obtained when using the price of 26-30 count shrimp.

DEPICTING THE RESULTS

The estimated coefficients of the model are shown in Table 2. The system was estimated using a technique called three-stage least-squares.[9] A detailed discussion of these results, while academically interesting, would not serve the purpose of this paper. However, it is worthwhile to note two aspects of the results in Table 2. First, all coefficients of the model have the expected sign. If this were not the case on might want to explore the validity of the model, data, and/or theory. Secondly, most variables appear to be important (as indicated by the t coefficient) in

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[8]Personal communication with Dr. John Vondruska, Fisheries Development Division, NMFS, Washington, D.C.

[9]There are many econometric textbooks that provide a discussion of three stage least squares (3SLS) and other methods of solving simultaneous systems of equations (see footnote 4).

TABLE 2. Coefficient estimates and t-statistics from 3-SLS solution of model I

P	I	L	TR	S	C	S_{t-1}	P_{t-1}	P_{t-2}	X_{t-2}	Y	r	F	PR_{t-2}	T_{t-2}	Q_2	Q_3	Q_4	M	M^2	M^3	M^4
1.0	-5.9×10^{-5} (2.65)	-1.6×10^{-5} (1.93)			5.8×10^{5} (3.32)	-5.4×10^{-6} (2.92)	1.10 (9.10)	-0.09 (0.72)			-0.021 (2.04)				-0.201 (2.06)	-0.414 (3.67)	0.193 (1.35)				
	1.0							1210 (3.17)	30.2 (3.40)						-28.03 (0.03)	168.8 (0.19)	6117 (6.93)				
		1.0	0.288 (7.59)										-117 (1.56)	90.7 (1.35)				-2382 (0.97)	1011 (1.43)	-121.7 (1.68)	4.47 (1.77)
737.3 (0.91)		2.45 (14.69)	1.0									-714 (1.95)			214.6 (0.10)	-5127 (1.78)	-1284 (0.50)				
	1.20 (4.22)	0.828 (3.37)		1.0	-0.763 (1.65)	0.855 (16.32)															
					1.0			-700 (0.84)		10.0 (0.75)					4152 (2.69)	6451 (4.07)	4639 (3.03)				

TABLE 3. Reduced form equations from model I

JOINTLY-DEPENDENT VARIABLES	S_{t-1}	P_{t-1}	P_{t-2}	X_{t-2}	Y	r	F	PR_{t-2}	T_{t-2}	Q_2	Q_3	Q_4	M	M^2	M^3	M^4	1
P	-5.3×10^{-6}	1.09	-0.20	-.0018	.0006	-0.02	.0011	.006	-.0049	0.04	0.03	0.12	0.13	-0.05	.0066	-.0002	1.02
I	0	0	1210	30.25	0	0	0	0	0	-28	169	6117	0	0	0	0	4760
L	-.00387	792	-143	-1.28	.418	-15.4	-69.4	-394	306	238	-5018	-1176	-8028	3407	-410	15.1	-4821
TR	-0.013	2745	-496	-4.45	1.45	-53.3	-241	-961	746	826	-17403	-4078	-19584	8312	-1001	36.8	-16177
S	0.852	655	1874	35.4	-7.3	-12.7	-57.4	-326	253	-3005	-8873	2855	-6646	2821	-340	12.5	-11924
C	0	0	-700	0	10.1	0	0	0	0	4152	6451	4639	0	0	0	0	17830

explaining the variation in the model.[10] Variables which are not significant in the model may serve to highlight equations which deserve further study.

The reduced form equations are presented in Table 3. As discussed earlier, these equations are derived from manipulation of the estimated structural equations (equations 6-11) so that each jointly-dependent variable is expressed as a function of only the exogenous variables. Relationships which are implied but not explicitly stated by the structural equations are explicitly shown by the reduced form equations. Income (Y), for example, affects the level of shrimp consumption which in turn has an impact on price. This indirect impact of income on price is apparent upon examination of the reduced form equations in Table 3. One may also trace out this chain of "events" on the flow chart (Figure 4) starting with Y. Forecasts of the jointly-dependent variables are generated by plugging in forecasts of the exogenous variables for the desired month. Predictions of the exogenous variables are available from several sources (e.g., the Wharton E.F.A., Inc., Data Resources, Inc., and the U.S. Department of Energy).

The accuracy of the shrimp price forecasting model was tested during two periods. In the first period actual values of all exogenous variables, including lagged jointly-determined variables (e.g., P_{t-1}) are used to generate forecasts. In the second period forecasts are made by using actual values of all exogenous variables with the exceptions of the lagged jointly-dependent variables. Instead of using actual values for these variables the predicted values from the model are used (e.g., the predicted price for the preceeding month is used for P_{t-1} in the reduced form equations instead of using the actual value of P_{t-1}). The second period forecasts more closely reflect the limited knowledge available to the price forecaster since the model uses its' own forecasts from previous months to generate additional predictions. The first period begins with the first observation of the data set, September 1974, and ends with June 1980. Period II runs from July 1980 to September 1981.

The comparison between actual and predicted prices is made by calculating the root mean square error (RMSE) between the two values.[11] The

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[10]Although statistical tests of significance (such as the t coefficient) are not applicable to 3SLS results, they serve as a guide in applied work.

[11]The formula for calculating RMSE is:

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (P_i - \hat{P}_i)^2}$$

where: P_i = actual price in month i

$\hat{P}_i$ = predicted price in month i

n = number of months

The RMSE is a common measure of the accuracy of forecasts (Klein and Young 1980, p. 147).

RMSE shows a type of average or standard forecasting error. Before presenting an evaluation of the accuracy of the model, two variations of the model can be compared. These variations are introduced because they have in some aspect outperformed the model defined by equations 6 through 11. The RMSE of predicted price is shown in Table 4 for each model where the models are defined as:

MODEL I: as defined by structural equations 6 through 11 in the text.

MODEL II: differs from model I in that the price lags (P_{t-1} and P_{t-2}) are not included in the price equation.

MODEL III: differs from model I in that the one month price lag (P_{t-1}) and the prime rate of interest (r) are not included in the price equation.

The percent error, defined as the percent RMSE is of the average price during the period, is also shown in Table 4.

TABLE 4. Comparison of the Price Forecasting Accuracy Between Models

MODEL	RMSE		% ERROR	
	Period I	Period II	Period I	Period II
I	0.17	0.66	5.2	18
II	0.62	0.46	19.1	12.5
III	0.24	1.04	7.4	28.3

Inspection of the RMSE's listed in Table 4 indicates that during period one model I had the lowest standard error. This is not particularly surprising considering that model I has been chosen as the "best" model based on the value and statistical significance of the estimated coefficients. On the average the "predicted" price of model I has been 5.2 percent from the actual price during period one. During period two, however, model II has been more successful in predicting price than model I and III. This result is somewhat surprising when one considers that the error in model II during period I is roughly three times as large as the error in the other two models during the same period. Also, the estimated structural coefficients from Model II leave much to be desired in terms of their conformance with prior expectations and the level of statistical significance.[12] The obvious question which comes to mind is: Why does model II outperform the other models during period II? Two possible

[12]The estimated equations of model II and III are not presented in this paper.

answers are offered here: 1) it was a matter of chance that on the average will not be repeated, and 2) the model which behaves the best over the range of data (period I) used to estimate the model (i.e., model I) is not the best model to use for actual forecasting. The correct answer will most likely come to light as a track record of the predictive accuracy of each model is accumulated.

In addition to the magnitude of the error the number of correctly forecast turning points is also of interest in evaluating the effectiveness of a forecasting model. Actual and predicted prices during the 15 months of period II (i.e., July, 1980 through September, 1981) are plotted in Figure 5. There were 3 positive reversals (including the increase in August price over July) and two downturns in actual prices from July 1980 to September 1981. Model I failed to accurately predict any of these turning points. It was, however, only a month late in forecasting the last downturn in prices which occurred in May, 1981. The up and down moves in price in August and September, 1980, were accurately predicted in model II. It is clear from inspection of Figure 5 that model II forecast far more turns in price than actually took place. The increase in prices in August, 1980, was the only month in which a turn in prices was accurately forecast by model III.

FORECASTING WITH ESTIMATED VALUES FOR ALL EXOGENOUS VARIABLES

Although the forecasts for period two approximates a "true" forecast, it is not since actual values of income, interest rates, precipitation, temperature, the exchange rate, and fuel prices were used to make the predictions. Estimates for these variables in future months have to be made and inserted in the reduced form equations to make a true forecast. Comparing the track record of forecasts made by the models using estimated values for all exogenous variables is required before a choice of the best forecasting model can be made. A forecast of the monthly price of 26-30 count shrimp from August 1981 to June 1982 was made in August 1982. Predicted values of the exogenous variables had to be used to derive the forecasts. These results, along with the actual prices when available, are printed in Table 5. The RMSE can only be calculated on the basis of five months. Model II has a lower RMSE than the other two models. However, model III was closer to actual prices in four of the five months. A graph of the prices in Table 5 is presented in Figure 6. With the exception of the initial month of August, model III comes closest to the actual price. Models II and III forecast the same number of ups and downs in shrimp price although model II predicts the second fall and rise to happen a month earlier than model III. Predicted prices from model I are rising throughout with the exception of the initial decline and rise in the second and third month. The model I results may at first glance appear unrealistic. However, the results of the analysis of seasonal variation presented earlier indicates that it is not unusual to see an uninterrupted increase in prices between October and May. Actual price magnitudes and movements over the August 1981 to June 1982 period remain to be seen. When this information becomes available the relative accuracy of the forecasting models will be better known.

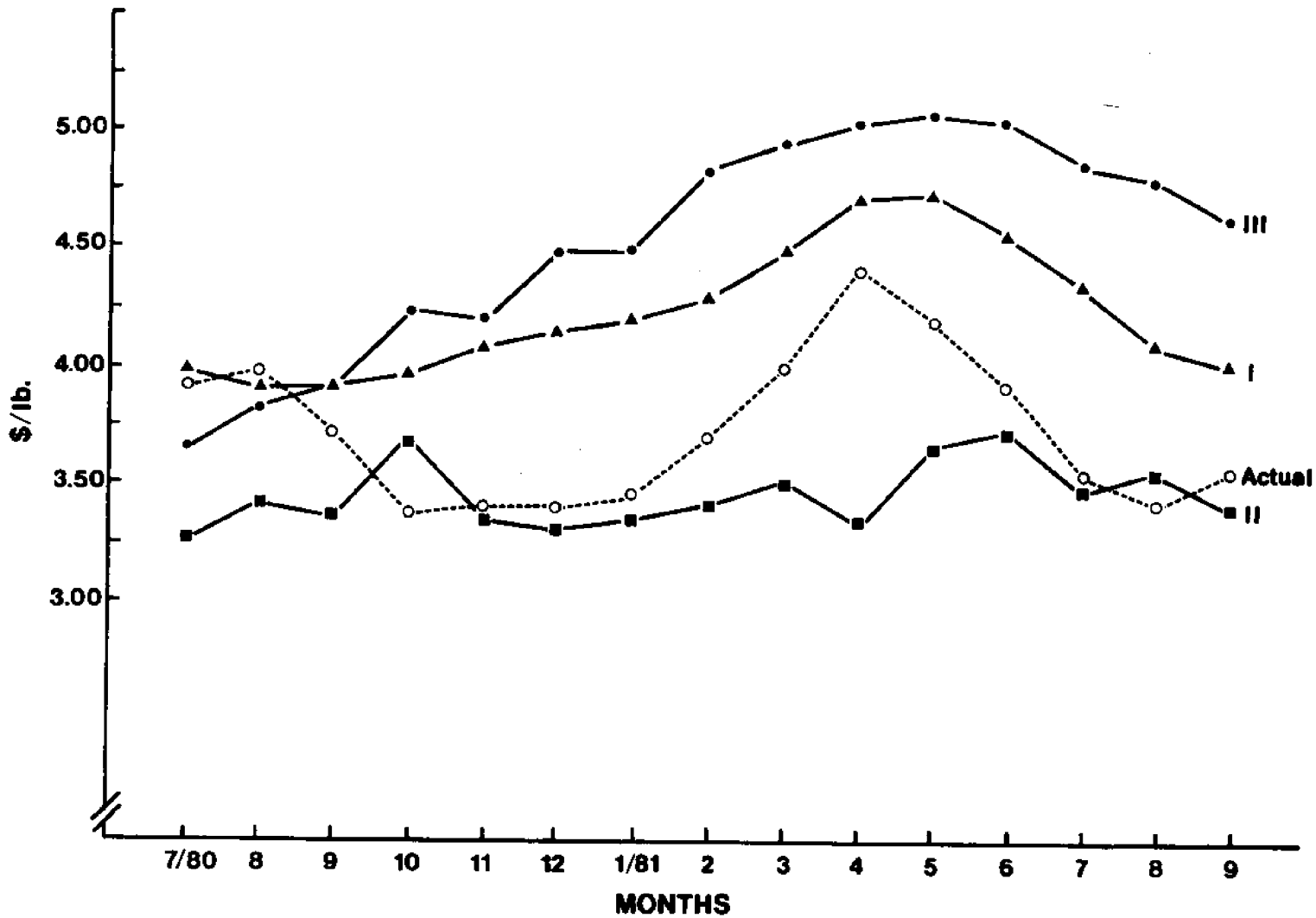

FIGURE 5. Plot of actual and predicted exvessel prices, 26-30 count shrimp, July 1980 through September 1981.

TABLE 5. Price Predictions, 26-30 Count Shrimp, Made in August 1981

Month/Yr	Actual	MODEL I	II	III
8/81	2.91	3.07	3.50	3.89
9/81	3.32	2.79	3.14	3.43
10/81	3.45	2.87	3.35	3.44
11/81	3.72	3.02	3.37	3.77
12/81	3.69	3.17	3.43	3.73
1/82		3.25	3.42	4.00
2/82		3.39	3.51	4.04
3/82		3.58	3.59	4.34
4/82		3.81	3.52	4.28
5/82		4.04	3.67	4.56
6/82		4.21	3.68	4.51
RMSE (n=5)		.53	.34	.44

SOME PRELIMINARY FINDINGS

Additional specifications of the model and a longer track record are required before additional conclusions can be drawn. Some implications that have come to light deserve mention, however. One such implication is that the most accurate predictive model for the price of one count size of shrimp may not be the best model for a different size. The reason for this discrepancy is not clear. There is evidence from price elasticities developed in the model that prices of shrimp at different count sizes respond differently to equal changes in such variables as disposable income, imports, and landings.

There are indications that today's market for shrimp may be substantially different from the market that existed ten to fifteen years ago. Previous research dealing with the market for shrimp during that time period indicated that shrimp prices were very responsive to changes in the level of disposable income. Our analysis indicates the shrimp prices are in fact far less sensitive to income changes than previous studies suggested. Changes in the quantity of shrimp consumed also appear to be less responsive to changes in shrimp prices than earlier research indicated. The lower measures of response calculated from our models may be related to the fact that per capita shrimp consumption is no longer trending upward over time as it was during the period which earlier studies dealt with (i.e., late 50's to early 70's). When price, consumption, and income all tend to increase with time a model may overstate the response of price to income changes and consumption to price changes. For this reason response measures estimated using data from no later than ten to fifteen years ago are likely to be more accurate than the response measures calculated using earlier data.

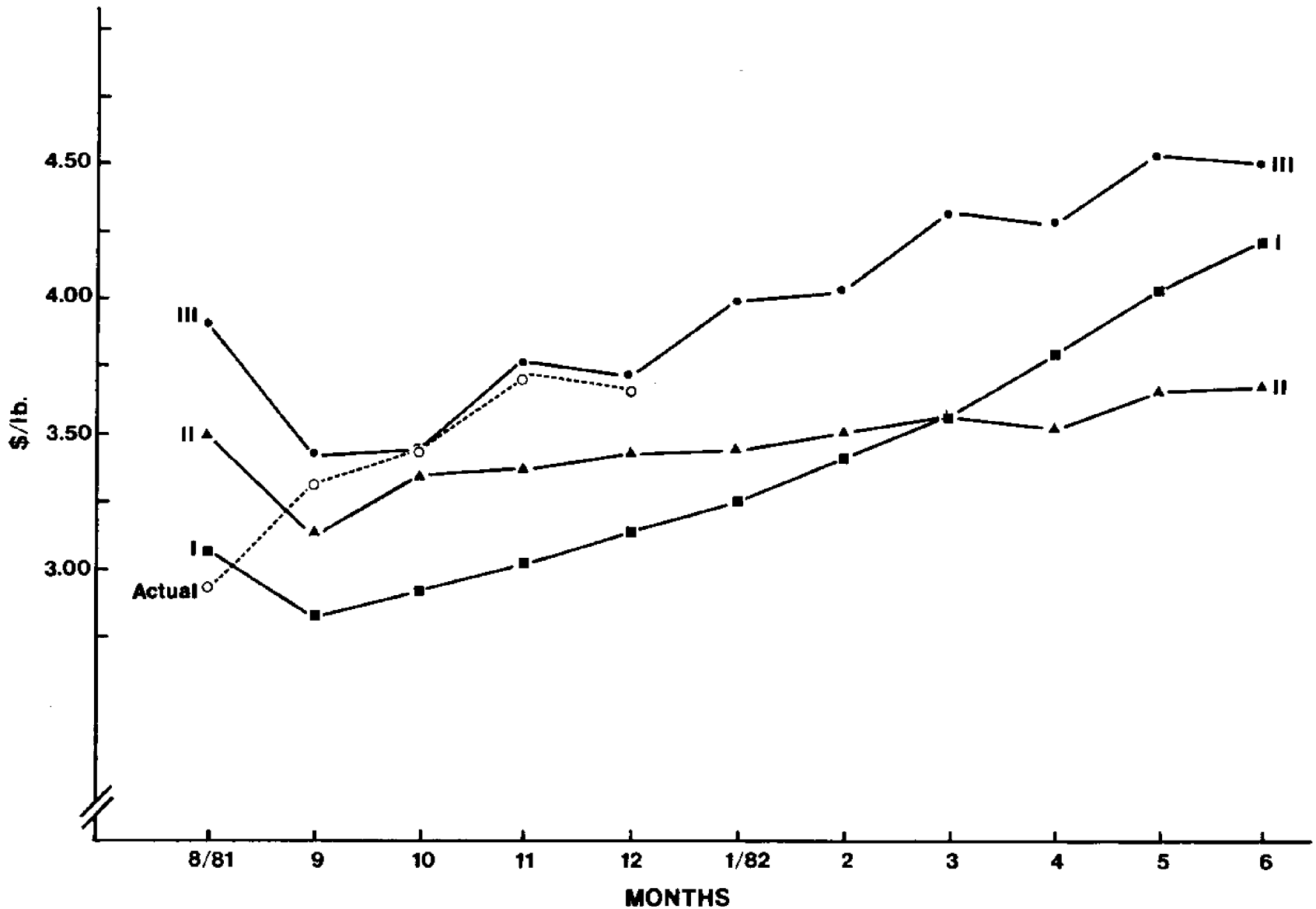

FIGURE 6. Plot of predicted exvessel prices, 26-30 count, August 1981 through June 1982. Prediction made in August 1981. Actual prices are plotted when available.

SUMMARY AND CONCLUSIONS

Exvessel shrimp prices were analyzed for stable seasonal, cyclical, and long-run trend movements. A distinct seasonal pattern was established which showed monthly prices to be steadily increasing from January, where they were about average. Prices tend to peak in May and then decline through September. Prices tend to rise during the last quarter of the year after hitting a low in September. A quadratic trend line was found to provide a good approximation of long-term trend. However, it was not possible to identify a consistent pattern of cyclical price movements.

A system of simultaneous equations was developed for the purposes of forecasting exvessel prices. The forecasting model is more complete than previous attempts to model the shrimp market in the sense that landings, fishing effort, and imports are determined within the framework of the model. Previous research has treated these variables as exogenous to the system. Based on a limited amount of testing for predictive accuracy, the best model on theoretical and statistical grounds does not appear to generate the most accurate predictions. However, the testing period has been short and a longer track record is needed.

A larger set of actual predictions to compare with actual prices will allow several questions to be addressed. Among these are:

- can the model which is most appealing for theoretical and statistical reasons be relied on for accurate forecasts?
- is it possible to accurately predict turning points in the shrimp market?
- can an increase in accuracy be realized by modifying the model so that it is geared toward a particular size of shrimp?
- does the most accurate model vary depending on the count size of shrimp?

In addition to a continual monitoring of the predictive performance of the models developed to date, the researchers intend to formulate new specifications of the model. Some of these specifications are intended to allow the questions put forth above to be addressed. Others are simply envisioned as possibly providing a closer approximation of the impacts of events in th "real world." Modifications which will be examined in the near future include the addition of a wholesale price equation and incorporating the prices of complimentary and substitute products. It is thought that a wholesale price equation will bring the model closer to the actual market for shrimp. Prices of substitute and complimentary products have not been found to be a significant determinant of shrimp prices in past studies. These same variables may now prove to be important because the shrimp market has changed over time. An attempt to subdivide landings, imports, and stocks into small, medium, and large size of shrimp is being considered and may lead to greater accuracy in predicting prices within the respective size classification. The system of equations put forth in

this paper represents a continually evolving model viewed at one point in time. As research continues the system will surely take on additional improved specifications.

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ECONOMIC, MARKET, AND TECHNICAL CONSIDERATIONS FOR PRAWN FARMING IN SOUTH CAROLINA[1]

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INTRODUCTION

Commercial farming of prawns (Macrobrachium rosenbergii) has been established in tropical areas such as the Hawaiian Islands and Puerto Rico. A major reason for success in the tropical areas is the high water temperatures which permit a year round growing season. In temperate climate areas, there is also substantial interest in farming prawns. In South Carolina prawn farming currently consist of a two-phase operation: the nursery phase during which postlarvae are reared to juveniles; and the grow-out phase during which the juveniles are pond-reared to marketable size prawns. By incorporation of the nursery system the growing season in South Carolina is extended about 2-3 months. This paper examines the economic aspects of prawn farming under two different conditions: (1) existing ponds and associated facilities including a water source; (2) new construction of facilities. In addition, the market and technical aspects associated with the development of small-scale prawn aquaculture in temperate areas is reviewed using South Carolina as a model.

ECONOMIC CONSIDERATIONS

Nursery Phase

Nursery systems under development at the Marine Resources Research Institute are simple in design and operation. Basically they consist of tanks containing prawn habitats which are connected to recirculating water management systems (Sandifer and Smith, 1977 ; Smith and Sandifer, 1981). Rearing is typically conducted in two stages. Prawns are initially stocked at densities of 150/ft^2 tank bottom area and then the density reduced to 50-100/ft^2 after a month. Feeds consist of Purina Marine Ration 25 supplemented with a variety of foods (i.e. hen eggs, fish roe, squid, spinach, etc.). Total nursery cost for feed, water, electricity, labor, and tanks was estimated to be $15.70/1,000 juveniles for a 10 week nursery cycle. Of this, electricity ($0.05/kwhr) comprised 34% of the rearing expenses while labor charges ($4.00/hr) accounted for 48% of the estimated production cost. Thus, the use of inexpensive heat sources (i.e. heated effluents, geothermal water, etc.) Coupled with automated feeders should result in lower nursery costs.

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Grow-out Phase

Annual costs were estimated for a hypothetical production unit consisting of ten 0.41 ha ponds. Annual costs were categorized as either fixed or variable costs. Annual fixed costs are associated with items of fixed investment such as ponds, wells, equipment, etc. Such fixed costs per pond was estimated to be $773.93 per year for new facilities and $258.55/pond/yr in situations where there were existing facilities. The fixed cost estimates were derived from known parameters as possible, but they should not be regarded as precise (Bauer, Smith, Sandifer, and Jenkins MS).

Total variable costs, including seed stock, feed, nursery costs, labor, etc. were estimated to be $1860.13 per pond for new and existing facilities. Seed stock cost was based on the purchase of 19,166 post-larvae at $30 per thousand to produce 17,424 juveniles (90% survival) for stocking one 0.41 ha pond at 4.3 prawns/m^2 (recommended densities 4.3-6.4 prawns/m^2, Smith et al, 1981).

Production level at the stocking density of 4.31 juveniles/m^2, based on 1978-1980 experimental trials at MRRI, was 185 kg of tail weight per pond. At this production level and the current price for prawns at $11.03/kg (i.e. $5.00/lb-based on direct consumer sales by farmers in 1981), the net revenue per pond for farms with facilities was $-66.01. If prawns can be sold at $11.58/kg (i.e. $5.25/lb), net revenue per pond for farms with existing facilities was $23.62 while farms with new facilities still experienced losses. This indicates that a marketing method which includes sale of a portion of the crop to specialty outlets at higher prices may be needed to provide a reasonable return to investment.

MARKET CONSIDERATIONS

Market Outlets

From the foregoing information it is obvious that for prawn farming to become an attractive agricultural enterprise in South Carolina a high market price will be needed. Price for the crop varies depending on the market outlets and prawn sizes. In South Carolina, three possible markets have been investigated for prawn growers: (1) seafood retail markets, (2) seafood restaurants, and (3) direct sale to consumers (Liao and Smith, MS, 1982).

In seafood retail markets, fresh prawn tails in the medium size count category (i.e. 78-110 count/kg) were preferred. Thus, grading may be required for this market, and the grower would need to deliver prawns to the retailers. Such steps would increase the production costs to the grower.

Seafood restaurants have more requirements in regard to product size uniformity, frequency and amount of supply, packaging, and quality. Thus, grading, processing, packaging, and shipping are required in marketing prawns to restaurants. Most restaurateurs indicated a preference for a consistent product size in the 35-55 or less tail/kg count category which represents "jumbo" or larger market classes. Depending on entree, prawns might be marketed as tails or as whole animals.

In direct consumer sales, producers may advertise in local newspapers that fresh prawns would be available for purchase at time of harvest. The advantage of this marketing approach is that there are fewer requirements in regard to the size of the product, the volume of orders, and packaging as compared to that of seafood restaurants and retail markets. At this time the direct consumer sale marketing approach appears most appropriate for small producers.

Market Demand

Since 1960, marine shrimp imports have provided more than half of the shrimp consumed in the United States. Further, these imports have exceeded domestic shrimp landings in 15 of the last 20 years. In 1980, it was estimated that 55% of the 464 million pounds of shrimp utilized in the U.S. were imported. Prawns are closely competitive with saltwater shrimp in the market place, and so it appears that there is a ready market for this product. Based on 1973-74 consumption data, per capita consumption of shrimp in South Atlantic states was higher than that of the U. S. Furthermore, per capita consumption of shrimp in South Carolina (2.16 lbs) was higher than the consumption level in other South Atlantic states. Thus, there may be a substantial market demand for prawn products in South Carolina.

TECHNICAL CONSIDERATIONS

Site Selection

The selection of a suitable site is of paramount importance. The farm should be constructed on reasonably flat land and the pond soils should be relatively impervious to water (e.g. clay). Water should be readily available and of sufficient volume and quality for filling and flushing the ponds. This water may come from a well, stream, or reservoir. Ponds should be completely drainable within 24 hours. The pond acreage developed will depend on the volume of production desired. However, larger farms usually benefit from greater efficiency in resource utilization, especially labor. Small farms usually succeed when operated as a family venture and when combined with other business activities. In Hawaii, an 8 ha (20-acre) farm is considered to be the minimum size for successful commercial production of prawns (Shang, 1981).

Pond Management

Sound management of ponds is essential for success in prawn farming. Prawns should be fed daily and the water quality regularly monitored. In most cases low dissolved oxygen levels associated with dense phytoplankton blooms have been the major reason for prawn mortalities in ponds. Dense blooms should be avoided. Also, the farmer must control predation by birds, fishes, small mammals, etc. to avoid low production rates. Finally, macrophytic plants should be strictly controlled to prevent difficulties during harvesting.

CONCLUSIONS

Present analysis of costs and returns of prawn farming indicates a near break even condition exists in situations where pond and associated facilities are already available. There appears to be no product acceptance problem from buyers in seafood retail markets and direct consumer sales. In addition, there appears to be a ready market for prawns in the U. S. Additional research is needed to investigate ways of reducing production costs (i.e. lower seed stock and feed costs, labor costs, etc.) as well as increasing the value of the crop (i.e. development of specialty markets, increased production of larger more valuable prawns).

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FAO MARKETING INFORMATION AND ADVISORY SERVICES FOR FISH PRODUCTS

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Differences in resources availability and market preferences between countries and between regions encourage international trade in fishery products, and in many developing countries rapid development in fishery industries is largely based on export-oriented operations. Major handicaps to trade expansion have been difficulties in identifying marketing opportunities to enable present and potential producers to take advantage of them, insufficient knowledge of product requirements and inflexibility of national administrations regulating export trade which could have been avoided if they had been alerted to the existence of such problems. Marketing information which would help producers to direct shipments to the economically most rewarding markets and to obtain better prices is often inadequate or not available.

These shortcomings can be remedied by appropriate marketing information services supported by advisory assistance on product specifications, processing standards and export promotion.

Early in 1981 FAO started INFOFISH, a fish marketing service for Asia and the Pacific. The new service, which is funded by Norway initially, is the second in what eventually will be a network covering the major fish producing and consuming regions of the developing world. Its establishment owes much to the success of INFOPESCA, a similar service which has been helping the countries of Latin America and the Caribbean to find new markets for their fish and fish products.

The main reason for setting up these services is the knowledge that the biggest obstacle to the trading efforts of developing nations is simply a lack of communication between those who want to buy and those who can supply fish. Only a handful of large trading companies can afford the sophisticated market intelligence systems necessary to overcome this communication gap. One result has been that large quantities of fish that could have been caught and sold have remained untouched. Another is that some nations, particularly in the developing regions, have been importing fish that they could have bought more cheaply closer to home.

The results achieved so far with INFOPESCA, which began operating in June 1977, are very encouraging. In its first year, it helped to conclude deals involving $100 million U.S. In the first six months of 1980 alone, INFOPESCA assisted in the export of over 125,000 tons of fish products worth an estimated $160 million U.S. The marketing information service, which has its headquarters in Panama City, is now believed to be assisting with 20 to 25 percent of fish exports from the Latin American region.

Financed through the United Nations Development Program during its development phase, and operated by FAO, INFOPESCA is supported by 22 member countries from the region. In formal terms, its mission is threefold:

1. To find new markets for its members' fish products.

2. To encourage processors of fish to meet the specifications set by importers in these new markets.

3. To help train marketing echelons in its member nations.

Basically, INFOPESCA is a clearing house for information. Government contact points have been designated in all member countries and these, together with about 100 companies, keep a continuous stream of information flowing into Panama City about fish available for export. INFOPESCA, in turn, channels the information--and other material--selectively to importers who have signaled an interest in particular products.

INFOPESCA is a small, tightly-organized group, headed by a director assisted by three experts and a staff of 16. Urgent requests to buy or sell move by cable, telephone or telex. Routine inquiries and information go by mail. At present, the data are stored manually, but the system is designed for quick adaptation to computer storage and retrieval. INFOFISH is using computer systems from the start. Its experience should be useful in helping INFOPESCA to make the transition from manual to computer processing.

The success of INFOPESCA depends on the careful market studies made by its staff and the routine, day-by-day, maintenance of its information base. INFOPESCA has records not only of names, types and quantities of fish products for sale or in demand, but information about the multitude of factors that can make or break a successful deal--local import/export duties and regulations, freight charges, market trends and tips on advertising, packaging and quality control. INFOPESCA also receives and passes on information about investment projects in the region, financed by international organizations, governments or private enterprise.

Some information is channelled out via regular newsletters and bulletins. The newsletter with its price reports is a valuable reference for those engaged in the immediate tasks of buying and selling fish, both within and outside the region. The bulletins and other technical reports produced by the INFOPESCA staff generally take a longer term view. They might consider, for example, trends in the market for tuna or give a report on the possibilities of exporting sea urchins to Japan. In all cases, however, the information focuses on the needs of fish traders, processors and fishermen within the INFOPESCA region.

Much of the group's work consists of playing "honest broker" to potential purchasers and would-be sellers, but, just as often, INFOPESCA takes the initiative in building new trade; it has helped to open up new markets in developing nations in other regions of the world. INFOPESCA is also helping countries within the region to realize more of the potential of their unexploited fish resources. For example, it has demonstrated the possibility of using Sardinops sagax, a large South American sardine, as a substitute for herring. INFOPESCA has also promoted the introduction of Latin American hake into the lucrative markets of the industrialized world.

The emphasis of INFOPESCA's marketing efforts has been to bring Latin American fish products onto the world markets. Some of its member countries, particularly in the Caribbean, import sizeable quantities of fish, but, for the most part, the problem is to take advantage of rich fishery resources that far exceed the demand for fish within the region. INFOFISH has a somewhat

different problem. Although the Asian and Pacific regions have substantial fishery resources, because of the high demand for fish by the local populations, the emphasis is much more on trade within the region.

INFOFISH, which has a membership of 20 nations, started with a valuable store of information built up prior to moving to the region. It has data on over 400 fish products, more than 300 producing or exporting companies in the region, and almost 2,000 buyers and importers in 80 countries. INFOFISH also has on its staff experts who have had direct experience of INFOPESCA during its formative period.

Each of the operational and planned FAO marketing information services can be expected to have its own identity, depending on the needs of the regions they serve. When INFOSAMAK, the service for the Arab world, and INFOPECHE, planned for West Africa, also come into service, there will be the basis for a powerful new trading group in world fish markets. Over the past decade, the developing countries' share of fish exports has risen from 30 to 40 percent. In terms of value this means an almost fourfold increase from around $1 billion U.S. to $3.8 billion U.S. It will be interesting to see the impact on these figures of FAO's efforts to stimulate regional fishery development and trade during the present decade.

TRENDS IN EXPORT OF FEDERALLY INSPECTED FISH

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INTRODUCTION

The Southeast Inspection Office of the United States Department of Commerce conducts inspection and certification of fish exported out of the country. This is performed on a voluntary fee-for-service basis. Best estimates indicate that in 1981 over 50 percent of fish exported out of the southeast were federally inspected. The species, quantity and country of import of inspected product is a good index of what is occur commercial export market and give good indices of which products are more important for export to foreign nations, which nations are importing the largest quantities of fish from the southeast United States and which ports the fish are being shipped from.

PROCEDURE

Data were taken from official export certificates issued by United States Department of Commerce inspectors throughout the southeast for the period October 1, 1980 through September 30, 1981. The data were organized to show the kinds of fish being exported, their quantity, the countries to whom they were being shipped, which countries received the largest quantity and what were the kinds of fish they received and what quantities of each kind of fish.

RESULTS

In 1981, exported federally inspected product amounted to 22,424,100 pounds. This is an increase of eight and one half million pounds since 1980 and eighteen million pounds since 1979.

TABLE I. Quantity of Export Federally Inspected Fish

(In Pounds)

1979	1980	1981
4,713,710	13,854,095	22,424,100

The 22,424,100 pounds inspected for export was 69.5% of the total product of 32,254,400 inspected on a lot basis in the southeast in 1981.

Location of product inspected ranked by quantity was in Miami, Tampa, Mobile, New Orleans and Panama City.

TABLE 2. Location of Fish Inspected for Export

	City	Quantity (lbs)
1.	Miami	13,115,425
2.	Tampa	7,354,833
3.	Mobile	1,340,843
4.	New Orleans	279,540
5.	Panama City	222,515

Rank listing of product exported from ports of embarkation in the southeast shows Miami leading followed by Tampa, Mobile, Savannah, Jacksonville, Charleston and Long Beach.

TABLE 3. Ports of Embarkation

	City	Quantity (lbs)
1.	Miami	13,023,682
2.	Tampa	5,807,431
3.	Mobile	1,234,831
4.	Savannah	477,248
5.	Jacksonville	258,000
6.	Charleston	137,100
7.	Long Beach, CA	124,344

Mullet with 7,741,008 pounds led all the other fish in quantity by 5 million pounds over squid which was second. See Table 4.

TABLE 4. Fish Inspected for Export

	Species	Quantity (lbs)
1.	Mullet	7,741,008
2.	Squid	2,243,772
3.	Drum	2,231,433
4.	Kingfish	1,673,235
5.	Mackerel	1,449,204
6.	Bluefish	1,447,274
7.	Jack Crevelle	992,131
8.	Ladyfish	554,764
9.	Whiting	524,031
10.	Mullet Roe	520,568
11.	Grouper	461,232
12.	Redfish	416,992
13.	Turbot	293,330
14.	Octopus	212,170
15.	Spiny Lobster	89,480
16.	Cod	80,300
17.	Skipjack Tuna	79,094
18.	Red Snapper	58,887
19.	Catfish	55,378
20.	Sole	40,215
21.	Salmon	30,000
22.	King Crab	28,370
23.	Pompano	17,250

Venezuela led all other countries in quantity of fish imported from the southeast with 10,593,552 pounds. Egypt was second with 4,207,131 pounds and Nigeria third with 2,882,130 pounds. See Table 5.

TABLE 5. Quantity of Export Federally Inspected Fish By Country

	Country	Quantity (lbs)
1.	Venezuela	10,593,552
2.	Egypt	4,207,131
3.	Nigeria	2,882,130
4.	Turkey	1,487,011
5.	West Indies	524,584
6.	Israel	395,548
7.	Spain	329,230
8.	Taiwan	241,786
9.	France	212,469
10.	Columbia	180,840
11.	Portugal	79,094
12.	Greece	45,906
13.	England	40,000
14.	Kuwait	39,000
15.	Japan	37,400
16.	Lebanon	8,000

Mullet, squid, kingfish, spanish mackerel and bluefish were the primary kinds of fish exported to Venezuela.

TABLE 6. Inspected Export Product to Venezuela

Species	Quantity (lbs)
Mullet	2,134,143
Squid	2,087,012
Kingfish	1,595,235
Mackerel	1,334,924
Bluefish	1,032,060
Jack Crevelle	839,115
Whiting	524,031
Grouper	461,232
Turbot	293,330
Octopus	212,170
Cod	80,300

Mullet again was the leading species exported to Egypt.

TABLE 7. Inspected Export Product to Egypt

Species	Quantity (lbs)
Mullet	4,150,081
Sole	40,215
Mullet Roe	9,050
Rock Fish	7,785

Mullet, drum and ladyfish were important fish exported to Nigeria.

TABLE 8. Fish Exported to Nigeria

Species	Quantity (lbs)
Mullet	938,530
Drum	744,422
Ladyfish	554,764
Redfish	416,992
Bluefish	77,092
Catfish	55,378
Jack Crevelle	39,574

Only one important species was inspected and exported to Turkey. They received 1,487,011 pounds of drum.

TABLE 9. Fish Inspected for Turkey

Species	Quantity (lbs)
Drum	1,487,011

The West Indies received 524,584 pounds of inspected fish. Bluefish had the highest volume with 338,122 pounds.

TABLE 10. Fish Inspected for West Indies

Species	Quantity (lbs)
Bluefish	338,122
Jack Crevelle	74,045
Red Snapper	58,887
Mackerel	36,280
Pompano	17,250

Israel concentrated on mullet and mullet roe receiving 355,948 pounds of mullet and 39,600 pounds of mullet roe.

TABLE 11. Fish Inspected for Israel

Species	Quantity (lbs)
Mullet	355,948
Mullet Roe	39,600

Spain received mullet roe, kingfish, mackerel and Florida lobster.

TABLE 12. Fish Inspected for Spain

Species	Quantity (lbs)
Mullet Roe	92,100
Kingfish	78,000
Mackerel	78,000
FL Lobster	52,760
King Crab	28,370

Taiwan, as could have been expected, received a large quantity of mullet roe.

TABLE 13. Fish Inspected for Taiwan

Species	Quantity (lbs)
Mullet Roe	229,146
FL Lobster	12,640

France also liked mullet roe.

TABLE 14. Fish Inspected for France

Species	Quantity (lbs)
Mullet Roe	142,672
Jack Crevelle	39,397
Salmon	30,400

Columbia preferred squid.

TABLE 15. Fish Inspected for Columbia

Species	Quantity (lbs)
Squid	156,760
FL Lobster	24,080

Various countries; Portugal, Greece, England, Kuwait, Japan and Lebanon received smaller quantities of fish mostly mullet and mullet roe.

TABLE 16. Fish Inspected to Various Countries

Country	Species	Quantity (lbs)
Portugal	Skipjack Tuna	79,094
Greece	Mullet	45,906
England	Mullet	40,000
Kuwait	Mullet	39,000
Japan	Mullet	37,400
Lebanon	Mullet Roe	8,000

DISCUSSION

As can be seen by the results, definate trends can be perceived in the commercial export market of fish from the southeast.

1. Volume federally inspected export fish is rising rapidly increasing 475% in two years.

2. Mullet is the principle species of export and should steadily increase in volume.

3. South American, African and mid eastern nations are the largest recipients of federally inspected fish.

Marketing, extension and promotional efforts should be directed toward the species and countries shown by this study to be most favorable for the importation of underutilized southeast fish species.

PROBLEMS REGARDING EXTENSION OF FISHERIES TECHNOLOGY IN DEVELOPING COUNTRIES

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INTRODUCTION

The communication of technological knowledge, sometimes called extension or transference of technology, is a rather complex phenomenon involving not only knowledge, but cultural and economic aspects as well. This makes it difficult to analyse the phenomenon as a whole.

In the field of fisheries this problem is even more evident because the problem of fisheries extension is usually treated as a special branch of agricultural extension but, except for some similarities (mainly in aquaculture), the situation is different when we start to apply technology to marine catch, its utilization and marketing. Although some of the problems faced in the field of technology transfer in marine science and fisheries had been analysed (4), (9), (20), a more in-depth analysis seems necessary to complete the chain representing fisheries.

The problem becomes a practical one when we analyse our current training programmes and the impact that governments and trainees are expecting from them. The general indication that technology should be appropriate, not sophisticated, to a country's needs and level, etc., deals only with the philosophical point of view and ignores practical ways of tackling the problem of transference of technology. If we train people from developing countries by giving them only technical knowledge and no indication why extension activities may or may not be successful, we are communicating only part of our message. Many papers have been written about the relationship between training and the spreading of knowledge; in the special case of fish utilization in developing countries, we can mention the paper by James (10).

This paper discusses some aspects of transference of technology within developing countries, paying special attention to fish utilization and the situation in Latin America. Although there are some references to transference of technology on fish utilization at subsistence and artisanal levels, this paper focuses on the industrial level found today in Latin America, as well as in other developing countries around the world.

TECHNOLOGY AS A COMMODITY

Past analysis of cultural implications of alien technologies in developing countries revealed that many of these problems were probably caused by a massive transference of heavy technology. 'Appropriate technology' then sprout up, which endorses a soft, more viable technology suitable to conditions in developing countries (5), (6). And even though the idea of 'appropriate technology' is a very good one, in practice it may not have

the desired results. For example, despite all multilateral and bilateral aid, as well as national and regional efforts to introduce more effective fish-smoking ovens in Africa, consumption of firewood for such purpose as reported by governments was just as high as that consumed in open fire - the most primitive and inefficient way to use firewood (16). As a matter of fact, many attempts to transfer technology to developing countries were conducted on an idealistic basis.

An interesting possibility is to use the model of demand and supply to analyse the transference of technology, even in developing countries. As any other merchandise, technology has its own market and thus its supply and demand. But the technology market in developing countries is not necessarily the same as that in developed countries.

DEMAND FOR TECHNOLOGY IN DEVELOPING COUNTRIES

Is there really a demand for technology on fish utilization in developing countries? This is the first question that needs answering. Although the demand for technology is usually treated together with the demand for food produced with this technology, it is useful to disclose both demands because more than one technology can exist to produce the same kind of product. In developing countries the technology used to produce a product is very often considered unique for all practical purposes. Say a country wants to develop its shrimp fisheries for export in exchange for hard currency. Once this goal is reached (normally with outside help), the question remains: do they now want to improve their processing methods? The answer to this is usually a weak yes or no. Their goal was reached, why change? It is not uncommon to find in Latin America fish-canning machines 20, 30 and even 40 years old. They never had an incentive to change or improve their processing methods. Canned fish is produced and sold - full stop. Industrial production is carried out in developing countries normally (when national) as a superficial mimetic of the same activity carried out in developed countries (e.g., no own developments added and this results in production lines remaining static for years and years).

The static conception regarding technology becomes more evident when we analyse the artisanal or subsistence fish utilization techniques (15). There is no single explanation to this phenomenon, but some of the reasons may be: cultural (artisanal level), panic to fall into the philosophy of change for change's sake only (both artisanal and industrial levels), economics (e.g., sometimes it is easier to ask for increases in import tax than to look for a better technology; no purchasing power, etc.).

And this is the point. Sometimes technology is adopted by a country because their market demands a particular product. And their pursuit of technology starts and finishes right there.

But why is it necessary to always look for ways to improve existing processing methods? I faced this question several times in Latin America.

Changes are necessary to improve quality, reduce costs, save energy, raw materials, water, etc., consumption, wastage, avoid pollution, improve storage and transport, extend product's keeping time, meet new public regulations, increase safety factor, improve relationship between the worker

and the machines or job, improve productivity, etc. This list seems endless and it is by no means a static one. Fisheries around the world are now facing new challenges as petrol costs and the need to utilize unconventional species increase.

Theoretically, to close the so-called 'technological gap' between developed and developing countries, technological improvements or changes in developing countries should be made at a higher rate than were made in developed countries when they were at the same stage of development. I am not going to discuss here if this is feasible in every field and country, but it does seem feasible in the field of fish utilization in Latin America.

This same development and know-how makes an appropriate technology unappropriate with time. If we start our development with a soft technology, a lot of changes are necessary to improve it. For example, it is one thing to have an 'appropriate technology' to produce canned fish but it could be quite another process to can fish properly.

The lack of technological demand is not always related to governmental policies or political decision, but to people responsible for the management and technical operation of the industries. A dynamic point of view regarding technology cannot be compulsory, but it forms part of the root of any true and self-sustained development. The lack of technological demand can sometimes be related to the lack of appropriate fish-industry management as a whole.

This problem is even more acute when large losses occur from fish handling, processing or storage. The feeling arises that it is not always economic constraint which precludes fish waste reduction, but the rejection of new methodology even when it is 'appropriate'. Sometimes the simplest things are ignored. A case in point is a drying rack which keeps fish off the ground and reduces loss by up to 15 percent (7). But despite all efforts to install their use and even though they can be constructed locally almost everywhere - from timber, bamboo, vegetal fibres, etc., - fish continue to be sun-dried on sandy beaches world over in developing countries.

TECHNOLOGY SUPPLY IN DEVELOPING COUNTRIES

Perhaps the most frustrating fact to a fish technologist is to know that technology, which could help avoid fish waste or improve fish products, already exists in developing countries or is easily available.

Before analysing characteristics and problems of technology offered in developing countries, we should remember the main channels through which industries obtain information and make innovations in developed countries, and they are:

(a) innovations introduced by the industries themselves as a result of research, suggestions, etc.;

(b) innovations introduced by university research and extension services, public research centres, etc.;

(c) innovations resulting from specific research or studies contracted with private technical consultants (individuals or companies);

(d) attendance at technical congresses, meetings, etc.;

(e) subscriptions and analyses of specific periodicals;

(f) sharing information within industrial associations.

The chart in Figure 1 shows how the offer of technology generates from the know-how of existing special bodies: extension services, consultant companies R and D branch of the same industry, and machine companies.

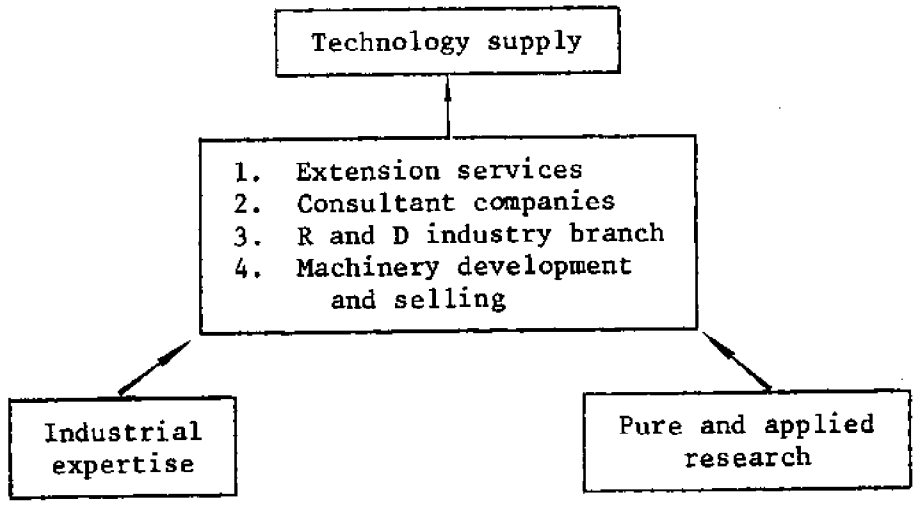

Figure 1 Scheme of the supply of technology

Extension services were perhaps the first services to offer technology. They exist in developed, as well as in developing countries. In agriculture they have been in existence for a long period, but in fish utilization extension services were set up slightly over 50 years ago, even in developed countries.

Extension services usually receive their main revenue from the public budget although in its more developed stages a fraction of the revenue comes from services rendered. Public support of extension activities in fisheries could be justified, even in developed countries, for the following reasons:

(a) importance of fish as food to consumers;

(b) nature of producers or industries. No individual producer or private company can afford to finance all technological development in a country. This is where fish utilization differs largely from other industries, say for example, electronics, which considers research part of the whole. This is particularly valid in developing countries;

(c) to ensure that pure and applied research is used in a given length of time to a useful purpose (Feedback I);

(d) to ensure the right problems are studied in universities and research centres (Feedback II);

(e) to ensure training in schools is in tune with industries necessities (Feedback III).

From the above it is obvious that only institutes which possess technical know-how or intermediate institutes which have a good relationship with the chain of production should undertake extension. But extension services, like any other 'business', needs customers to survive. And if the customer does not want the merchandise the enterprise fails. Many extension services organized in developing countries, modelled along the same lines as those in developed countries, failed because producers or industry did not ask for their services as expected. The extrapolation of the offer does not necessarily mean the extrapolation of the demand. An extension service in a developing country should be more active and try to sell not only its know-how, but its purpose.

In Latin America an extension service in the field of fish utilization is not unusual although many institutes do not always include the word extension. There are official and private institutes, universities or research centres and some foundations. Although the purpose of this paper is not to analyse their effectiveness, the most active in the fishery field seems to be located in Chile and Peru.

This is consistent with the importance allocated to fisheries in these two countries, past and present. In the case of Chile, two institutes, Instituto de Fomento Pesquero (IFOP) and Fundación Chile, seem to have passed from the first stage, i.e., spreading know-how to the second stage, i.e., showing their effort on an economic basis. They are, in fact, selling services. Maybe this is the first sign of things going well - industry and producers willing to invest their money in applied or specific research. See as example References (2) and (8).

But industry is not the only branch in Latin American fisheries. Artisanal fisheries are important if we consider extension essential, not only to provide know-how, but also to stimulate demand. Brazil is one of the countries in Latin America which has given importance to extension activities in artisanal fisheries in a large scale. There are several institutions performing extension at artisanal level in Brazil, and specific technical publications are available - see References (1), (12) and (13) - even comic books have been published there to introduce new techniques, to help fishermen to obtain bank loans, etc. But in a general view the field of extension at artisanal level is wide open in most parts of Latin America.

Consulting Firms: Private firms or individuals who sell their services for profit should not be listed under extension services, but since they compete in the technology market their role should be defined to have a whole picture. As a matter of fact, some fishery companies are in an advanced stage of development in Latin America. At such a stage there is a definite request for expertise, know-how or assessment of technology. And even though these companies cannot afford their own specialized experts, they are willing to pay for external services. Very often their very existence is a direct consequence of the problems and necessities which crop up when exporting fish.

Chilean and Peruvian service companies usually advertise in special technical magazines. See References (2), (18), (19). The services offered vary widely: quality control, feasibility studies, industrial organization, training, etc. To a lesser extent, these companies are also in Argentina, Brazil, Mexico and Uruguay. But to state that this is the typical situation in Latin America would be too general.

R and D Branch of the Industry: Although many fishing companies in Latin America have their own laboratories for quality control of products and raw material they are not up to the standard of R and D laboratories found in developed countries. Nevertheless, from time to time standard quality labs undertake activities that correspond to those of an R and D laboratory (product development, testing new raw materials or analysing methods or packaging, etc.). But too often only a technician is in charge of a very well equipped laboratory.

This might prove to be one of the most fruitful fields in which to work on industrial extension in Latin America. Joint arrangements with laboratories at universities or research centres with access to industrial installations, part-time fellowships for students, participation by industry in research projects, special training, etc., are all possible alternatives to expand R and D within the fishing industry.

Machines and Their Sale: This is an easy way to introduce technology. A machine is not only a device for performing a specific task but it is also a condensation of technology. It should, however, be acknowledged that an imported machine does not reflect the stage of development of the importing country and is thus not a complete technology transfer.

In this paper we will refer only to machines developed and produced locally in Latin American countries and used in fish handling, processing and storage. Argentina is probably the leading country in this field. They produce washing machines (fish, fillets, boxes), fillet skinning machines, bone separators, fillet V-cut machines (with electronic adjustment of the cut according to fillet size), plate freezers, refrigeration machines in general, ice plants, plastic boxes (different sizes and types), can sealing machines, retorts, different types of conveyors, and even complete fish meal and oil plants (excluding centrifuge). Argentina is now exporting its fish processing machines to other South American countries and Brazil is also producing and exporting some machines for fish processing, also in the field of refrigeration, and fresh-fish processing.

Certainly such machines were not invented in Argentina or Brazil. The first step was to copy machines already imported, but then several steps of adaptation were made. To copy machinery is not well viewed by the developed world today, but from a historical point of view such a procedure was utilized to transfer knowledge after the Industrial Revolution from England to the rest of Europe (14) and to the USA (11), and probably from Europe to England before the Industrial Revolution (3). To copy on a continuing basis should not be encouraged, but could be analysed as a development starter.

But you need a combination of factors to develop machinery. In the case of Argentina, the fishing industry took advantage of a well-established

refrigeration industry (for meat) and an equally well-established light-mechanic industry. One example is worthy of note. An automatic can-sealing machine originally developed in Argentina to meet the needs of its fish-canning industry is now produced in France under Argentinian license. Finally, it is necessary to point out that industries which produce machinery in Latin America are usually diversified. They also produce machinery for industries other than the fishing industry because the market demand for the fish processing machines is not so high. Offering technological assistance to produce machinery is also a valid extension service and should be studied further in universities and research centres in developing countries.

REMARKS

From the situations outlined above, it is obvious that extension and more specifically the adoption of new technologies by developing countries depends not only on governmental decisions and activities such as training research and extension, but also on the interest of producers of fish products, services and machinery. A complete technology market profile could help identify areas where government action is most needed and areas which could be taken over by private or public enterprises. This is very important because if development occurs within a few years, government institutions (extension services, universities or institutes) could be tempted to manage the market without sharing or transferring the responsibility to independent concerns or individuals. For example, job opportunities are muted if university laboratories for monetary reasons only continue to perform routine chemical analyses or work that could be performed equally well outside by qualified people, usually trained by the same universities. If people are trained to handle certain jobs in industry they should have the opportunity to use their expertise.

The above is only one of the many problems universities and research institutions are now facing in Latin America in the field of fish utilization. Certainly a market approach to extension of technology does not sound idealistic, but it helps pave the way - from Leonardo's studio to the Watt workshop and the Edison laboratory to the new R and D laboratory concept in developed countries.

CONCLUSIONS

There are no clever conclusions because many questions still remain unanswered and many aspects call for more in-depth analysis. Nevertheless, in view of the relationship that exists among universities and research institutes in North and South America, some suggestions could be presented to the US participant attending the Conference, bearing in mind the number of Latin American students enrolled in undergraduate and graduate courses in US universities.

(a) Call to the attention of Latin American students the extension services performed by US universities.

(b) Some US universities hold special courses on extension (e.g., EM 411 and EM 412 Extension Methods and EM 514 Marine Extension Methods from Oregon State University) (17). I

think student counsellors should make interested students aware of these courses and advise them accordingly. I realize that extension in the USA and in Latin America are two different things, but it is better to have some idea on the subject than none at all.

(c) A third step could be the involvement of Latin American graduate students in extension activities or research linked with extension activities.

Although these three paragraphs do not appear to have any direct connection with the rest of this paper, they will make Latin Americans aware of the problems regarding transference of technology and extension. Moreover, it will help them and their countries when they return home.

Of course, Latin American institutes should try their best to clarify realistically their situation and goals regarding transfer of technology in the field of fish utilization. The interchange of experience and knowledge within institutes of the region should be also encouraged.

Because of the special fisheries situation found in Latin American today, all the above mentioned possibilities will help her in her efforts to attain self-sustained technological development.

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FAO REGIONAL COOPERATIVE PROGRAMS ON FISH UTILIZATION IN LATIN AMERICA

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INTRODUCTION

Cooperation among developing countries in the field of technology is one of the targets being pursued by the UN system as a decision of member countries. The basic idea is to have countries gather together to solve common technical problems by exchanging experiences and results, to share facilities and funds and eventually to utilize jointly foreign expertise or advice. This alternative differs from the traditional international or bilateral aid programs and calls for more active participation of developing countries.

This paper deals with the experience of the Fish Utilization and Marketing Service (FIIU) in attempting to start TCDC in Latin America among institutes working in different countries in the field of fish utilization.

The first regional program on the quality of frozen hake started in 1978 effectively and dealt with initial goals. The second program on the utilization of small pelagics was outlined in 1980 and 15 institutes have shown interest in this program's development.

FAO, through regional programs, is not only looking for a link among institutes from different countries but also a link among research centers or laboratories at universities, with the fish industry in the countries concerned as a means to plan research in a more appropriate and realistic way.

BRIEF DESCRIPTION OF THE PROGRAMS

Hake Program

As a consequence of the FAO Technical Consultation on the Latin American Hake Industry held in Montevideo in 1977 (1), a cooperative regional program of research was started relating to the quality of frozen South American hakes under the following conditions of processing:

Experiment 1 - Chilled (ice or CSW) without delay and then frozen.
Experiment 2 - Chilled with delay (5 h) and then frozen.
Experiment 3 - Chilled without delay and double freezing.

The program was carried out because the Technical Consultation disclosed a lack of basic information on storage quality of stored fresh and frozen Latin American hakes (2). Research analysis, performed on hake before the Consultation, showed that Latin American institutes had little experience in handling hake while on board or in storage.

Three countries have agreed with FAO to carry out experiments: Chile

(Escuela de Ciencias del Mar, Universidad Católica de Valparaíso), Peru (Instituto del Mar, El Callao) and Uruguay (Instituto Nacional de Pesca). Although Argentina has not signed the agreement, an institute (Centro de Investigaciones de Tecnología Pesquera, Mar del Plata) carried out experiments under conditions similar to those in Experiment 1.

The program involved institutes from countries with appreciable hake fisheries. These institutes have the possibility to influence current industrial practices and official regulations as well as to extend knowledge.

The program held three meetings to discuss the results achieved: Viña del Mar (Chile) in 1978, Montevideo (Uruguay) in 1979, and El Callao (Peru) in 1981. The last meeting in Peru concluded that "a sufficient amount of information about the keeping time of chilled and frozen South American hakes is now available".

But technical information was not, in practice, the only result achieved. In three of the countries the results, or part of them, were utilized by governmental bodies or industry to set up better regulations or start better practices. The results were included in seminars given to people from industry, and in regular university courses, and papers were presented at congresses and/or published in national/international journals. This resulted in improved general knowledge about industrial utilization of hake in these countries. Certainly it is very difficult for FAO to keep track of all the resulting activities, but their very existence shows us a self-supported interest in the subject. Moreover, we know from the technical results that skilled staff now exist in four Latin American countries on fresh and frozen fish handling and storage trials which did not exist in 1977.

Of course, the program could be improved. The extension activities were not planned beforehand, industry participation in the project itself was minor and many problems arose when carrying out experiments under practical conditions. The planning inside each institute was made without regard to its own human resources or time available, etc., although, in this connection, some positive points were learned by the institutes and by the Service and were used to plan the regional program on small pelagics.

FAO was only responsible for the coordination of the program and its total cost less than $25,000 (U.S.) These funds paid operative costs of the project within the institutes (Chile and Peru), technical meetings (travel expenses, tickets, etc.) and foreign advisors who assisted in the technical meetings.

Foreign participation was reduced to one Danish consultant attending the technical meetings and to the cooperation of NORDRECO AB (Sweeden) and UNILEVER (UK). These European firms did an excellent job on storing and analyzing samples sent from Latin America. And even though they do have an interest since Latin American hakes are exported, their invested effort surpassed expectation. Their results helped correlate the institutes' results and clarify different points of view. In general, the institutes tended to be more severe in judging the samples than the firms.

The hake program has shown that it is possible to create a Latin American network of research institutes working on fish utilization marketing and extension. There are many things still in store for the hake

program. We tried to benefit from the experience of the first program to formulate a new, more ambitious one covering nearly all of Latin America with focus on a common and very well-known problem--the utilization of small pelagics for human consumption. The institutes which until now have either participated or demonstrated an interest in the regional programs are listed in Table 1.

TABLE 1. Latin American Institutions Interested in Regional Programs

Country	Institution
Argentina	Centro de Investigaciones de Tecnología Pesquera (CITEP), Mar del Plata (1) (2) Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata (3)
Brazil	Instituto de Tecnologia dos Alimentos, Campinas (2) Fundacão Universidade de Rio Grande, Rio Grande (2) Faculdade de Veterinaria, Universidade Federal Fluminense, Niteroí (2)
Chile	Universidad Católica de Valparaíso, Valparaíso (1) (2) Instituto de Fomento Pesquero (IFOP), Santiago (2) Universidad del Norte, Antofagasta (2)
Colombia	Universidad del Valle, Cali (2) Instituto de Investigaciones Tecnológicas, Bogotá (3)
Costa Rica	Centro de Investigaciones en Tecnología de Alimentos, Universidad de Costa Rica, San José (2) (3)
Dominican Rep.	Instituto Dominicano de Tecnología Industrial (INDOTEC), Santo Domingo (2) (3)
Ecuador	Instituto Nacional de Pesca, Guayaquil (2)
Mexico	Instituto Nacional de Pesca, Mexico (2)
Peru	Instituto del Mar (IMARPE), Callao (1) (2) Instituto Tecnológico Pesquero (ITP), Lima (2)
Uruguay	Instituto Nacional de Pesca (INAPE), Montevideo (1) (2) Instituto de Investigaciones Pesqueras, Universidad de la República, Montevideo (2) (3)
Venezuela	Departamento de Tecnología de Alimentos, Universidad Central de Venezuela, Caracas (2) (3) Departamento de Tecnología de Procesos Biologicos y Bioquímicos, Universidad Simón Bolivar, Caracas (3)

(1)--Interested/participating hake program.
(2)--Interested/participating small pelagics program.
(3)--General interest on regional cooperation.

Small Pelagics Program

The utilization of small pelagic fish for human consumption is undoubtedly

one of the greatest challenges facing fish technologists. At present, around the world some 11 million tons of these fish are used directly as food and 17 million tons are reduced to fishmeal and oil--the latter only making an indirect contribution to human nutrition, principally in the developed countries. A further 25 million tons are currently not caught due, at least in part, to a lack of suitable catching, handling and processing methodologies and facilities.

The efficient utilization of small pelagic fish has received relatively little attention in the past, possibly since the technologically advanced countries have large resources of small pelagic fish which are either poorly exploited or converted into fishmeal, and considerable effort will be required to utilize these resources for human food.

The Latin American catch of small pelagics is around 6.5 million tons and increasing. Following the catch delay of Peruvian anchoveta, Chile increased its landing of small pelagics and other countries such as Uruguay decided to also expand their small pelagic fisheries.

Whereas the world's average of small pelagic utilization for human consumption is around 40 percent of the catch, in Latin America it reaches only 20 percent. These figures are chiefly determined by the 5 million-ton catches of Chile and Peru. Table 2 gives a more detailed account of the catches. The catches of the USA and Canada are also given for comparison.

In 1976 the Fisheries Development of FAO prepared a global project to tackle these problems. At the same time, DANIDA offered to consider funding some preparatory activities, and the handling of small pelagic fish was identified as a particular bottleneck to improved utilization. A project proposal was prepared by FAO--Project to Improve the Handling of Small Pelagic Fish--approved by the Danish Government in March 1977 and became optional in late 1977. The component relating to processing and marketing aspects of this proposal was accepted by Norway and this project became operational in 1979.

In 1980 a technical meeting was held in Montevideo to review the situation in Latin America (3), and 15 people participated from five countries. A cooperative program was outlined, presented and later discussed with several institutes in various countries of the region. Fifteen institutes indicated interest in the small pelagics program and presented their own programs and comments. Several of these institutes already have their own small pelagics program. To them, the regional program is a venue to discuss results and exchange information with other institutes having similar problems.

We can say that the reply was enthusiastic and went beyond FAO's highest hopes. Although a wide participation is desirable, it would also raise the previous budget estimate. But despite acute budget cuts and funding difficulties for Latin American projects, six institutes in the region received funds or advice from FAO during 1981 for their small pelagic projects. Nevertheless, development rate of this project will be very slow in the future if appropriate funding is not identified.

TABLE 2. Landings ('000 kg) and Utilization (%) of Small Pelagic Fish in Latin America (1978-79)

Country	Total Landings (1979)	Utilization (1978)		Main Small Pelagic Species Landed
		for Human Consumption	for Reduction	
Argentina	19,853	99[c]	a	E. anchoita
Brazil	239,181	82	18	S. aurita/Anchoviella spp./ S. japonicus
Chile	2,596,883[a]	7	93	S. sagax/T. murphyi/ S. japonicus/E. ringens
Colombia	4,258	10[c]	90	Cetengraulis mysticetus
Costa Rica	4,653	100[c]	-	Opisthonema spp.
Cuba	30,000	80	20	Caranx spp./O. oglinum/ Harengula spp.
Ecuador	593,132[a]	27	73	O. libertate/S. japonicus/ C. mysticetus
Mexico	440,934	16	84	E. mordax/S. caerulae/ O. libertate
Panama	132,103	30[c]	70	O. libertate/Cetengraulis mysticetus
Peru	2,393,771[a]	23[b]	77[b]	S. sagax/E. ringens/ T. murphyi/S. japonicus
Uruguay	1,183	80	20	Brevoortia spp./E. anchoita
Venezuela	40,471	86	14	S. aurita/C. edentulus/ O. oglinum
Canada	217,117	95[c]	5	C. harengus/C. pallasi/ S. scombrus
USA	1,374,115	9[c]	91	B. patronus/B. tyrannus/ E. mordax

[a]1980
[b]1979
[c]estimated

Sources: FAO Yearbook of Fishery Statistics, Catches and Landings, Vol. 48 (1979).

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Oficina Sectorial de Estadística, Ministerio de Pesquería, Lima Peru.

Sistema Economica Latino Americano (SELA)

Ministerio de Agricultura y Ganadería, San Jose, Costa Rica

CONSTRAINTS

The most obvious constraint is lack of funds (we can say a general lack of funds). FAO funds are already scarce and even scarcer as far as funding programs for Latin America are concerned, due to the comparatively high degree of development achieved by this region when compared to other regions. This does not mean that Latin America is not in need of help. It only means that priority is shifted to more urgent food problems in other regions.

On the other hand, Latin America not only holds an important part of today's fisheries but also holds a significant part of resources that, in the future, could be caught in a more or less conventional way. Unconventional resources call for large investments to be operative (e.g., krill, oceanic squid or mesopelagics), and it is not likely that a ton of these unconventional resources could be landed in the near future at a lower cost than, for example, a ton of small pelagics now being landed in Latin America.

Moreover, the situation in Latin America differs according to country, and certainly regional programs could effectively help transfer knowledge within the region.

In setting up the programs, there are two basic points to consider. The first is that FAO can only play a coordinating role and the second is that institutes should use, as much as possible, their own resources. Although FAO can facilitate program implementation and development within a country and the region, it is clear that is does not want to manage unilaterally "FAO business". The institutes in turn should draw up their own priority scale. It is not easy to convince institutes of this necessity. As a matter of fact, it is something like FAO versus fashionable scientific subjects. But this will encourage institutes to face reality if they are not already aware of it. We think this point was successfully achieved with the hake program. In fact, FAO funds were only a small fraction of its total cost. And this philosophy is also behind the small pelagics program.

Even though institutes regard regional programs as priorities, there are always costs that cannot be covered by the institutes themselves. Such costs are: contracting advisors (to be used jointly), import/export wages (e.g., in connection with samples, special equipment, etc.), special equipment or reactives (not always available in the country and very difficult to import in small quantities), travel costs (participating in technical meetings), etc.

Given the very limited project funds, the approach based on an international research network among national institutions makes sense in principle. It allows a wide coverage of activities, both geographically and by subject, and it is particularly suitable for the collection and exchange of technical information and the monitoring of research activities. However, this approach has some important limitations which have serious implications for project implementation. Its effectiveness depends heavily on the capabilities and quality of the participating national institutions which bear most of the costs of research activities. It requires a great deal of organization and coordination by the project staff to harmonize the diverse interests of various institutions in order to have an integrated

program. Precisely because of the wide coverage, the project's resources may be too thinly stretched on too many activities. Finally, a global or regional project such as this one could not be expected to produce, all things being equal, as many results and as much impact as a national project.

In terms of technical activities, the project design should have its targets clearly identified, e.g., in the small pelagics project, methods to be tested for specific bulk handling and storage. In order to guide the implementation of the network approach, it would have been desirable to establish a broad program of research which would provide a coherent framework for integrating and synthesizing individual country experiments, thereby avoiding undue duplications. Furthermore, since a feasibility study would be a logical end-product of these technical experiments, the program should contain a plan for linking technical investigation with economic and commercial feasibility. In practice, coordination is very difficult at this stage since many countries are potential or actual competitors in the international market of the products to be improved or developed.

Finally, fulfilling the implementation phase as scheduled is not easy since each institute works at its own pace. It is advised that every institute be responsible for doing what is within its reach.

From past experience, it is clear that personal contact is very important both between people from institutes with FAO and between the institutes themselves so that many of the above mentioned problems can be overcome.

WAYS TO HELP PROGRAMS

Funding large programs is becoming more difficult every day because of economic constraints. Although funds are always necessary, the participation of NORDRECO AB and UNILEVER in the hake program has shown us the possibility of receiving assistance other than direct funding.

Listed below are possible alternatives to direct funding that can be offered through FAO to development programs:

1. Direct contribution to some specific national institute (twin arrangements).
2. Participation in sample evaluation.
3. Review, comment and advice on results.
4. Distribution of printed material on subjects related to the program.
5. Finance exchange visits among Latin American institutes and among these institutes and U.S. institutes to carry out experimental work, particularly as regards equipment not available at home.
6. Invitation to researchers working in the programs to present results achieved in technical congresses.
7. Professors can call to the attention of Latin American graduate students attending U.S. universities the cooperative programs.

Even though in practice such heterogenous ways might be very difficult to coordinate, new attempts are necessary to expand technical cooperation. Unlike other regions, Latin American fish utilization institutes are mature and have enough technical know-how to influence fisheries development in the region.

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BIOLOGICAL TREATMENT OF FISH PROCESSING WASTES IN THE TROPICS

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INTRODUCTION

The biological treatment of any organic waste, whether in the tropics or the temperate zone, follows the same biological and engineering principles. There are few problems that are unique to the treatment of fish processing wastes. Those that do occur are usually caused by the seasonal occurrence of the wastes, nonuniform and intermittent loadings, and the location of the waste generating sites near the sea or navigable streams.

In the United States (and most other parts of the world), fish processing wastes have historically been returned to the sea. When large amounts of fish processing wastes are generated in one location, however, adverse environmental impact may occur if the wastes are discharged into small part of the sea. When the fish processing takes place on land in populated areas in the United States, the solid wastes are usually disposed of in municipal landfills, and the liquids are usually screened and disposed of in municipal sewers. Small operations in isolated areas usually do not cause adverse environmental impact if the wastes are discharged in a large receiving body of water with adequate mixing.

The location of waste treatment facilities in the tropics may encounter special problems relating to the general lack of infrastructure, the lack of trained personnel for construction and operations, and the presence of a distinct wet season and dry season which causes operating difficulties in certain biological processes.

This paper will briefly outline the various biological processes that can be used to treat fish processing wastes and identify the negative or positive effects of a tropical location on these processes.

TREATMENT OF ORGANIC WASTES

The organic wastes from fish processing sites are in the form of liquids, sludges, or solids. Biological treatment is applied to all three forms of wastes.

Organic wastes are a mixture of water, dissolved solids, and suspended solids. In liquid wastes, the organic fraction maybe as low as 100 mg/l (0.01% by weight) to as high as 10,000 mg/l (1% by weight); in sludges the organic fraction may be approximately 1000 mg/l (0.1% by weight) to 100,000 mg/l (10% by weight); in solids the organic fraction may be approximately fifty percent (50%) by weight.

It is not economical to dispose of the water along with the solid in liquid wastes. It is necessary to release the water to the environment and to dispose of the solids. In the treatment of solid wastes, the water is disposed of along with the solids. Sludges might be disposed of as a solid waste or a liquid waste depending upon water content, volume involved, and economics.

Liquid waste treatment may involve many steps as the liquid flows from one tank to another in series. Pretreatment and/or primary treatment may consist of one or more of the following:

a. pH adjustment, pre-aeration, and pre-chlorination are used to remove gases and/or odors or to make the processes that follow more efficient.

b. Grit removal, screening, and sedimentation are used to remove suspended solids.

c. Chemical coagulation and sedimentation are used to remove colloidal solids.

Organic dissolved solids are removed by secondary treatment processes, usually biological processes. Inorganic dissolved solids and the organic dissolved solids not removed by secondary treatment are removed by tertiary treatment processes such as evaporation, ion exchange, electrodialysis, reverse osmosis, ultra-filtration and centrifuging.

The solids and sludges removed during the treatment of liquid wastes may be treated by physical, chemical or biological processes before they are disposed of. The water (effluent) that leaves the liquid waste treatment plant is usually disinfected by chlorination before it is discharged from the plant.

The biological processes used for liquid wastes are:

a. Activated sludge and related processes

b. Trickling filters and rotary bio-contactors

c. Oxidation ponds and aerated lagoons

d. Land application

The biological processes used to treat or modify sludges are:

a. Anaerobic sludge digestion

b. Aerobic sludge digestion

The biological processes used to dispose of solid wastes (and some sludges) are:

a. Sanitary landfills

b. Land application

BIOLOGICAL WASTE TREATMENT

Biological waste treatment processes are usually either predominantly aerobic or anaerobic processes. Aerobic processes are used to convert organic dissolved solids (these cannot be removed by sedimentation) to organic suspended solids that can be removed by sedimentation. Anaerobic processes will also convert organic dissolved solids to organic suspended solids (but not as fast or as effdciently) and they will convert a significant proportion (10-50 percent) of the organic materials to gases, predominantly carbon dioxide and methane.

Biological waste treatment processes, aerobic or anaerobic, are similar whether they occur in liquids, sludges or solids. The micoorganisms use the waste stream as a source of the following essential elements and substances required for nutrition: (1) major elements, (2) minor elements, (3) trace elements, and (4) growth factors. The major elements are carbon, hydrogen, oxygen, nitrogen, and phosphorus. The minor elements are sulfur, potassium, sodium, magnesium, calcium and chloride. The trace elements are iron, manganese, cobalt, copper, boron, zinc, molybdenum and aluminum. The growth factors are substances required in trace amounts that the cells cannot synthesize. These include vitamins, essential amino acids and precursors for the synthesis of required synthesized compounds.

Municipal wastes normally have all of the essential elements and growth factors required for biological waste treatment. Some fish processing wastes maybe lacking in nitrogen and phosphorous. Experience indicates that the nitrogen to 5-day BOD ratio must be at least 1:32 and the phosphorous to 5-day BOD must be at least 1:150.

Microorganisms are classified according to their respiration requirement as aerobes, anaerobes, and facultative anaerobes. Respiration produces the energy required for the life processes; it essentially consists of removing hydrogen atoms from a hydrogen donor substance and eventually uniting the hydrogen with a hydrogen acceptor. In aerobic respiration, the hydrgen acceptor is molecular oxygen and the end product is water. In anaerobic respiration, the hydrogen acceptor may be combined oxygen in the form of radicals such as carbonate, nitrate, and the sulfate ion, or it may be an organic compound. The end products of these acceptors are methane, ammonia, hydrogen sulfide or a reduced organic compound. Facultative anaerobes use aerobic respiration if molecular oxygen is available; if it is absent, they will use anaerobic respiration. Obligate anaerobes cannot live in the presence of free oxygen.

Environmental factors that are of importance in the waste treatment plant are:

a. pH (normally best between 6.5 to 9.0).

b. Temperature (normally 45° to 75°C). Above 75°C, the microorganisms are killed; below 75°C, the microbial activity doubles with each 10°C increase in temperature.

c. Presence or absence of molecular oxygen.

d. Presence of certain toxic chemical agents and compounds. Toxic compounds usually have no effect at low concentrations. They stimulate growth as the concentration increases. If the concentration is further increased, theyinhibit growth. If the concentration is increased more, they kill the organism.

e. Mixing of the microorganisms and the waste being treated.

f. Recycle rate.

g. Detention time.

ACTIVATED SLUDGE AND RELATED PROCESSES

The activated sludge process is an aerobic process that depends upon a biological slime that develops naturally in aerated organic wastes. The process utilizes a biological reactor that is mixed and aerated (aeration tank) followed by a solid-liquid separator (settling tank) and recirculation pumps. The sludge in the biological reactor is kept at about 2,000 to 4,000 mg/l of suspended solids by recirculating the sludge (or a portion of the sludge) that is collected in the solid-liquid separator. When the activated sludge is maintained in the logarithmic phase of growth by recirculation and aerobic conditions, the sludge will absorb and adsorb the soluble organic substances found in the waste stream. The aeration period varies from about six hours to 20 hours, depending upon the temperature, characteristics of the wastes, and the degree of treatments required.

The conventional activated process uses a rectangular biological reactor, and the aeration and mixing is applied uniformly along the reactor. The flow through the reactor is plug flow (no mixing along the longitudinal axis of the reactor).

There are many modifications of this process. The modifications can be to the location of the aeration devices, resulting in tapered aeration and step aeration processes. This provides more oxygen where it is needed, at the upstream end of the reactor. The shape of the biological reactor can be changed from rectangular to round or square to cause the reactors to be completely mixed rather than plug flow. The major advantage of the mixed flow is that it is less susceptible to shock due to toxicity and variable loads, and it is more flexible in its operation. Another modification is to provide for two biological reactors, resulting in contact stabilization or biosorption processes. One reactor is for the sorption of organic materials by the activated sludge, and the other is for bio-oxidation of the sorbed materials. The extended aeration process has a long reaction time so that no excess sludge is formed. The reactors for extended aeration can be rectangular, round, square or race-track layout. Other modifications possible involve the recirculation rate, the food-to-microorganism ratio, and the mixed liquor/suspended solids concentration. The selection of the appropriate process normally requires bench scale or pilot scale studies of the waste.

Most of the activated sludge systems will remove 90 to 95 percent BOD_5 and 85 to 95 percent of the suspended solids. Good nitrification results if the mean cell residence time is greater than ten days. There are no adverse effects caused by a tropical environment. The high temperatures of the tropics will decrease reaction times required for a given degree of treatment.

TRICKLING FILTER AND ROTARY BIO-CONTRACTORS

A trickling filter consists of a filter bed with microbial growths through which the wastewater passes. The filter bed is a tank filled with gravel, crushed stone or slag having a size of about three inches. The bottom of the tank has an underdrain system for removing the wastewater as fast as it enters. The filter media might also be a synthetic media when stone is not available locally.

The microbial growths on the gravel sorb the organic matter as the water flows through the filter bed. The water passes through in minutes; the organic matter takes several hours or longer to pass through. Most of the microbial growth is aerobic. The growth adjacent to the gravel is usually anaerobic. As the microbial slime grows and gets thicker, some of it sloughs off the gravel and leaves the filter bed. The growths that have sloughed form the filter media (the humus) is settled in the final clarifier. This final sludge is collected and sent to the primary clarifier, where it is collected for sludge treatment and disposal.

Trickling filters are classified as low-rate filters or high-rate filters depending on loading rate. Low-rate filters have high (up to 90 to 95 percent) BOD_5 removal when treating municipal wastes, and nitrification is higher if low-rate filters have an effluent of about 12 to 25 mg/l of BOD_5. A single stage high-rate trickling filter will have an effluent of about 35 to 50 mg/l of BOD_5. Two-stage high-rate trickling filter plants will produce an effluent of about 15 to 35 mg/l of BOD_5. Very little nitrification occurs in high-rate trickling filters. Odors may be a problem in low-rate filters, but they seldom are a problem in high-rate filters.

The rotary bio-contractor is similar to the trickling filter. In the trickling filter, the media is stationary. The rotary bio-contractor employs media disks that are rotating through the waste water. The advantages of the rotary biological contractor are its ability to handle shock loads and its ability for multi-stage units to achieve a high degree of nitrification.

Trickling filters and rotary biological contractors need little maintenance, laboratory control and minimum operator time. They require little energy, but they do use a large land area.

There are no adverse effects caused by a tropical environment. The high temperatures should increase the degree of treatment.

OXIDATION PONDS AND AERATED LAGOONS

The oxidation pond (stabilization pond) is a pond through which the waste water flows. The microorganisms in the pond bring about the bio-oxidation of the organic matter to form carbon dioxide, ammonia, inorganic radicals such as sulfates and phosphates, and new microorganisms. The algae, utilizing the inorganic radicals, carbon dioxide and the sun's energy, produce dissolved oxygen and new algal cells. Many of the biological solids settle to the bottom of the oxidation pond and undergo anaerobic digestion similar to that in a digester. The effluent might go directly into the nearest water body, but some use intermittent sand beds to remove algal growths before being discharged. The oxidation pond must be drained after several years to remove the accumulation of sludges.

Oxidation ponds can be classified as aerobic, anaerobic, or facultative. Aerobic ponds have dissolved oxygen present throughout the depth of the pond; anaerobic ponds do not have dissolved oxygen at any depth; facultative ponds have oxygen near the surface and are anaerobic near the bottom.

In order to increase the organic loading to the pond, mechanical aeration can be added. Another modification includes the addition of another oxidation pond in series or a settling tank to remove the sludge.

The increased temperatures of the tropics should increase the reaction rates for these ponds. During the rainy season, drainage will have to be controlled carefully or detention times will be decreased by increased flow.

ANAEROBIC SLUDGE DIGESTION

Anaerobic sludge digestion is the biological oxidation of degradable organic sludges under anaerobic conditions. Most of the microorganisms in the anaerobic digester are facultative anaerobes. The organisms that produce the methane are obligate anaerobes. Small amounts of molecular oxygen are toxic to them.

The digestion process can be considered as three stages: (1) liquefaction of solids, (2) digestion of the soluble solids, and (3) gas production. The organic-acid-forming microorganisms in the digester utilize the carbohydrates, proteins, fats and their degradation products to produce organic fatty acids. The methane-producing microorganisms use the fatty acids as substrates and produce methane and carbon dioxode. The methane producers grow more slowly than the acid producers and require a more narrow pH range of about 6.7 to 7.4 . A properly operating digester produces 55 to 75 percent methane to about 25 to 45 percent carbon dioxide plus trace amounts of other gases.

Two-stage digesters have been successfully used. The first stage is a continuously mixed high-rate digester followed by an unmixed-low-rate digester. Most of the biodegradation takes place in the first stage; the purpose of the second stage is to allow stratification to take place so the digested sludge and the supernatant can be separated and withdrawn from the digester.

Digesters work best up to a temperature of about 35°C, the optimum temperature for the mesophilic bacteria. The optimum temperature for the thermophilic microorganisms is 54°C, but thermophilic digestion has not been successful in the U.S. because the thermophiles are very sensitive to environmental changes in the digester. This could cause the efficiency of anaerobic digesters to be lower in the tropics than in cooler areas.

AEROBIC DIGESTION

Aerobic digestion is the biological oxidation of organic sludges under aerobic conditions. Aerobic digestion closely resembles the activated sludge process since the aeration equipment and tanks are similar. A thickener-clarifier usually follows and aerobic digester to reduce the suspended solids concentration in the supernatant and to reduce the high recycled sludge flow rate. The advantages of aerobic digestion (followed by thickener-clarifier) over anaerobic digestion are less operational problems, less laboratory control, the supernatant liquor has a much lower BOD concentration, lower capital costs, and the thermophilic microorganisms are less sensitive to environmental changes. The disadvantages of aerobic digestion compared to anaerobic digestion are higher energy requirements, no methane is produced, and the digested sludge has a lower solids content so the volume of digested sludge is higher. (Changing the solids concentration from 2 percent to 4 percent reduces the volume of sludge by one-half.)

SANITARY LANDFILL

Solid wastes can be disposed of in a sanitary landfill. A sanitary landfill consists of a trench or area excavated in a clay soil. If the soil is not a relatively impermeable clay, then the trench or area will have to be lined with a suitable lining. The trench or area is filled with solid wastes that are compacted in two-foot lifts. At the end of each day, a cover of soil of at least six inches is placed over the wastes deposited that day, which might consist of several compacted two-foot lifts. When the trench or area is filled to its design height, a final cover of at least two feet of soil is added. The purposes of the daily cover and the final cover to minimize vector breeding areas and animal attraction, control water movement, control (but not stop) gas movement, and minimize fire hazard.

Sludges can be successfully disposed of in a landfill if they are mixed with enough solid wastes so that no free water occurs in the working area (the area where the solid wastes are unloaded and compacted). A sludge with ten percent solids will require about two to three times its volume of municipal waste or equivalent to absorb the water.

The organic material buried in the landfill undergoes anaerobic digestion. Approximately half of the organic solids are converted to carbon dioxide and methane in about equal volumes. Biodegradation can be speeded up in the landfill if the moisture content and temperature are increased (up to a maximum of about 75°C), the wastes are shredded, the

leachate is collected and recirculated through the solid wastes, and the solid wastes are seeded with anaerobic sludge from a waste treatment plant.

The two most common problems with operating a sanitary landfill in the tropics are caused by the lack of enough suitable clay in many tropical areas and the existence of a long rainy season followed by a long dry season. This can result in an unfavorable water balance; more water will infiltrate the final cover than will evaporate from the surface. This can cause a severe leachate problem unless the leachate is removed from the landfill during the dry season and evaporated on the landfill surface.

LAND APPLICATION

Solid wastes and sludges can be plowed into a soil surface as a fertilizer or a soil conditioner. Land application should be operated as an aerobic process. For best results, the waste material is placed within the root zone, and a crop (not necessarily a revenue crop) is maintained. Only heat-dried sludges should be used on crops that are eaten raw by humans. The soil is not allowed to be saturated with water except for brief periods of time. During the dry season, a favorable water balance can be maintained; during the wet season, the wastes may have to be stored until the dry season or the application rate will have to be reduced.

One of the operating difficulties of land application is the build-up of salts in the root zone. The salts can be removed by collecting the leachates for disposal or by natural leaching during the wet season.

Liquid wastes also can be disposed of by land application. The liquid can be used as irrigation later during the dry season. The wastes will have to be stored or disposed in another manner during the wet season. In some land application projects, the water is reclaimed by sub-drains or ditches and released into the environment. The organic material trapped in the root zone is aerobically decomposed. Care must be taken not to saturate the soil for long periods of time because the vegetative cover will be destroyed, and anaerobic conditions will occur causing low rate biodegradation and odors.

CONCLUSIONS

Several factors that affect the treatment of wastes from the fish processing industries are:

1. Lack of experience in treating fish processing wastes under the regulations.

2. Variation in the locations of fish processing plants from remote locations with no infrastructure to municipal facilities.

3. Small volumes of wastes are produced by many fish processing industries on a seasonal or intermittent basis.

4. As more of the fish is harvested for market, the remaining waste stream becomes more complex.

All of these factors make cost estimates of waste treatment difficult to make. Small waste treatment plants that are operated intermittently or seasonally are normally more expensive per unit volume to construct and operate. In general, it is more economical to use public waste treatment facilities than to build and operate a small plant. Of course, it is more economical also to use the simplest process that provides the desired treatment and that satisfies the regulations. In general, a sanitary landfill or application process should be considered first for solids and sludges, while the oxidation pond should be the first choice for liquid wastes.

As more parts of the fish are processed for marketing, the disposal of the remaining fish and the chemicals used for separation become increasingly complex and expensive. These additional costs of waste treatment can grow faster than the additional revenues that the processing provides.

SPINY DOGFISH FOR YU-SONE, SURIMI, OR KAMABOKA: AN EXTENSION REPORT

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PROBLEM

The catches of spiny dogfish or sandshark have been reported as being a few million pounds per year from areas ranging from Maine to the Mid-Atlantic States as well as the Pacific coast waters in the U.S. (1). Domestic markets for dogfish are limited. Currently, big markets are in the German schillerlocken product (smoked restaurant item) for the belly flap and the British "fish and chips" market for the back meat. However, more outlets are being requested for this tremendous dogfish fishery by producers, seafood processors, as well as related industries such as manufacturers of dogfish processing machines. Machines, such as the one developed by MIT (5), can greatly speed up the processing of dogfish. Yet, the capital investment also requires justification by an increase in market volume. These dogfish related industries began to search for additional foreign market outlets, some asking whether dogfish is good for Oriental products such as surimi and kamaboko that have well recognized market volume in Japan, or the yu-sone product that has great market potential in China. A short-term study was then conducted in this laboratory to investigate the suitability of dogfish for making good yu-sone or kamaboko products.

In previous experiences, we learned that many types of shark were excellent materials for yu-sone, a seasoned-dried fish flake product (12). They were also very common raw materials for the surimi type of product as reported in some literature (4,11,13). However, dogfish is unique for its small size as compared with other shark species--high fat content (40% dry basis), fine muscle fiber, and less urea content than other types of shark. Unique problems in making a good yu-sone product from spiny dogfish are skinning, dressing, adjustments on the fat content, and preservation of the fiber integrity. As for surimi, limited success with California dogfish had been obtained by Kudo et al. (7). It was rated as a substandard to poor raw material for surimi application depending on the freshness and flesh treatment. Approaches for optimization can be studies that include minimizing the effect of excess fat, optimizing the chopping procedure, and finding the proper formulation to obtain the good "ashi" property acquired by the kamaboko product (13).

BACKGROUND AND METHODOLOGY

Yu-sone products. Yu-sone processing involves separation of pure fish flesh, boiling the flesh, dewatering, addition of ingredients such as oil, starch, sugar, salt, and spices, and stir-frying until dry (15).

Amounts of ingredients in the finished product need to be in a certain ratio to meet the traditional taste preference. Thus, additives are always metered based on the dry weight of the fish used. Adjustments of the quantity of ingredients, oil in particular, should be made according to the fat content already present in the fish. The quality of finished yu-sone products made from different fish species depends on the color, the fat stability against rancidity, the inherent fish odor, and especially the fibrous texture property of fish flesh. Although fish oil can be stabilized by the antioxidants present in the added commercial oil (12), the rancid oil already present in aged raw material cannot feasibly be salvaged. This is the special quality concern to be emphasized when frozen stored fatty fish such as dogfish is to be used as the raw material for the yu-sone product.

In this study, the yu-sone processing method and machinery developed for the shark yu-sone reported in the previous study (15) was used. The product rancidity was measured through the TBA colorimetric method (14).

Surimi and Kamaboko types of products. Basically, the flesh is first recovered by deboning the fish. It is in minced form before treatments of soaking, washing, and dewatering take place. Blood, fat, pigments, and undesirable soluble protein extracts are washed away in this step. If the treated minced fish is to be stored for future use, it is usually mixed with 0.2% sodium tripolyphosphate and 5% sucrose to preserve the protein gelling property during frozen storage (7,10). To process the surimi further into a kamaboko type of product it is typically ground with about 3% salt, less than10% starch, and other desirable flavoring ingredients such as MSG, wine, colorants, and spices (10). The weight percentages of ingredients are based on the flesh weight. The mixture is then shaped, preset, and cooked by either boiling, steaming, broiling, frying, or combined cooking methods depending on the product variety.

Factors determining the gel quality and strength can be classified in two catagories; the quality of the materials and the processing conditions. The material quality is affected by 1) species of fish: mainly related to the composition and concentration of its salt soluble proteins. It is also affected by the content of undesirable components such as fat, blood, amino compounds, and small peptides; 2) freshness of fish: mainly associated with the pH, degree of autolysis, and spoilage; or 3) quality and quantity of additives such as water, salt, starch, egg white, oxidizing agents, sugar, soy proteins, and others. The factors in processing conditions refer to 1) freezing or not freezing the raw material before use; 2) washing or not washing the minced fish; 3) means of chopping; 4) chopping time and temperature; 5) presetting time and temperature; and 6) cooking time and temperature. Detailed discussion on the effect of these factors is available in several references (7,8,9, 10,11,13). Although adjustment of processing parameters should be made on each different fish species used, the condition for each parameter is usually within a certain range. Thus, a systematic approach to optimize the kamaboko process for new fish species is usually possible.

In this study, chopping was done on a Hobart silent cutter (M-84181) and cooking was done in sub-boiling water for about 10 minutes after the

fish paste was shaped into the Chinese style fish ball configuration. Evaluation of gel strength in this study was based on the folding test (7) using an average of three measurements and the compression/penetration (c/p) force with five runs for each sample. The c/p force was measured by an Instron Universal Testing Machine (Type TM 1130, Instron Engineering Corp., Canton, Mass.) using the appropriate attachment to accomodate a 1/4 inch diameter plunger (2,6). The head and the chart speeds were 5 and 10 inches/minute, respectively. The c/p modulus $(G)=(F/A)/(\Delta L/L)$, where $A = 0.04909$ in^2, $L = 0.75$ in., F = load in pounds at the gel breaking point. The unit of G was in lb/in^2. Each of these three parameters: G, F/A, and $\Delta L/L$, was used to judge the springiness or cohesiveness of the gel (9).

APPROACH TO PROBLEM SOLVING

The objectives of this investigation are: to produce quality yu-sone and kamaboko products from dogfish, using the best known technology with necessary process modification, to find the good and bad properties of dogfish for these applications, and to provide recommendations on areas necessary for further study in this subject.

For the yu-sone application, questions which needed to be answered first were: whether dogfish fiber could survive the process, whether the fish oil could be stabilized by the added commercial oil with its antioxidants, and how to adjust the formulation to produce an acceptable product. Two 50-pound batches of fresh round dogfish were obtained from the New England area and processed with two formulations; one was the standard and the other was the adjusted.

For the surimi application, several batches of fresh and frozen dogfish were obtained from the same source. They were divided into ten batches for producing kamaboko type product with different treatments, testing the effect of 1) amount of water added, 2) amount of starch added, 3) chopping time, 4) iced storage and 5) frozen storage. Yet, most importantly, a qualitative answer should be given to say whether the best possible kamaboko product made from dogfish is satisfactory or not for industrial scale application (5).

RESULTS AND DISCUSSION

Yu-sone application. In the first batch, when the standard formulation (15) was used, the finished product was oily and dark in color. The fat content was 42% which was much higher than the 30% desired level. This is due to the fat content of the raw material being 38% dry basis, while the standard formulation was designed for fish containing about 10% fat. Thus, the oil addition was reduced to half of the original amount to be 22.5% of the dry weight of the fish used. The final formulation was adjusted to oil 22.5%, sugar 26.2%, salt 6.55%, soy sauce 11.3%, flour 8.57%, licorice 0.52%, and MSG 0.2%. The finished product was lighter in color. Yet, the fiber texture was much finer than the product from other shark, although it was still comparable with commercial samples. After being packaged in molded polyethlene cups and stored for two months,

it ramained palatable and a rancid odor had not developed. The TBA value was very low at the level of 3.04 mg malonaldehyde per kg dry flesh after stored at ambient temperature for four months. The yield was 28% of the round fish. Due to its fine fiber, further investigation may be needed to find a new drying mechanism, where drying can be done in a shorter time with milder agitation so that fish fiber can be better perserved. Added oil seems to stabilize the product as expected (12).

Surimi and kamaboko application. The formulations used in this study were designed to compare the effect of water and starch contents on the gel strength. Thus, various amounts of water ranging from 6% to 0% and starch ranging from 10% to 0% were added. Fixed amounts of salt (3%), sugar (4%), and spices (0.3%) were used for each batch. The summary of formulations used is shown in Table 1. Although different formulations have been documented for other fish species (7, 10, 13), the ratios used in this study were chosen based on the previous experience with other types of shark in this laboratory. Washing of fish flesh was not done for all treatments because unwashed dogfish flesh resulted in a better gel (7). Some phenomena learned from this study are as follows:

1. Chopping time: When the fish flesh was chopped with salt and other ingredients for 20 minutes (Treatment P), the fish gel was very poor. When the chopping time was shortened to 10 minutes using the identical formulation (Treatment A), the resultant gel was much better. It exhibited 31.8 lb/in^2 of breaking force and 0.47 inches deformation/in at the breaking point, which was equivalent to about 69.10 lbs/in^2 c/p modulus (Table 2). When the chopping time was reduced further to 5 minutes as used in the rest of the treatments, a good gel was always obtained. It is suggested that the dogfish flesh is so fine that the salt-in process can be completed in an unusually short period. Excess chopping by the silence cutter destroys the functionality of the fish protein.

2. Effect of water content: The amount of water added into the mixture of Treatment A was 6%. In Treatments B and C, addition of water was reduced to 3% and 0%. The slight differences in texture data obtained from these three treatments were not statistically significant (Table 3). The variation in final water content was actually within 1.6% based on a 75% moisture content for fish flesh. Addition of water up to 6% may not be enough to weaken the gel strength as suggested by other studies (8,9). Further study is needed to see the effect of greater than 6% water additions and its relationship with starch addition.

3. Effect of starch: When starch addition was varied between 0 and 5% (Treatment E,F,G in Table 2), no significant difference in texture strength was observed (Table 3). Yet, when 10% starch was added (Treatment I) the breaking force and the c/p modulus of the gel was significantly

Table 1. Formulations and various treatments in chopping time and storage conditions used in making fish gel products*.

TREATMENTS	P	A	B	C	E	F	G	I	H	J
Freshness (days)	4	4	5	5	9	9	9	16	16	38
Chopping Time (min.)	20	10	5	5	5	5	5	5	5	5
Water Added	6%	6%	3%	0	0	0	0	0	0	0
Starch	5%	5%	5%	5%	5%	0	2.5%	10%	5%	5%
Storage Conditions	iced	iced	iced	iced	iced	iced	iced	frozen	frozen	frozen

* other ingredients added in same quantities for every batch included salt 3%, sugar 4%, and spices 0.3%.

Table 2. Texture parameters for fish gel products; data are average of five measurements except the folding test using three measurements, treatments are designated in Table 1.

PARAMETERS:*		TREATMENTS								
		A	B	C	E	F	G	I	H	J
F/A (lbs/in^2)	mean	31.77	27.50	27.30	33.41	26.50	22.00	48.82	23.22	24.24
	SD	1.83	3.06	5.91	3.46	1.43	6.93	14.10	0.41	0.41
ΔL/L (in/in)	mean	0.47	0.37	0.41	0.48	0.52	0.49	0.41	0.35	0.43
	SD	0.07	0.04	0.05	0.05	0.04	0.11	0.08	0.04	0.05
G (lbs/in^2)	mean	69.1	73.1	66.2	69.2	51.4	49.1	113.8	66.8	56.9
	SD	11.8	9.0	12.2	3.9	2.6	27.9	42.1	7.5	7.3
Folding test	mean	3.0	3.3	3.3	4.3	4.3	4.3	3.3	3.0	2.6
	SD									

* F/A: breaking force
ΔL/L: deformation at rupture
G: compression/penetration modulus

Table 3. Statistical analyses on texture strength data of fish gel products, superscripts indicate the significant level and ns means $p > 0.1$.

Factors:	Water	Age-in-Ice	Starch	Freezing	Frozen Storage
Treatments:	ABC	CE	EFGI	EH	HJ
F Values:					
F/A	1.98^{ns}	$3.80^{0.1}$	$7.61^{0.005}$	$23.75^{0.005}$	0.58^{ns}
ΔL/L	$3.91^{0.05}$	$4.01^{0.1}$	1.83^{ns}	$11.72^{0.025}$	$4.41^{0.1}$
G	0.65^{ns}	0.28^{ns}	$6.97^{.005}$	0.40^{ns}	3.37^{ns}
Ranking:					
F/A	ns	E>C (?)	IEFG	E>H	ns
ΔL/L	ACB	E>C (?)	ns	E>H	J>H (?)
G	ns	ns	IEFG	ns	ns

increased to 44.8 and 113.8 lbs/in^2, respectively, while the score of the folding test dropped from 4.33 to 3.33 inches/in. It suggests that too much starch will toughen the gel and degrade its resilence property.

The compression/penetration breaking force, F/A, was commonly used by many reports besides the folding test to interpret the gel strength (2,6,9). However, deformation, $\Delta L/L$, needs to be referred in the case when hardness is to be distinguished from resilence. The folding test seems to relate to the cohesiveness property of the gel, which may be closer to the "ashi" property acquired by the kamaboko type products. Several extensive studies in correlating sensory and instrumental data of sausage types of products have been completed (2,3, 6). Since the kamaboko type of product has a slightly different gelling system, gelling with protein-water-starch instead of protein-water-fat, similar studies may be needed to examine correlations between sensory scores and various objective data.

4. Effect of ice storage: When the gel made from the five day sample (Treatment C) was compared with the one made from the sample stored in ice for nine days (Treatment E), the nine-day product was slightly better than the five-day product in terms of the breaking force and deformation. This phenomena agrees with the observation by Kudo et al. (7). It is possible that undesirable components in the fish flesh bleach out continuously during storage in ice so that the relative share of salt soluble protein in the fish is increased which will enhance the quality of the gel. Large amounts of milky water was released from the flesh during storage. Further study is needed to verify this observation. Treatment E produced the best product in this study, having 33.4 lbs/in^2 breaking strength and 0.48 deformation.

5. Effect of frozen storage: When the dogfish, dressed and skinned, was frozen and stored at -10°F for one week (Treatment H), the breaking force of the resultant gel was reduced from 33.4 lbs/in^2 (Treatment E) to 23.2 lbs/in^2 and the deformation from 0.48 to 0.35 inches/in. Further changes in the breaking force and the deformation were not statistically significant (Table 3) when the fish was stored at the same condition for three more weeks (Treatment J), although G dropped slightly to 56.85 lbs/in^2 and the folding test score was 2.6. This degradation effect by freezing suggests that protective measures such as mixing the dogfish with sucrose and polyphospate before freezing may be needed to extend its shelf life (10).

SUMMARY

Spiny dogfish produced yu-sone, the seasoned-dried fish flake product, with good color, flavor and stability. The fiber texture was finer than other types of shark, yet was still comparable with many commercial products. The formulation after being adjusted for the excess fat content of the fish became cottonseed oil 22.5%, sugar 26.2%, soy sauce 11.3%, flour 8.57%, salt 6.55%, liquorice 0.52% and MSG 0.2%, based on the dry weight of the fish flesh as 100.

Kamaboko produced from unwashed dogfish fillets after storage in ice for nine days produced fish gel with very good textural properties. The breaking force was 33.4 lbs/in^2, and the folding test score was 4.33. Rheological data was measured by a textrometer using a 1/4 inch diameter compression/penetration plunger and a head speed of 5 in/min (2). Scores from the folding test seem to be the best single objective parameter to grade the fish gel in terms of the "ashi" property acquired by the market. Storage with ice for nine days increased the gel quality and was probably due to the leaching effect. The freezing of fish fillets degraded the gel quality, yet no further significant decrease in protein gelling quality was detected when the fillet was stored for an additional three weeks at -10°F. The addition of sucrose, tripolyphosphate, or antioxidants to the fish flesh before freezing seems to be needed to preserve its gelling property. The best formula for making a good fish gel product from spiny dogfish found in this study is to first leach out undesirable components from the flesh, followed by using a formulation with less than 6% added water, starch 5%, salt 3%, sugar 4%, and some spices as desired. The fish is then chopped with the ingredients for less than 10 minutes at a low temperature. The resultant sol is then shaped and cooked under sub-boiling temperatures, approximately 10 to 15 minutes or until the gel is set. Although the spiny dogfish has been shown to make acceptable kamaboko products, further improvements may be achieved by searching for optimal conditions for factors such as additives, means of chopping, presetting, and cooking.

In conclusion, the spiny dogfish seems to have great potential for both of the tested products. Judging from the size of the catch, this species seems to be worth a more systematic investigation to determine its maximum untilization.

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OPTIMIZATION OF DRYING CONDITIONS FOR STOCKFISH PRODUCED FROM UNDERUTILIZED FISH ---- DESIGN OF A FISH DRYER MULTISTAGED BY A PROGRAMMABLE CONTROLLER

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INTRODUCTION

Because of limited fishery resources, the uncertainty of the market outlook, and ever increasing operating cost, the utilization of all catches in any profitable form becomes more and more important to all fishing industries. In recent years, efforts had been concentrated in this laboratory on searching for feasible processing methods for producing dried finfish products to be exported to foreign markets. Selected products are both traditional and have high demand. The obvious advantages to this approach over the development of new products for domestic market include (i) the lack of need to develop new food preferences and eating habits, (ii) filling the need for high protein food stuffs in other countries, and (iii) developing additional means of reducing the U. S. trade deficit.

To develop a drying system that is economically feasible for the international market place, one should consider a capital intensive approach with reasonable capital investment. The best process was concluded from the previous study as being multistaged, having recirculating hot air, and using means such as puffing to accelerate the mass transfer property of the product (5). However, a well designed dryer is still the most important piece of equipment in the whole drying system. In this report, the authors try to summarize energy saving features of dryers on the market and design one dryer that can incorporate those useful approaches.

GENERAL DESCRIPTION

Basically, the dryer provided a heat source necessary for evaporation and internal mass transfer of the moisture. The transportation of this heat was assisted by the moving air controlled by a centrifugal blower. The product was placed in a rack truck with the air traveling across the racks horizontally. The air carrying the moisture taken out of the fish was recycled after passing through a dehumidifier. Temperature, relative humidity, and velocity of the air govern the drying rate.

Due to the delicate proteinaceous nature of the fish and the internal transfer rates being so slow, the external rates can easily surpass the internal rates, causing hastened denaturation at the product surface and resulting in case-hardening. This tendency is usually expressed on the traditional drying curve as having a constant rate followed by a falling rate period. Measures such as slowing down the air circulation rate and maintaining the optimal humidity in the dryer were required to decrease

the difference between the internal and external transfer rates, thus delaying the falling of the drying rate. The recirculation of air without frequent exchange with outside air results in a net saving on energy cost even though there is an additional need for dehumidification. The savings on energy cost can be extended when a heat pump is used to provide the dryer with both the heating and dehumidification capacities. Further, the heating and dehumidifying systems were built inside a by-pass duct that branched out of the main circulation path, so that a fraction of the air stream would be led through this by-pass only when needed. This may reduce the restriction in the main circulation path, thus saving energy used for the air blower as well as reducing the maintenance cost for the heating and dehumidifying system.

Since the drying force should be just enough to match the need at the product surface, the control of the staged drying was based on the dryness at the product surface, which was monitored by measuring the wet bulb depression of the surface temperature (1,4). A thermocouple sensor was placed on the surface of a representative sample for each batch being dried. The basic rule of control should be to set drying power initially at maximum as permitted, i.e. increased air speed, decreased relative humidity, and relatively high drying temperature. As the fish was dried, the surface moisture decreased, the effect of wet bulb depression diminished, and the surface temperature increased until it reached the first set point of the temperature controller. The drying conditions were then shifted to the second stage and maintained there until the second set point was reached. At this point, the diffusion property started to deteriorate. The drying conditions were changed to the third stage with even milder conditions so that permeability of the fish surface and continuous migration of the internal moisture could be maintained. A programmable controller was incorporated into the system to control the sequence of shifting and to facilitate the study in finding the optimal sequence to product quality products.

DESIGN AND PHYSICAL ARRANGEMENT

1. Dryer Construction:

The design of the dryer is shown in Figure 1. It has a main drying chamber of 48W x 46D x 70H inches with baffled inlet and outlet plenums on both sides to create horizontal air flow across the chamber. These two plenums were connected from above by a circulation duct of 46 x 12" cross section. A 22" forward-curved centrifugal blower was installed on the outlet plenum, pulling the return air from the chamber and discharging the air upward through an adaptor plenum connected to the return duct on the top of the chamber. A by-pass was installed parallel to the upward adaptor plenum. It had an accessable space of 30W x 24D x 22H inches for accomodating different heating and dehumidifying devices.

2. Decision Making for Stage Shifting:

The temperatures of the surface and interior of a probe sample were monitored by dual-point temperature controllers to create on-off signals at preset temperatures. These on-off signals were fed into the input side of the programmable controller (PC) to instruct at which stage a

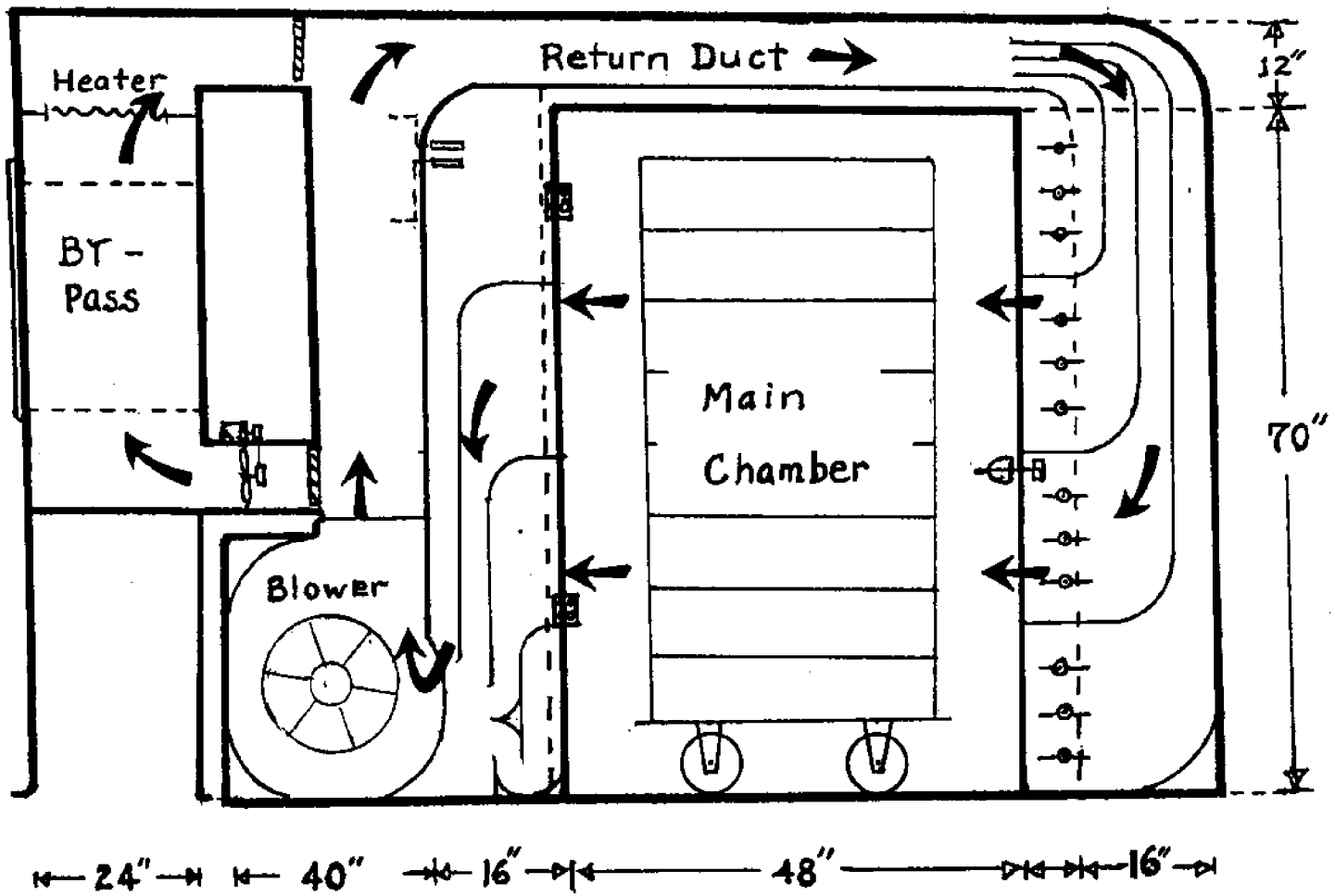


Figure 1. Physical arrangement and dimensions of the air dryer.

component should be on (Figure 2). The PC will be described in a later section. The weight changes of the probe sample were not used to control drying conditions because they represented the average dryness of the whole fish, not the condition of the fish surface. However, it may be useful to indicate the internal condition. The effectiveness of these two types of tools to control the stages will be compared in future study.

3. Heating, Dehumidification, and Air Circulation:

Heat is provided for maintaining the drying temperature, the diffusion rate inside the product, and evaporation of water from the fish surface. The evaporated moisture needs to be disposed of by certain means. If the discharge of moisture is through the exchange with outside air, there will be loss of heat energy. If the discharge is through condensation and recirculation of the air, the heat energy can be recycled. Although the extra dehumidification will consume energy, it is usually less than the heat lost to the outside. The difference is between the heat of evaporation of moisture taken away from the product versus the enthalpy needed to recover the heat loss of the drying air. The former catagory uses much less energy than the latter catagory. This was demonstrated by Strommen's work (3) indicating that 2/3 of energy costs were saved by using a heat pump for drying salt fish to 45% water content.

The electrical wiring of the heating system was designed to have three selectable regulation loops (Figure 2). Each loop used a separate thermoregulator preset at a different temperature. The regulated temperature was shifted automatically from one loop with high temperature to one with a lower temperature setting, depending on the status of the PC outputs, which were programmed to react according to the PC's input status. The heater consisted of a strip heater and the condenser coil of a heat pump. The heat pump was used because its evaporator coil was also needed for dehumidification. The use of the heat pump is illustrated in Figure 3. Physically, both coils of the heat pump were installed inside the by-pass chamber with the evaporator coil upstream. The compressor and one additional evaporator coil with a fan were installed outside. When only heating was needed the condenser and the outside evaporator were put in operation. The strip heater installed at the downstream of the condenser coil was also on to assist the heating. When the dehumidification was called for, the condenser and the inside evaporator were activated. When both heating and dehumidifying were needed both the dehumidifier and the strip heater were used. These sequences were carried out by switching the solenoid valves that controlled the flow of refrigerant to either the inside or outside evaportaor. A humidifier was also installed for controlling the relative humidity and supplying extra moisture needed in the later stage of drying.

For air circulation, a 22" forward-curved centrifugal blower was used, which had a capacity to deliver 9000 CFM at a low static pressure condition. This was selected because it would result in close to 360 ft /min linear velocity through the 4 x 6 ft^2 cross section area. This velocity has been suggested as the upper limit that can thermodynamically increase the drying rate (2,3). Further increase in air velocity can not significantly speed up the drying process. The actual measurement of the

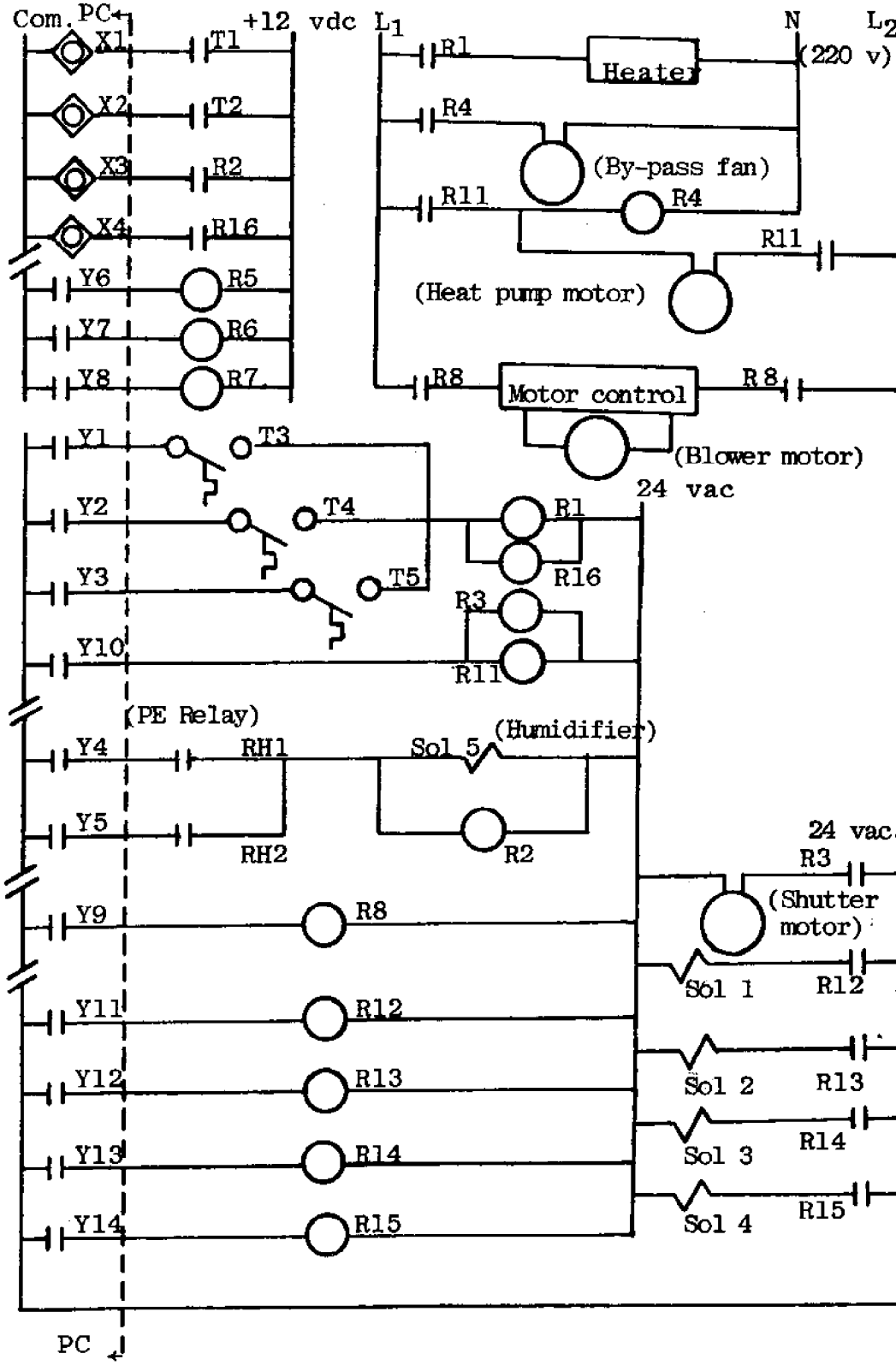
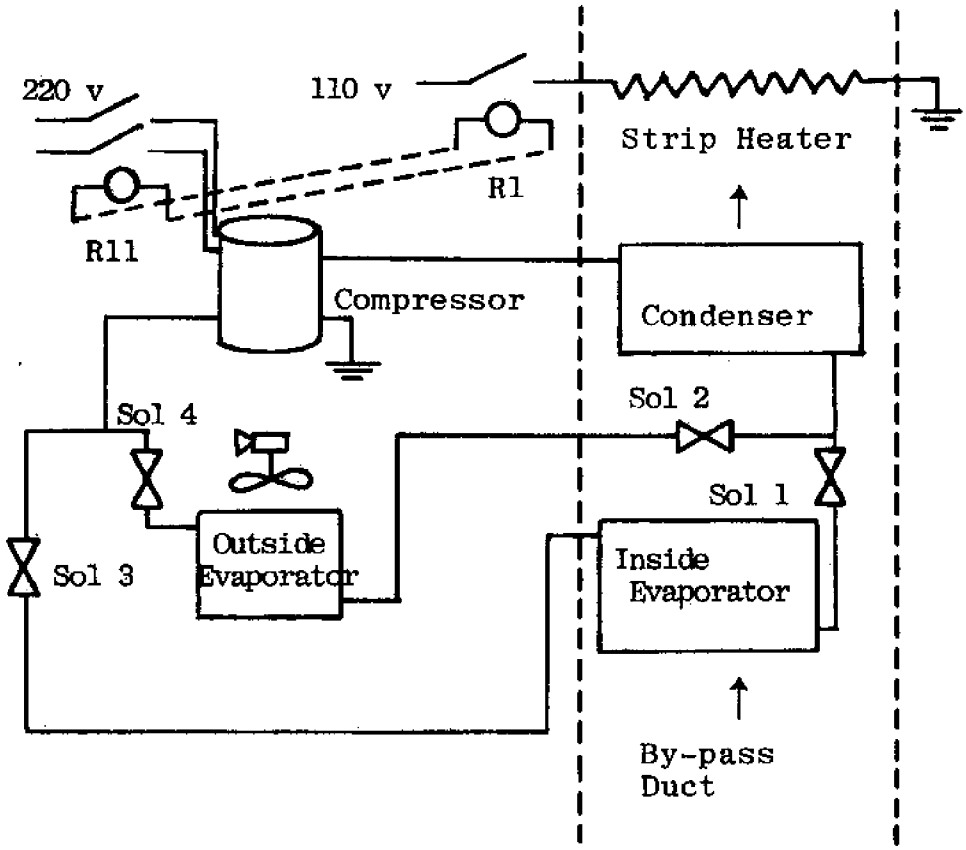


Figure 2. Electrical wiring for the dryer components.



DEMAND		COMPRESSOR	SOLENOID VALVE			
HEAT	DEHUMD		1	2	3	4
+	+	+	+	-	+	-
+	-	+	-	+	-	+
-	+	+	+	-	+	-
-	-	-	-	-	-	-

Figure 3. Illustration of arrangment and control sequence of the heat pump. Sequence is based on the use of normally closed valves. Inversed sequence is needed if normally open valves are used.

air velocity showed that the air distribution was relatively uniform throughout the chamber with an average of 300 ft /min when the blower turned at 300 RPM. The speed of the blower was controlled by a continuous variable speed controller. Three potentiometers with preset speed values were connected to the motor control circuitry through three relays which were controlled by outlet terminals of the PC.

4. Programmable Controller and Control Sequence:

The PC chosen for the study was an Omron Minature unit, CZ-1000W (Omron Electronics, Inc., Schambrug, Illinois) with a capacity of 16 input and 16 output terminals. It has 256 word RAM and 4 x 256 word EPROM capacity in relay ladder language. The application of the software in controlling drying stages is exemplified as follows.

The two surface temperature sensors (T_1,T_2), three thermoregulators (T_3,T_4,T_5), three blower speed potentiometers (A_1,A_2,A_3), and two humidity relays (RH_1,RH_2) are hooked up to the PC terminals, X_1,X_2,Y_1,Y_2, Y_3,Y_4,Y_5,Y_6,Y_7, and Y_8, respectively. The terminal designations and shifting sequence are summarized in Table 1.

One example of three stages of drying conditions is shown in Figure 4. The first stage is to warm up the dryer and the fish, as well as dry out the surface moisture. The chamber temperature is set by T_3, air velocity by A_1, and the dehumidifer is operated constantly. When the temperature of the fish surface increased to the first set point of the temperature sensor, T_1, the drying conditions are switched to the second stage with the T_4,A_2 and RH_1 control parameters. The set point of T_1 will be close to the temperature of wet bulb depression of the fish surface under the second stage. As the fish is dried during this stage, the surface temperature will keep climbing slightly due to the decrease in moisture throughout the fish. When this temperature reaches the set point of T_2, the drying conditions are switched to the third stage with the T_5, A_3,and RH_2 control parameters. T_1 and T_2 will be determined so that they can create staging sequences that bring about the optimal overall drying rate. At this point, the drying conditions become the mildest in the whole drying process. At any stage, one of the thermoregulators and one of the humidity controls are actually controlling the modulation of the heater, heat pump, and humidifier. The heat pump is also regulated by the status of the input X_3 to channel the evaporated refrigerant of the heat pump to either inside or outside evaporator. The status of X_3 is determined by the on-offs of the control relay R_2, which follows the on-offs of either one of the humidity relays, RH_1 or RH_2. Whenever dehumidification is called for, X_3 will be off, the inside evaporator valves on, and the outside evaporator valves off. The software program is as shown in Table 2. If a different heat pump configuration is required (3), the coil and control arrangement will be changed accordingly.

In summary, the use of a PC enables us to control complicated sequence of drying easily. The changing of experimental conditions can be done by simply changing the software program and control settings. The system can then be used to search for the optimal conditions to feasibly produce dry fish products.

Table 1. Connection and designation of I/O terminals to controls of different components in the dryer as well as status of controls at different drying stages.

Component Controls	PC I/O	Stage 1	Stage 2	Stage 3
Thermoregulator:				
Temp. SP-1 (T_1)	X_1(00)	-	+	+
Temp. SP-2 (T_2)	X_2(01)	-	-	+
Heater:				
Thermostat-1 (T_3)	Y_1(E0)	+(±)	-	-
Thermostat-2 (T_4)	Y_2(E1)	-	+(±)	-
Thermostat-3 (T_5)	Y_3(E2)	-	-	+(±)
Stripheater (R_1)	-----	±	±	±
Hygrometer:				
Humidity relay (RH_1)	Y_4(E3)	-	+(±)	-
Humidity relay (RH_2)	Y_5(E4)	-	-	+(±)
Humidifier solenoid (Sol 5)	-----	-	±	±
Heat Pump:				
Control relay-Heat (R_{16})	X_4(03)	±	±	±
Control relay-Dehum. (R_2)	X_3(02)	-	±	±
Compressor relay (R_{11})	Y_{10}(E9)	-	±	±
Valve solenoids (Sol 1-4)	Y_{11}-14 (EA-ED)	± (See Figure 3)	±	±
Air Blower:				
Blower Speed-1 (A_1) (R_5)	Y_6(E5)	+	-	-
Blower Speed-2 (A_2) (R_6)	Y_7(E6)	-	+	-
Blower Speed-3 (A_3) (R_7)	Y_8(E7)	-	-	+
Blower Relay (R_8)	Y_9(E8)	+	+	+
By-Pass:				
Fan relay (R_4)	-----	+	±	±
Shutter relay (R_3)	-----	+	±	±

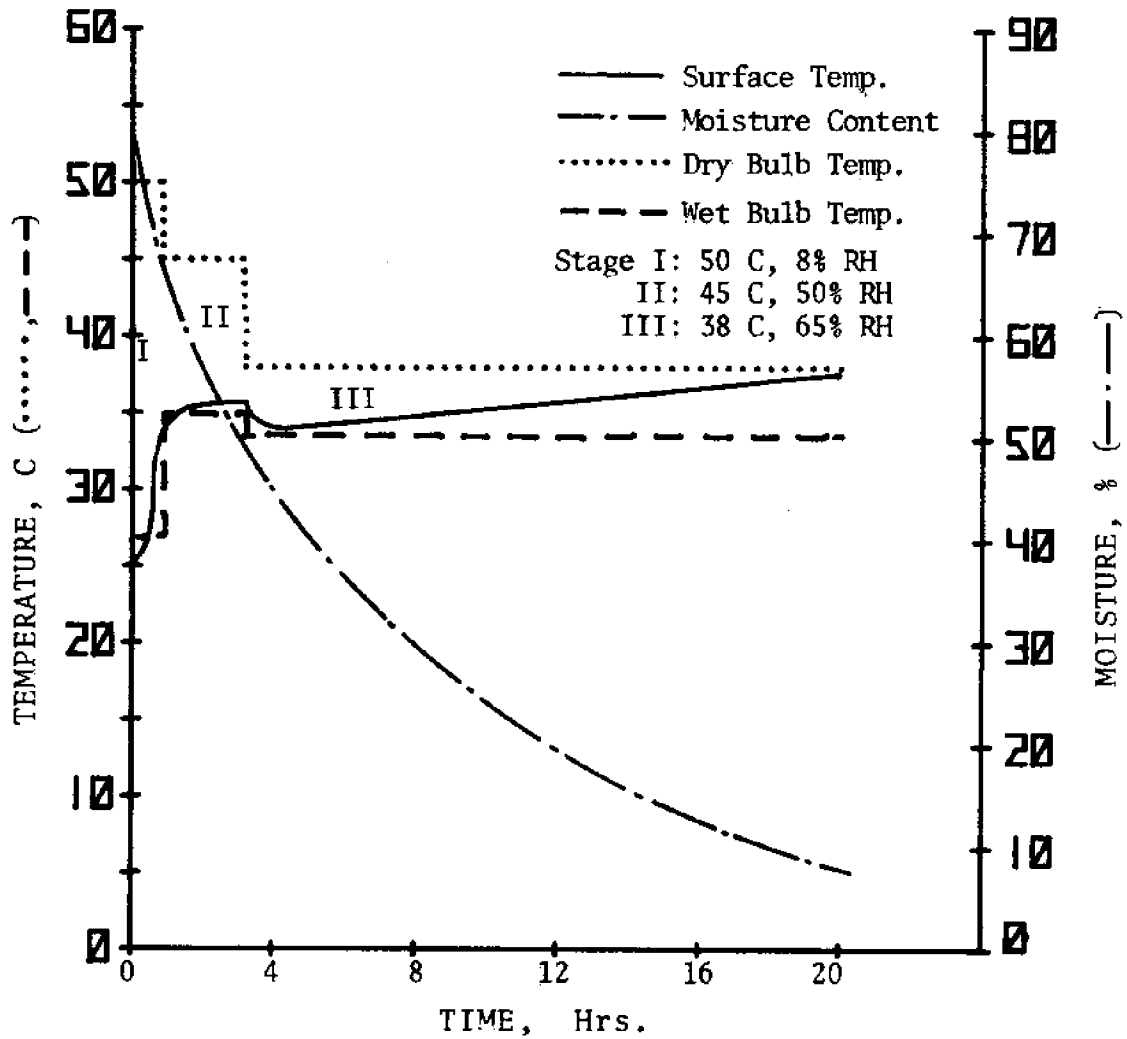


Figure 4. Temperature changes at the surface of mullet fillets going through staged drying.

Table 2: Portions of software for the programmer controller.

```
START:
00    LDNOT 00------(IF EITHER X1 OR
01    ORNOT 01       X2 IS ACTIVATED,
02    JUMPNOT 0B     THE PROGRAM JUMPS
STAGE I:             TO STEP 0B)
03    OUT E0--------(THERMOSTAT T3)
04    OUT E5--------(BLOWER SPEED 1)
05    OUT E8--------(BLOWER RELAY)
06    ---
--    ---
--    ---
0A    JUMP 00-------(RETURN TO STEP 00)
0B    LDNOT 01------(GOES TO STAGE III
0C    JUMPNOT 03      IF X2 IS ACTIVATED)
STAGE II:
0D    OUT E1--------(THERMOSTAT T4)
0E    OUT E3--------(HUMIDITY RELAY 1)
0F    OUT E6--------(BLOWER SPEED 2)
--    ---
--    ---
STAGE III:
13    OUT E2--------(THERMOSTAT T5)
14    OUT E4--------(HUMIDITY RELAY 2)
15    OUT E7--------(BLOWER SPEED 3)
--    ---
--    ---
```

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THE BACTERIOLOGY OF FRESH AND SPOILING TROPICAL FRESHWATER FISH

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INTRODUCTION

Investigation of the bacterial flora of fish and its influence on fish spoilage have been confined largely to marine species. Comparatively, little attention has been paid to freshwater fish, particularly in the case of tropical species.

The bacteriology of fresh and spoiling fish has been reviewed by several authors, however, data on tropical freshwater species are included only in some of them, (12,30,37), and a considerable amount of additional information has subsequently become available.

There are many references in the literature to the extended storage life in ice of certain tropical species compared to fish from coldwaters. It seems likely that marked differences in spoilage rates from different environments may in some measure be due to differences in the number, nature and behaviour of the bacterial flora (31). In the present work, an attempt has been made to describe the flora of newly caught tropical freshwater fish and the subsequent changes occurring therein during spoilage.

THE BACTERIAL FLORA OF NEWLY CAUGHT FISH

Quantitative aspects. The more important recent data are given in table 1. So far data are too few to generalize, the bacterial loads being somewhat within the range described for cold freshwater fish but lower than on marine fish compared with data given by Shewan (30).

Unlike the seasonal changes described for coldwater species (4), there is no seasonal variation reported for the bacterial load of newly caught tropical freshwater fish.

Confirming the general assumption of a small number of psychrophiles in the microflora of warmer waters, Watanabe (36), reported that only 5 to 20% of his isolates from Lakes Kariba and Tanganyika fishes could grow at 5°C.

Qualitative aspects. Table 2 shows that the bacterial flora of fish from tropical freshwater are composed of the same microorganisms reported for coldwater species, i.e., *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*/*Cytophaga*, *Vibrio*, *Micrococcus*, Coryneforms and *Bacillus*. It will also be noted, however, that there are marked differences in the percentage of groups. This is exemplified in figure 1 which shows the differences in the ratio of gram-negatives

TABLE 1. Bacterial loads of newly caught tropical freshwater fish

Species (Latin/common name)	Catching Area	Skin (per cm^2)	Gills (per g)	Guts (per g)	Source
CHANIDAE					
Chanos chanos (milkfish)	India	-	-	10^8	Mary et al (23)
CICHLIDAE (tilapias)					
Sarotherodon mossambicus	Zambia	10^4	-	-	Watanabe (35)
Sarotherodon nilotious	Uganda	10^3	-	-	Hoffman et al (11)
Sarotherodon spp.	Nigeria	10^2-10^3	-	-	Adebona (1)
CLUPEIDAE					
Hilsa ilisha (hilsa fish)	Bangladesh	10^4	10^6	10^4	Joarder (13)
CYPRINIDAE					
Carp (several species)	Bangladesh	10^5	10^5-10^6	10^5-10^6	Qudrat-i-Khuda (28)
SILUROIDS (catfishes)					
Brachyplatystoma vaillanti	Brazil	10^5	10^6	10^6	Lima dos Santos and Garbutt (21)
Callichrous bimaculatus Heteropneustes fossilis Mystus spp. Wallagu atu	Bangladesh	10^5	10^5	10^5	Qudrat-i-Khuda et al (28)
Clarias spp. Synodontis spp.	Zambia	10^4-10^5	-	-	Watanabe (36)
Crysichtys spp.	Nigeria	10^2-10^3	-	-	Adebona (1)

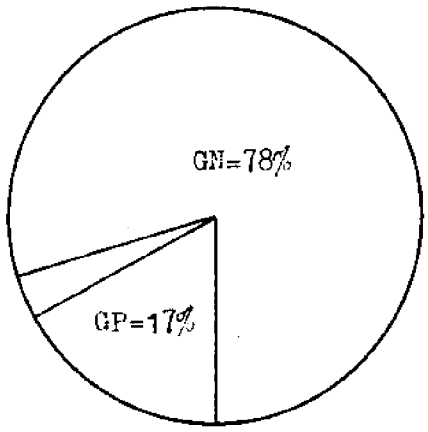
TABLE 2. Aerobic bacterial flora of newly caught tropical freshwater fish expressed as percentage of total numbers isolated

Species (Latin/common name)	Author	Sample place	Catching Area	Achromobacter*	Flavobacterium	Pseudomonas	Vibrio	Aeromonas	Enterobacteriaceae	Others	Total of Gram negatives	Micrococci	Streptococci	Corynetorms	Bacilli	Total of Gram positives	OTHER
CHANIDAE																	
Chanos chanos (milkfish)	Mary et al (23)	guts	India	50	–	17	–	–	–	–	67	33	–	–	–	33	–
CICHLIDAE																	
Sarotherodon mossambicus (Kariba bream)	Watanabe (35)	skin	Zambia								54	24	–	8	11	43	3
											46	36	–	9	9	54	–
CLUPEIDAE																	
Hilsa ilisha (hilsa fish)	Joarder (13)	skin	Bangla-desh	3	5	15	2	2	–	–	27	63	–	5	3	71	–
		gills		5	1	37	16	1	4	–	64	24	2	9	1	36	–
		guts		7	3	43	7	1	4	–	65	21	2	8	4	35	–
		flesh		6	3	33	4	–	2	–	48	37	–	8	3	48	4
Limnothrissa spp. (mudsucker)	Watanabe (36)	skin	Zambia								37	38	11	12	2	63	–
Stolothrissa spp. (kapenta)	Watanabe (36)	skin									60	23	2	13	2	40	–
CYPRINIDAE (carps)																	
Catla catla	Venkataraman and Sreenivasan (34)	skin	India	20	11	–	–	–	14	16	61	39	–	–	–	39	–
Cirrhina cirrhosa		flesh		25	16	21	–	–	16	–	78	16	–	–	4	20	2
Labeo fimbriatus		skin		7	43	7	–	–	7	–	64	14	–	7	14	35	1
SILUROIDS (catfish)																	
Brachyplatystoma vaillanti	Lima dos Santos (19)	guts	Brazil	12	–	8	39	–	–	–	59	33	–	2	–	35	6
Silonia silundia	Venkataranan and	skin	India	10	–	–	–	–	–	–	10	50	–	–	40	90	–

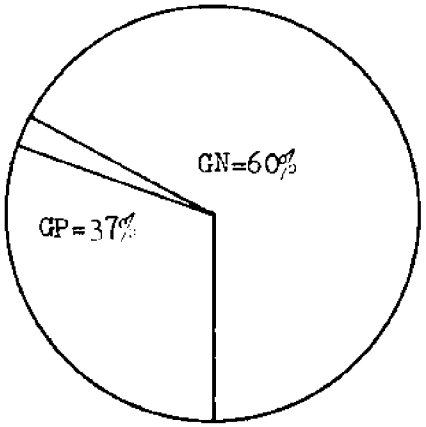
* Includes Moraxella and Acinetobacter

FIGURE 1. Distribution (%) of composition of gram-positives and gram-negatives in the microflora of newly caught fish from tropical and cold/temperate waters, (Lima dos Santos, 1978).

MARINE FISH

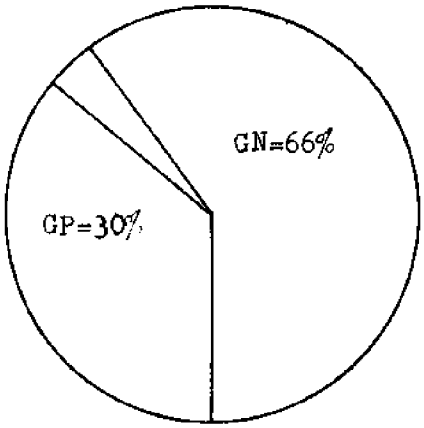


COLD/TEMPERATE WATER

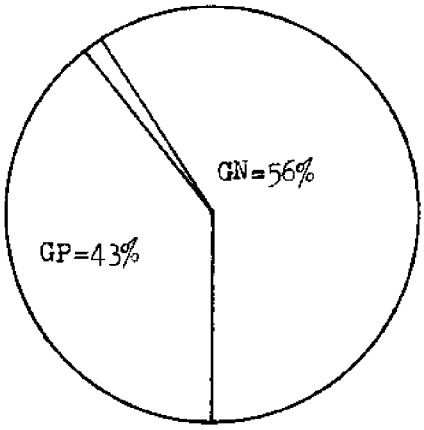


TROPICAL WATER

FRESH-WATER FISH



COLD/TEMPERATE WATER



TROPICAL WATER

and gram positives in the bacterial flora of fish from different environments. Figure 2 also shows a direct comparison between the average percentage of Pseudomonas reported to be present in the skin microflora of fish from different environments.

In the case of tropical freshwater fish, it can be seen that Pseudomonas contributes less than 10% of the skin flora, which might have some influence on their claimed extension of storage life in ice. In the intestines, as previously pointed out by Shewan (30), the Vibrio genus does not seem to be as predominant as in marine fish and the Flavobacterium-Cytophaga group appears to be practically absent.

CHANGES DURING ICE STORAGE

Quantitative aspects. Again, there is very little information on the quantitative changes occurring in the bacterial flora of tropical freshwater fish during iced storage. Investigations were mainly carried out with fish flesh samples and with plates incubated at room temperature (30-35°C). Few workers have used skin samples and incubation temperatures of 20-25°C. Some of the more recent data on these changes are shown in table 3 and 4. These results indicate that an initial lag phase is followed by an increase in population up to a maximum of 10^5 to 10^8 organisms per square centimetre of skin or gram of flesh. Accordingly to some authors (7,8), these levels contrast markedly with the higher figures of 10^8 to 10^9 per square centimetre of skin or gram of flesh published for cold/temperate marine fish. In order to explain these differences a number of workers have suggested that the surface slime (30) or the flesh (35) of tropical fish might contain anti-bacterial compounds which retard bacterial growth. The difference is not so evident, however, if a comparison is made with the bacterial changes reported for the flesh of cold freshwater species as shown in table 5. Another point to be considered refers to the lower counts, often one-tenth of the 20-25°C count, which may be obtained by incubation at 35-37°C, emphasizing the psychotropic nature of the fish spoilage flora (22). For instance, table 6 compares the differences reported for bacterial counts on the skin of the Amazonian catfish after incubation at different temperatures(19).

Qualitative changes. Qualitative bacteriological changes during iced storage of tropical freshwater fish have been studied by a very limited number of workers. Watanabe (35) working with iced Kariba bream (Sarotherodon mossambicus)reported that gram negatives increased from 50% at the first day in ice to 87% after 14-15 days of storage. Hoffman et al (11) confirmed Watanabe's results by reporting that after a few days of iced storage, the entire skin population of tilapia (Sarotherodon esculentus and S. niloticus)consisted of gram negative roads. The studies of these authors, however, did not include the determination of bacteria genus or species, but cultures were distinguished on the basis of gram reaction and general morphology. A more detailed picture is given in table 7 which shows the qualitative changes occurring in the skin flora of iced Amazonian catfish (Brachyplatystoma vaillanti) during storage (19).

FIGURE 2. Distribution (%) of Pseudomonas in the skin microflora of newly caught fish from tropical and cold/temperate waters, (Lima dos Santos, 1978)

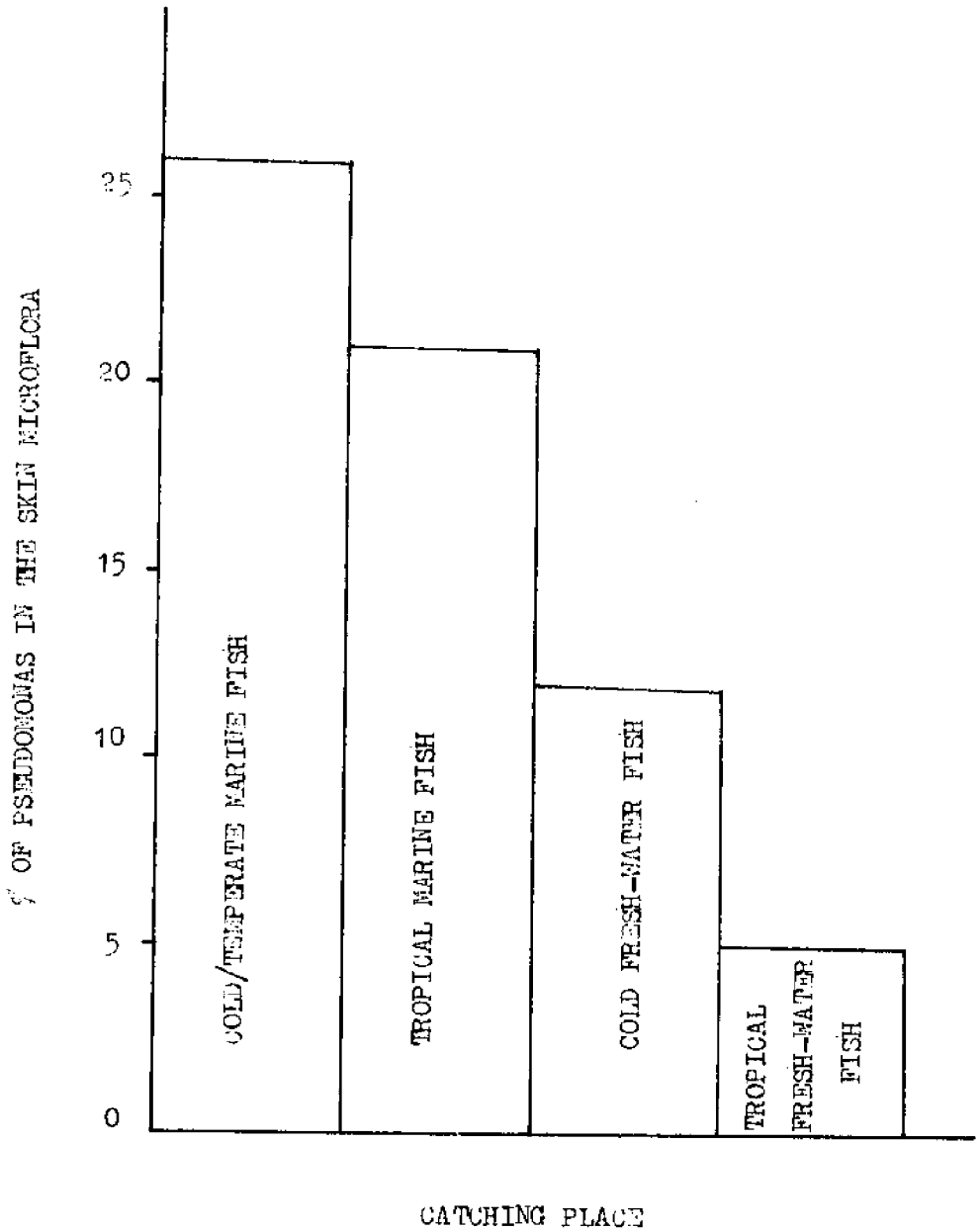


TABLE 3. Changes in the bacterial counts on tropical freshwater fish skin (per square centimetre) during storage in ice

Species	DAYS IN ICE						Author
	0–2	5–7	8–9	11–12	14–16	19–22	
TILAPIAS							
Sarotherodon spp.	10^2	10^3	–	–	10^5	10^6	Adebona (1)
S. esculentus	10^3	10^3	10^4	10^5	10^5	10^6	Hoffman *et al* (11)
S. niloticus	10^3	–	10^5–10^6	–	10^7	10^7	Hoffman *et al* (11)
S. mossambicus	10^4	10^5	10^6	10^7	10^7	–	Watanabe (35)
CATFISH							
Brachyplatystoma vaillanti		10^5	10^7	10^7	10^8	10^8	Lima dos Santos (20)
Chrysichtys spp.	10^2	10^3	–	–	10^4	10^5	Adebona (1)

TABLE 4. Bacterial populations in the flesh (per gram) of fresh and spoiled fish from tropical freshwaters

Species (Latin/common name)	Catching Area	Fresh (initial counts)	Spoiled	Source
Chanos chanos (milkfish)	India	10^2	10^5	Joseph *et al* (14)
	Philippines	10^2	10^7–10^8	Beza and Sison (2)
Sarotherodon esculentus (tilapia)	Uganda	10^3	10^6–10^7	Hoffman *et al* (11)
Sarotherodon niloticus (tilapia)	Uganda	10^3	10^6–10^7	Hoffman *et al* (11)
Labeo rohita (carp)	India	10^5	10^7	Bhattacharyya *et al* (3)
Cirrhina mrigala (carp)	India	–	10^5–10^6	Nair *et al* (25)
Barbus carnaticus (gende)	India	10^3	10^8	Moorjani *et al* (24)
Barbus dubious (kooralu)	India	–	10^5–10^6	Lahiry *et al* (18)
Brachyplatystoma vaillanti (piramutaba)	Brazil	–	10^6	Lima dos Santos (19)
Arapaima gigas (paiche)	Peru	–	10^7	Souness and Treurniet (32)

TABLE 5. Bacterial populations in the flesh (per gram) of fresh and spoiled fish from cold/temperate freshwater

Species	Catching Area	Fresh (initial counts)	Spoiled	Source
Salmo irideus (trout)	Spain	10^1	10^6	Tejada *et al* (33)
	Italy	-	10^6-10^7	Perna (27)
	Italy	-	10^6-10^7	Redaelli (29)
	Denmark	-	10^6-10^7	Hansen (10)
Coregonus clupeaformis	Canada	10^6	10^7-10^8	Gillespie and Ostovar (9)
	Canada	10^5	10^7	Ostovar (26)

TABLE 6. Bacterial counts on the skin of Amazonian catfish (Brachyplatystoma

BACTERIAL COUNT PER SQUARE CENTIMETRE OF SKIN		
Days in ice	At 35 - 37°C	At 20 - 25°C
6	2.4×10^4	5.7×10^5
19	6.0×10^5	3.3×10^8

TABLE 7. Microbial population change in the skin of Amazonian catfish (Brachyplatystoma vaillanti) stored in ice (Lima dos Santos, 1978)

Micro-organisms	Microbial population at 20-25°C (% of composition)				
	6 days	9 days	12 days	16 days	19 days
Pseudomonas	53.2	82.0	80.8	74.0	84.0
Group I	14.9	14.0	12.8	4.0	2.0
Group II	38.3	68.0	68.0	70.0	82.0
Moraxella-like	4.3	14.0	12.8	20.0	8.0
Flavobacterium-Cytophaga	31.9	0	0	0	2.0
Vibrio	2.1	0	0	0	0
"Coryneforms"	8.5	4.0	0	4.0	6.0
Micrococcus	0	0	6.4	2.0	0

From this table, the increase of the Pseudomonas Moraxella-like group at the expense of the rest of the flora can be seen. These findings are similar to those reported by Canadian workers (15,16, 17,26) for cold freshwater species as well as to Shewan's classical picture for North Sea fish (31). Therefore, they seem to contradict the view that freshwater fish have a different type of spoilage flora (5,6) and to confirm and extend the more general belief that Pseudomonas strains are probably the most important of the organisms concerned in the spoilage of fresh iced fish. However, Alteromonas putrefaciens and hydrogen sulphide producers do not appear to be described for tropical or cold/temperate freshwater fish.

CONCLUSION

The information available is far from sufficient. From the work done to date, it appears that some marked differences might exist between the bacterial flora of marine and freshwater fish, but further confirmation and careful consideration of the significance for the spoilage pattern of fish from the different environments is needed. There are no indications, except for the lower number of psychrophiles for warm water fish, of such marked differences in the case of tropical and cold freshwater species.

Freshwater fish constitute a very significant part of tropical fish landings and major developments in this fishery can be anticipated. The dearth of information available on these fish and their spoilage is the most striking problem confronting those concerned with tropical freshwater fish quality. The iced shelflife should be investigated for all commercially important species. Whenever possible, such investigations should be accompanied by quantitative and qualitative bacteriological studies.

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THE EFFECT OF PACKING ON THE MICROBIOLOGY OF SEAFOODS IN TRANSIT

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INTRODUCTION

When shipping foods for microbiological analysis, several factors are a consideration. First, what is the initial bacteriological status of the food in question? Next, how can the shipper by assured that this food will have representative microbiological counts when it arrives at its destination for testing? This study was concerned with determining the optimum packing conditions and shipping for seafoods destined for testing, be it for regulatory or quality control purposes. The specific objectives of this study were: 1) to measure the temperature changes during shipment of seafoods under different packing conditions, 2) to measure the changes in bacterial counts during shipment, and 3) to correlate this data and make recommendations as to the best packaging to insure representative counts on seafood samples after shipment.

MATERIALS AND METHODS

For this study, already processed and frozen seafood products were used: fish sticks and fish cakes, frozen breaded shrimp and frozen breaded fish fillets. Samples were obtained from various local supermarkets, placed in an ice chest and immediately transported to the Food Quality and Safety Laboratory, Texas A&M University. Upon arrival at the lab, one sample was removed from each of the products (eg., one frozen fish fillet or fish cake, 50 grams of breaded shrimp) for microbiological testing. The remaining products were resealed and placed in Styrofoam containers. Each of these containers was given a different cooling treatment: wet ice, dry ice, or a packaged chemical gel coldpack (Blue Ice). One Styrofoam container was simply closed and tightly sealed (no cooling mechanism added). All containers were closed and held at 25-27°C. (room temperature) in the lab. The internal temperature of each container was monitored continuously on a recording temperature monitor. Samples were quickly removed from each of the four containers at 12, 24, and 48 hours after initial microbiological testing. Tests conducted were aerobic plate count, APC, (using Standard Methods Agar), coagulase-positive staphylococci (using direct plating onto Baird-Parker Agar), coliforms, fecal coliforms and E. Coli (MPN multiple tube technique).

After many repetitions of this procedure, a mean temperature/time graph was constructed, the APC counts were analyzed for significance using Duncan's Multiple Range Test, and trends were noted among the coliform counts.

RESULTS AND DISCUSSION

The four methods of packing the samples were selected to represent ways a small processor or supermarket would send samples for testing when, due to

the small amount of sample, a freezer truck was not practical. The microbiological tests were selected as a general battery of tests often used in quality control and assessment.

The Temperature/Time Graph (Figure 1) represents the mean temperatures, during time, of each of the four packing treatments. The final temperature, regardless of treatment, was close to ambient (19-23°C.), with the exception of dry ice, which was slightly cooler (15°C.) The temperature of the container with no cooling treatment ("No Ice") rose the most steadily, reaching ambient within 10-15 hours. At 12 hours, most samples taken from this container were thawed and at 24 hours were quite warm. The containers with wet ice and Blue Ice maintained a more even temperature throughout the test period, with an average temperature between 0 and 5°C. for the first 10-12 hours. After 12 hours, however, the temperatures within these containers began to rise steadily, averaging a rise of 2°C. in 5 hours.

The temperature within the container with dry ice behaved in a different manner, dropping very rapidly to as low as -38°C. in the first 8-12 hours. After this initial drop, however, the temperature rose fairly quickly. At 28 hours the temperature had risen to 0°C., and rose steadily to near ambient. All samples taken from this container at 12 hours were thoroughly frozen and 8 of 10 samples were still frozen at 24 hours. Two of ten samples had begun to thaw, but were still cold.

After looking at the temperature curves, one would expect to see significant difference in the bacteriological counts for the different treatments. However, analysis of the data using Duncan's Multiple Range Test showed no significant difference, at the 5% level, in bacterial counts from 12 hour sampling. At 24 and 48 hours, there was significant difference between the counts from samples stored with no ice and those with some cooling treatment, but no significant difference between those treatments.

Figure 2 shows the mean APC counts for fish cakes. At 12 hours, for most samples, there was actually a drop in APC, probably due to shock to and/or loss of injured bacteria. At 24 and 48 hours, the counts for the "no ice" samples were significantly higher, but the other three treatments produced counts fairly close together. Samples stored on dry ice were most representative after 48 hours; after dropping somewhat at 24 hours, the counts rose to only slightly higher than the initial counts.

Mean APC counts for fish fillets (Figure 3) are almost identical to those of fish cakes. Dry ice still maintained very representative counts at 48 hours. The counts for wet ice are representative, but somewhat suspect, as in some cases the package was not watertight, and the washing action of the melted ice could have resulted in lower counts.

The mean counts of frozen breaded shrimp (Figure 4) again showed significantly higher counts by the non-iced samples as compared to cooled samples. Blue ice and dry ice both maintained fairly representative counts at 24 and 48 hours.

Aerobic plate counts were used for the statistical analysis as these are simplest to quantify. In testing for coagulase-positive staphylococci, using direct plating onto Baird-Parker Agar, there were so few initial counts that it was difficult to ascertain the effect of the different treatments. The few positive samples found initially seemed to indicate, upon testing, that

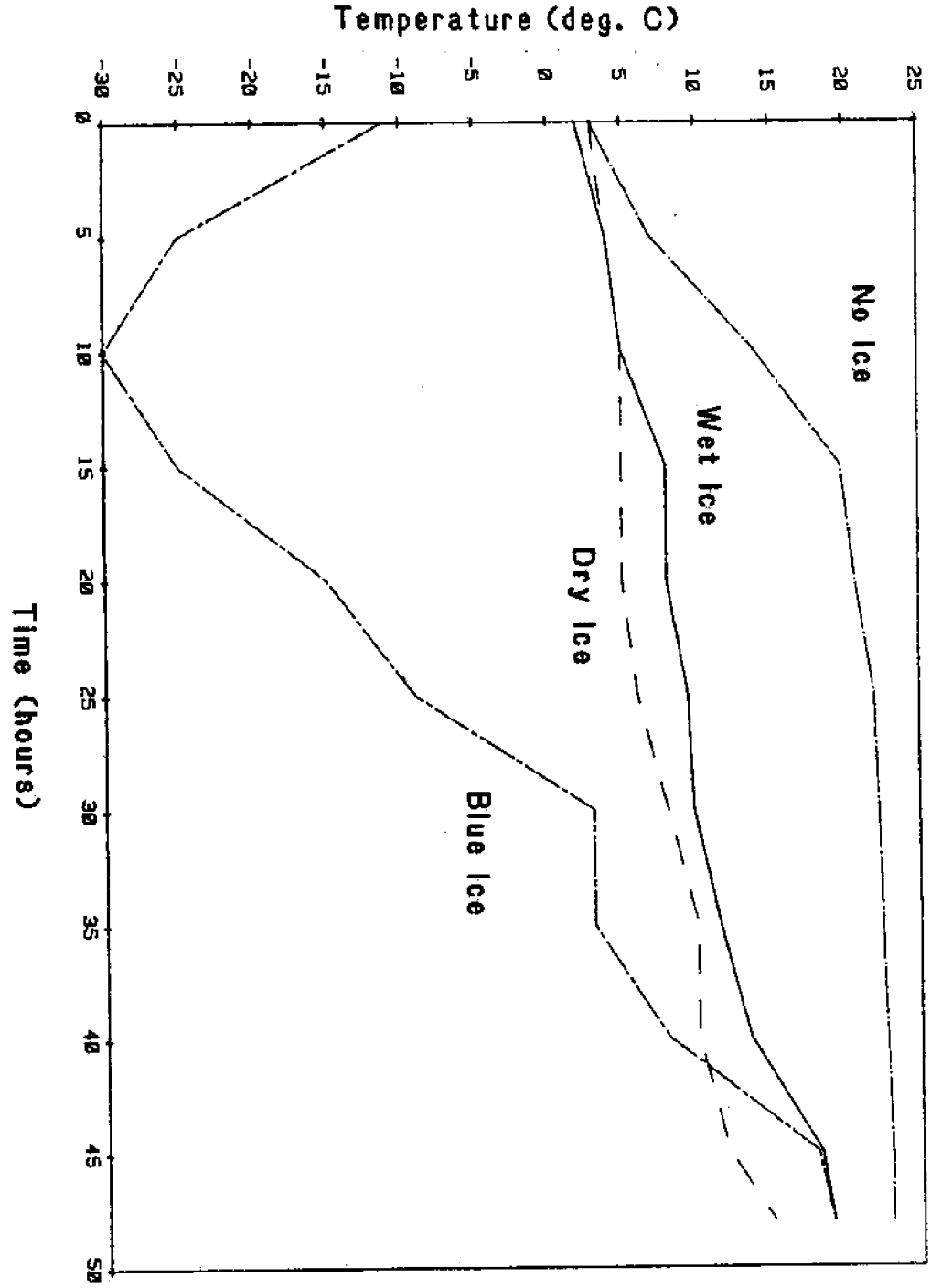

Figure 1. Temperature/Time Data

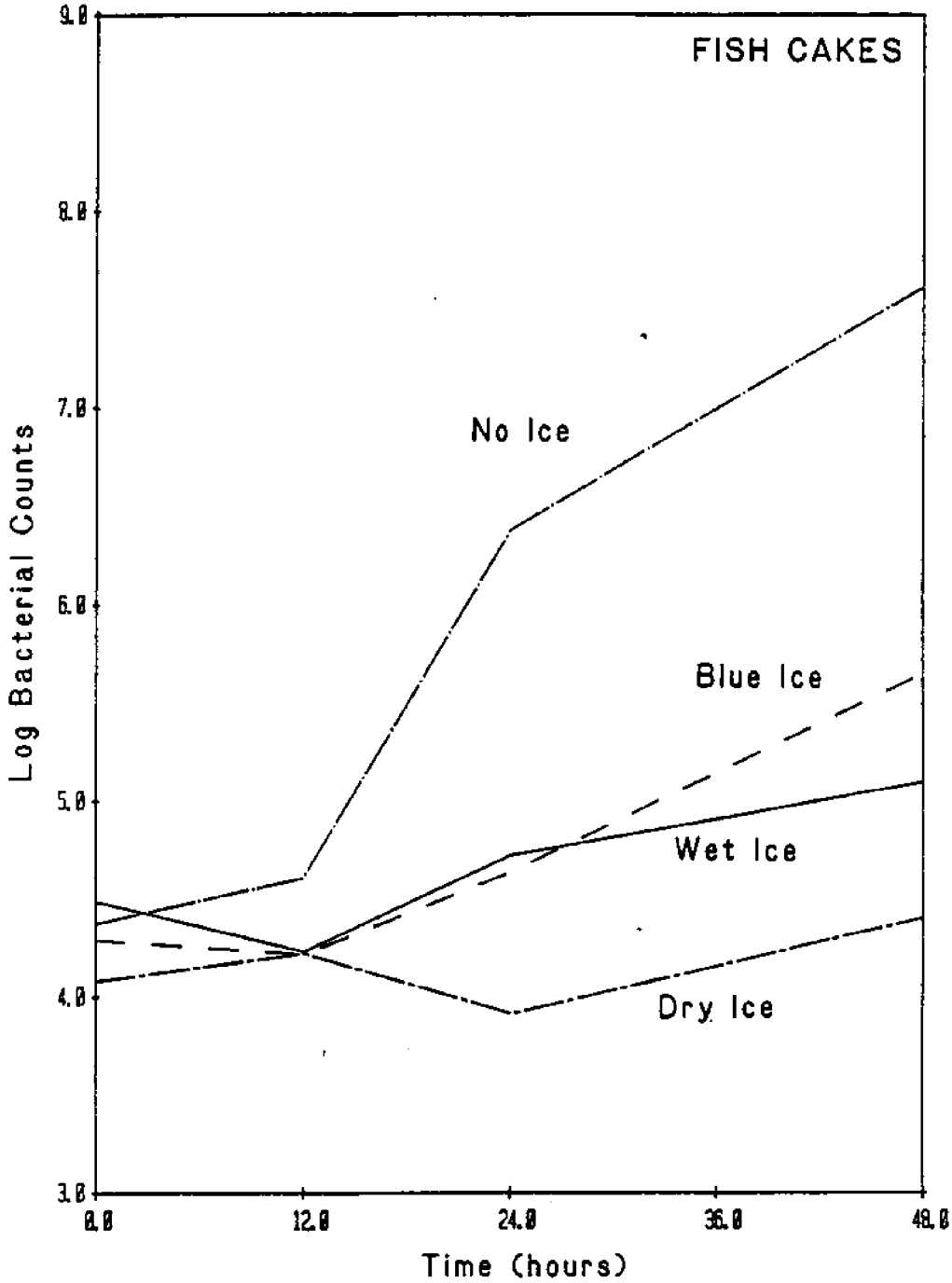

Figure 2. Mean log bacterial counts for fish cakes.

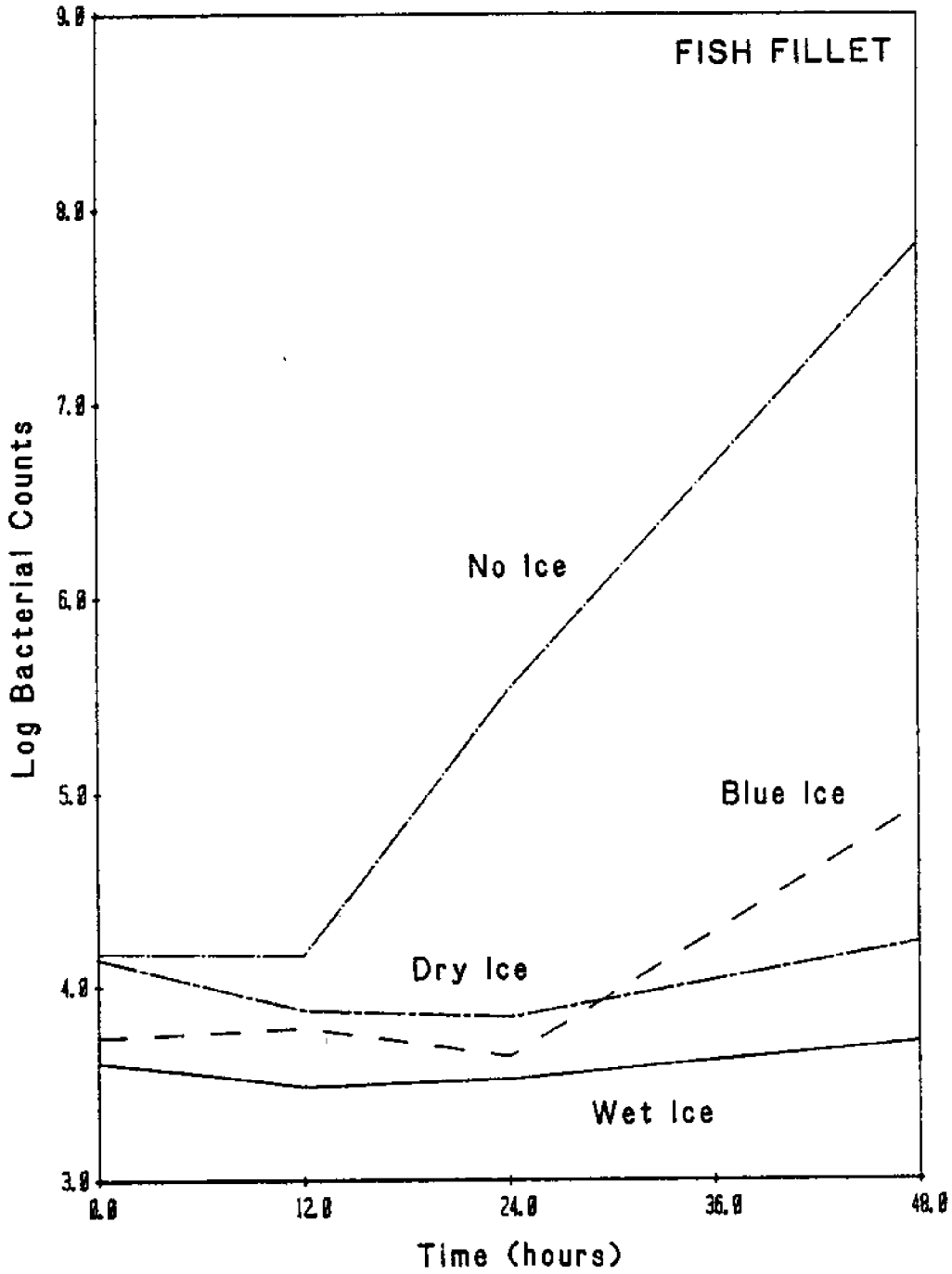

Figure 3. Mean log bacterial counts for frozen breaded fish fillets.

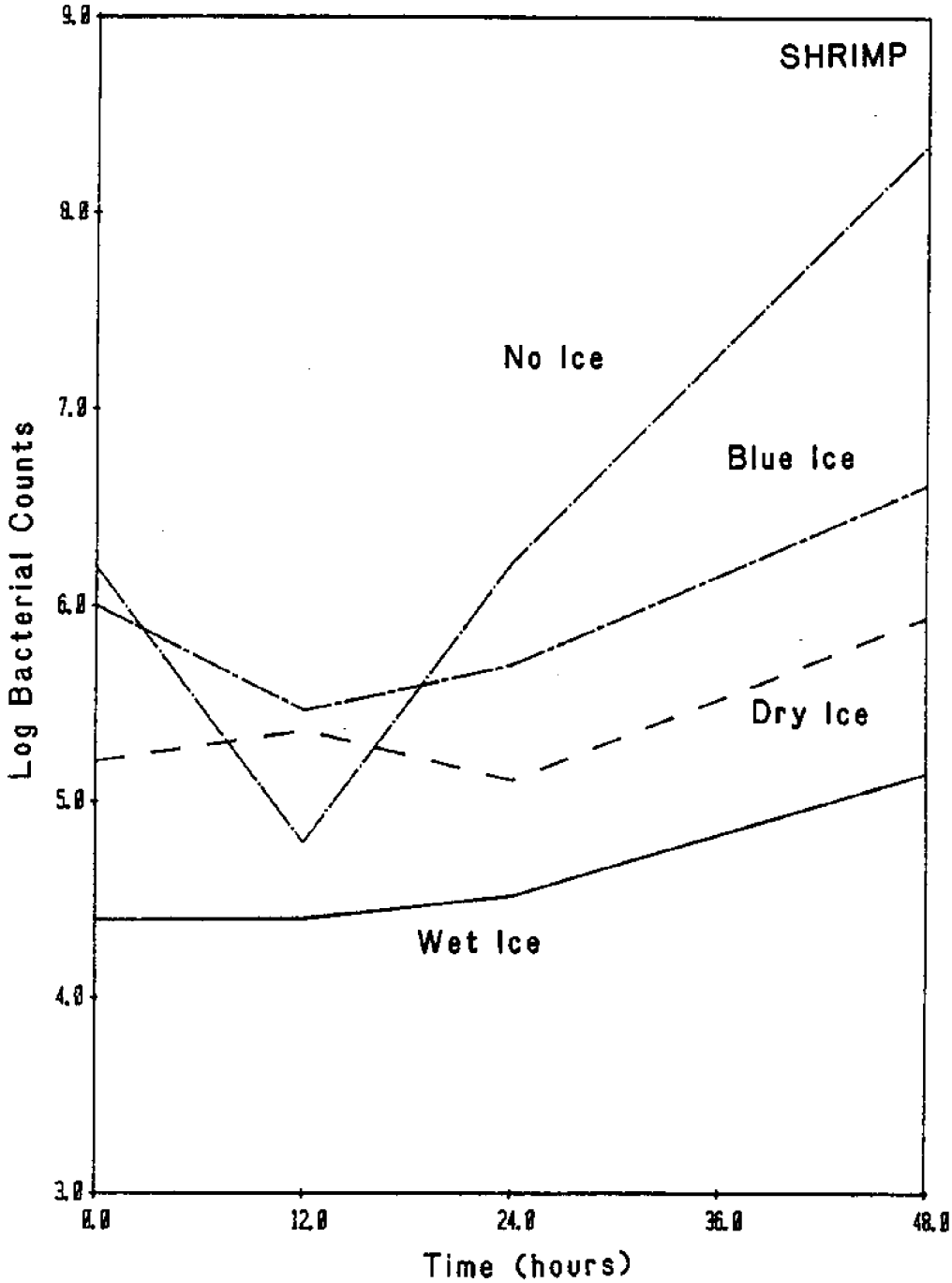

Figure 4. Mean log bacterial counts for frozen breaded shrimp.

cooling treatment has little effect on the growth of coagulase-positive staphylococci.

Looking at coliform counts showed some general trends (Table 1). Coliform counts for samples held under blue ice generally increased steadily. For example, one typical breaded shrimp sample had initial counts of 23/g. This increased to 43/g at 12 hours, 93/g at 24 hours, and >2400/g at 48 hours. Fish fillets showed much the same trend, as did fish cakes, although several samples had initial coliform counts of <3/g and remained at that level throughout the testing period.

Dry ice had a varying effect on coliform counts. Some samples increased, although at a much lesser rate than samples under other treatments. Some counts, primarily those of fish cakes, had initial counts of and remained at 3/g consistently. For the most part, however, counts on many samples tended to decrease over time. About 50% of our samples showed this trend.

Wet ice presented a problem in determining general trends, as the data tended to be variable, with little apparent consistency. Some increased during the first 24 hours and then decreased at the 48 hour sampling. Others showed a kind of rollercoaster effect.

The counts for samples stored with no ice treatment had a definite trend toward increasing with time.

CONCLUSION

After comparing the temperature curves with the statistical analyses and coliform trends, it is difficult to recommend an optimum pack for shipping seafoods. When looking solely at the temperature/time curves, dry ice would seem to the best cooling agent. More research,however, should be done on the count reduction apparently caused by the super-chilling effect of the dry ice. Blue ice, if properly frozen, appears to be a neat and fairly effective cooling agent. Different brands of blue ice, however, seem to have different thaw rates, which may account for some variability in the data. Wet ice is a less than optimum cooling agent, due to its melting and subsequent washing action. Shipping a frozen product with no cooling at all is definitely not recommended.

Further research on this project will involve actual shipment of samples and later inoculation of samples to evaluate recovery of specific organisms after storage and shipment abuse.

TABLE 1.

COLIFORM COUNTS FOR SELECTED SAMPLES

TREATMENT	SAMPLE	COLIFORM COUNT(CONFIRMED)/g 0	12	24	48
Blue Ice	Breaded Fish Fillets	23	9.1	23	23
	Breaded Fish Fillets	9.1	3.6	9.1	240
	Fish Cakes	<3	<3	<3	<3
	Fish Cakes	3.6	3.6	7.3	240
	Breaded Shrimp	3.6	15	9.1	>2400
	Breaded Shrimp	23	43	93	>2400
Dry Ice	Breaded Fish Fillets	<3	9.1	23	23
	Breaded Fish Fillets	43	9.1	<3	<3
	Fish Cakes	<3	<3	<3	<3
	Fish Cakes	23	7	11	9
	Breaded Shrimp	240	43	23	23
	Breaded Shrimp	93	240	43	23
Wet Ice	Breaded Fish Fillets	<3	<3	23	3.6
	Breaded Fish Fillets	23	23	43	23
	Fish Cakes	<3	<3	23	9.1
	Fish Cakes	23	23	3.6	7.3
	Breaded Shrimp	150	75	240	93
	Breaded Shrimp	<3	9.1	<3	93
No Ice	Breaded Fish Fillets	3.6	23	43	>2400
	Breaded Fish Fillets	23	9.1	23	23
	Fish Cakes	<3	9.1	93	>2400
	Fish Cakes	9.1	3.6	9.1	>2400
	Breaded Shrimp	150	240	>2400	>2400
	Breaded Shrimp	36	<3	23	43

EFFECTS OF SURFACE ASSOCIATION AND OSMOLARITY ON *VIBRIO CHOLERAE*

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INTRODUCTION

Vibrio cholerae has been isolated from a number of diverse aquatic systems, including the brackish waters of the Mississippi River delta and estuaries along the coast of the Gulf of Mexico and the Atlantic Coast (2-4,7). In order to account for its ubiquitous distribution in brackish and estuarine waters, Colwell *et al.*(3), hypothesized *V. cholerae* to be a member of the autochthonous estuarine bacterial community. This viewpoint has been expressed by other investigators working in the field (7,8,10).

Recent studies on the effects of varying environmental parameters on growth and survival of *V. cholerae* have shown that *V. cholerae* is able to grow under simulated estuarine conditions (10). Furthermore, at moderate temperatures, growth appears to be influenced primarily by nutrient concentration and salinity, as the optimum salinity for growth of *V. cholerae* was found to be between 15 and 25 $^{o}/_{oo}$ (10).

V. cholerae has been demonstrated to survive for extended periods in microcosms incubated at 10°C (11). In these studies, beginning with a low inoculum concentration, no culturable *V. cholerae* cells were recovered from microcosms incubated at 10°C; although after the microcosms were subsequently incubated for four days at 25°C, a large culturable population of *V. cholerae* developed. This indicates that either viable, but non-recoverable *V. cholerae* cells were present, as suggested by Singleton *et al.* (11), or the cells were removed from the liquid phase of the microcosm by way of association with container walls.

Since enhanced survival may be a function of association with surfaces due to the establishment of a more favorable microenvironment (12), association with glass container walls may reflect an affinity for association with surfaces in the environment. Particulate materials which might provide suitable surfaces in the estuary include detrital particles, chitin, sand, and other inert materials as well as organisms of higher trophic levels. The demonstration of whether a species can, in some manner, become associated with particulate material in microcosms, although not sufficient, is necessary in order to suggest that association with the surface of particulate materials enhances survival of a microorganism *in situ*. The objective of this study was, therefore, to

investigate the association of V. cholerae with particulate materials in an aquatic environment.

MATERIALS AND METHODS

Laboratory microecosystems, i.e., microcosms, were prepared by adding 50 ml of seawater of 24 $^{o}/_{oo}$ salinity, to acid-cleaned 125 ml Erlenmeyer flasks. The flasks were sealed with aluminum foil and autoclaved for 15 min at 121°C. In some experiments the seawater was filtered through a 0.2μm filter (Nuclepore Corp., Pleasantown, CA) prior to adding to the flasks. After cooling to room temperature (ca. 22°C), the microcosms were inoculated to a desired cell concentration with V. cholerae and incubated for selected times at 22°C.

V. cholerae, WF 110, an environmental isolate (LSU serovar BB), in two physiological states was employed. Non-stressed V. cholerae cells were harvested by centrifugation from an 18 hr Brain Heart Infusion broth culture (Difco Laboratories, Detroit, MI) and washed three times in artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH) to remove nutrients prior to inoculation. Stressed, or envirotolerant, V. cholerae cells were also employed. The envirotolerant cells, characterized by decreased cell size and reduced metabolic activity (unpublished data), were maintained for 35 days in a microcosm containing unsupplemented marine salts solution, salinity 24 $^{o}/_{oo}$, prior to inoculation.

Numbers of culturable V. cholerae in samples collected from the microcosms were determined by the spread plating procedure. In initial studies, the influence of osmotic pressure of diluent and plating media were evaluated. Samples for bacterial enumeration were aseptically collected and processed in three enumeration protocols. In one protocol, representing a "standard", the osmolarities of the physiological saline (0.85% NaCl) (220 mOsm/liter) diluent and the culture medium, Brain Heart Infusion Agar (Difco) (330 mOsm/liter), were not adjusted to that of the microcosm (525 mOsm/liter). The other two enumeration protocols included marine salts solution (525 mOsm/liter), as an electrolyte source, and a sucrose solution (525 mOsm/liter), as a non-electrolyte solution, for dilution blanks. Also, the culture medium was adjusted to 525 mOsm/liter with marine salts and sucrose.

To evaluate the influence of osmotic pressure on V. cholerae, microcosms were inoculated with non-stressed or envirotolerant cells and samples were collected aseptically after desired incubation periods. Samples were processed by each of the three enumeration protocols and culture plates incubated for 24 hr at 22°C before counting colonies. The selected enumeration protocol for subsequent experiments employed osmotically balanced diluents and culture media. In addition, seawater complete agar (SWC) was employed as the culture medium since this medium contains a lower concentration of nutrients and any potential alterations in bacterial counts resulting from nutrient stress would be avoided.

The influence of particulate materials on development of V. cholerae populations was first assayed by comparing the maximum population size attained in the microcosms. Autoclaved seawater

microcosms, with particle-free water, i.e., 0.2μm filtered, or water with the natural particulate materials were inoculated to an initial cell concentration of approximately 5 X 10^4 V. cholerae cells per ml and incubated at 21°C. Samples for bacterial enumeration were collected after selected incubation periods and the number of culturable cells in the presence or absence of particles compared.

In order to more accurately monitor any influence and association of particulates on V. cholerae, microcosms were established with approximately 1 X 10^5 V. cholerae per ml and incubated for 24 hr at 22°C. Samples for enumeration were collected and 10 g sterile calcium carbonate ($CaCO_3$) particles with an average diameter of 5 mm were added to the microcosms. Following an additional 10 min incubation period, the number of culturable planktonic V. cholerae was determined. In some experiments chitin particles were added to microcosms to evaluate the effect on V. cholerae.

RESULTS AND DISCUSSION

Initial studies on the influence of osmotic pressure on culturable counts of V. cholerae demonstrated that (i) physiological condition of the cells and (ii) the enumeration protocol can interact to significantly influence the results (Tables 1 and 2). When non-stressed V. cholerae cells were employed as the inoculum, no significant effect ($p<0.05$) on culturable counts was detected when culturable counts obtained from the osmotically balanced or unbalanced protocols were compared (Table 1). There was a marked effect of osmotic pressure of the diluents and culture media on culturable counts of V. cholerae when physiologically stressed, i.e., envirotolerant, cells were employed (Table 2). Although there was no immediate difference in culturable counts obtained by the three enumeration protocols, samples collected 1, 2 and 3 hr after inoculation and processed by the imbalanced protocol yielded significantly smaller ($p<0.05$) culturable counts than the osmotically balanced protocols. This effect was not detected after the 3 hr sample, which probably corresponds to the onset of the growth phase. It is evident, therefore, that V. cholerae cells which are physiologically stressed are more sensitive to osmotic pressure change than non-stressed cells.

The influence of particulate materials on the maximum population size attained by V. cholerae in the microcosms was first assayed in order to determine if particle association could lead to increased population sizes and, hence, enhance survival in the aquatic environment. Microcosms containing estuarine water from which the particulate materials had been removed by filtration yielded culturable populations approximately two-fold larger than microcosms containing water with particulates (fig. 1). This difference was found to decrease with increasing incubation time. Thus, it was apparent that either the growth rate of V. cholerae was decreased by the presence of particulates or the decreased culturable count was due to cells becoming associated with the particulates, hereafter termed "attached".

Table 1. Plating medium and diluent osmolarity effects on culturable counts of *Vibrio cholerae*[a]

HOURS AFTER INOCULATION	BALANCED OSMOTICALLY[b] (marine salts)	BALANCED OSMOTICALLY[c] (sucrose)	IMBALANCED OSMOTICALLY[d]
0	1.96×10^4	2.10×10^4 (Δ = 7%)	1.92×10^4 (Δ = -2%)
1	1.91×10^4	1.87×10^4 (Δ = -2%)	1.84×10^4 (Δ = -4%)
2	2.09×10^4	2.03×10^4 (Δ = -3%)	2.06×10^4 (Δ = -1%)
3	3.10×10^4	2.97×10^4 (Δ = -4%)	3.03×10^4 (Δ = -2%)
6	1.21×10^5	1.30×10^5 (Δ = 7%)	1.25×10^5 (Δ = 3%)
12	1.76×10^5	1.72×10^5 (Δ = -2%)	1.81×10^5 (Δ = 3%)
24	1.64×10^6	1.68×10^6 (Δ = 2%)	1.56×10^6 (Δ = -5%)

[a] WF 110 18 hr growth in BHI broth prior to inoculation
[b] 525 mOsm/liter
[c] 525 mOsm/liter
[d] diluent: 220 mOsm/liter
medium: 330 mOsm/liter

Table 2. Plating medium and diluent osmolarity effects on culturable counts of *Vibrio cholerae*[a]

HOURS AFTER INOCULATION	BALANCED OSMOTICALLY[b] (marine salts)	BALANCED OSMOTICALLY[c] (sucrose)	IMBALANCED OSMOTICALLY[d]
0	1.38×10^4	1.34×10^4 (Δ = -3%)	1.42×10^4 (Δ = 2%)
1	1.79×10^4	1.90×10^4 (Δ = 6%)	1.40×10^4 (Δ = -28%)
2	2.44×10^4	2.25×10^4 (Δ = -8%)	1.31×10^4 (Δ = -46%)
3	2.62×10^4	2.42×10^4 (Δ = -8%)	1.51×10^4 (Δ = -42%)
6	5.30×10^4	5.60×10^4 (Δ = 5%)	5.45×10^4 (Δ = 3%)
12	2.71×10^5	2.73×10^5 (Δ = 1%)	2.83×10^5 (Δ = 4%)
24	2.36×10^6	2.58×10^6 (Δ = 9%)	2.45×10^6 (Δ = 4%)

[a] WF 110 maintained in 20 ‰ marine salts for 35 days
[b] 525 mOsm/liter
[c] 525 mOsm/liter
[d] diluent: 220 mOsm/liter
medium: 330 mOsm/liter

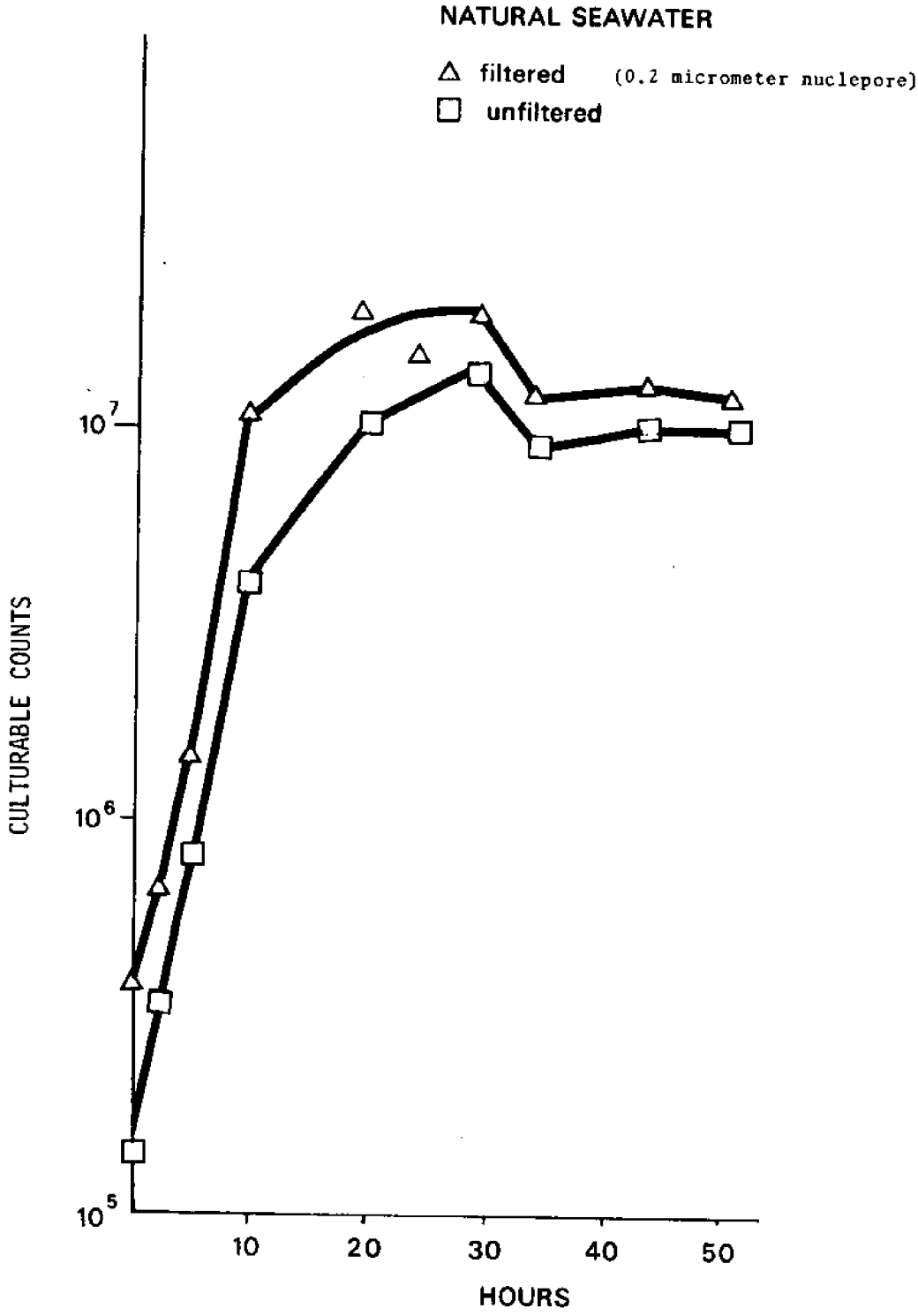

Figure 1. Development of V. cholerae populations in microcosms containing 0.2μm filtered and unfiltered seawater.

To more accurately evaluate removal of V. cholerae from the liquid phase of the microcosm by attachment to suspended particles, microcosms which were established with approximately 1 X 10^5 envirotolerant cells per ml and incubated for 24 hr at 22°C were sampled before and after addition of $CaCO_3$ particles (fig. 2). The addition of the $CaCO_3$ particles to the microcosms resulted in approximately 50% decrease in the number of planktonic bacteria.

A 50% decrease in the planktonic population was consistently detected. Therefore, the remaining planktonic cells (unattached) were employed as inocula for additional microcosms to determine if a newly developed population arising from these (non-attached) cells would consist of organisms with and without the ability to attach to $CaCO_3$. In these microcosms addition of $CaCO_3$ also resulted in approximately a 50% decrease in culturable counts (fig. 2).

The planktonic population which developed from non-attached cells contained organisms with the ability to attach. The attached cells were then employed as inocula for other microcosms to determine if the planktonic population, if any, developing from the attached population would also be decreased by the addition of particulates. $CaCO_3$ particles, with the attached V. cholerae, were collected aseptically from the microcosms after appropriate incubation, vigorously washed three times in 0.2μm filtered marine salts solution with salinity adjusted to 24 $^0/_{00}$. The individual particles were added to a sterile microcosm which was followed by a 24 hr incubation period at 22°C. The addition of $CaCO_3$ did not result in a detectable decrease in the number of culturable planktonic V. cholerae (fig. 2). Therefore, it was evident that two subpopulations of V. cholerae existed in the microcosms, one with and one without the ability to attach to $CaCO_3$. Furthermore, cells with the ability to attach, which were produced from those cells already attached, remained associated with the $CaCO_3$ particles, while those without the attachment capability entered the planktonic population.

Although it was evident that proportions of a V. cholerae population possessed attachment capability after incubation for specific time intervals, a series of microcosms was established and $CaCO_3$ added to a single microcosm after each interval to determine if the proportion of the planktonic population attaching changed with increasing incubation time. The proportion of the population attaching to added $CaCO_3$ was found to decrease logarithmically with time (fig.3). Using envirotolerant V. cholerae, the proportion of the population which was found to attach was observed within 20 min after inoculation, at which time 54% of the culturable population attached to the $CaCO_3$ particles. After 72 hr incubation, only approximately 4% of the culturable planktonic population attached to the $CaCO_3$ particles.

It was apparent that V. cholerae would attach to $CaCO_3$. Therefore, the degree to which V. cholerae would attach to chitin particles was also investigated. Samples collected from microcosms which were inoculated with envirotolerant (stressed) V. cholerae to a concentration of approximately 1 X 10^6 cells/ml and incubated for 24 hr at 22°C before addition of 1.0 g grade B purified chitin

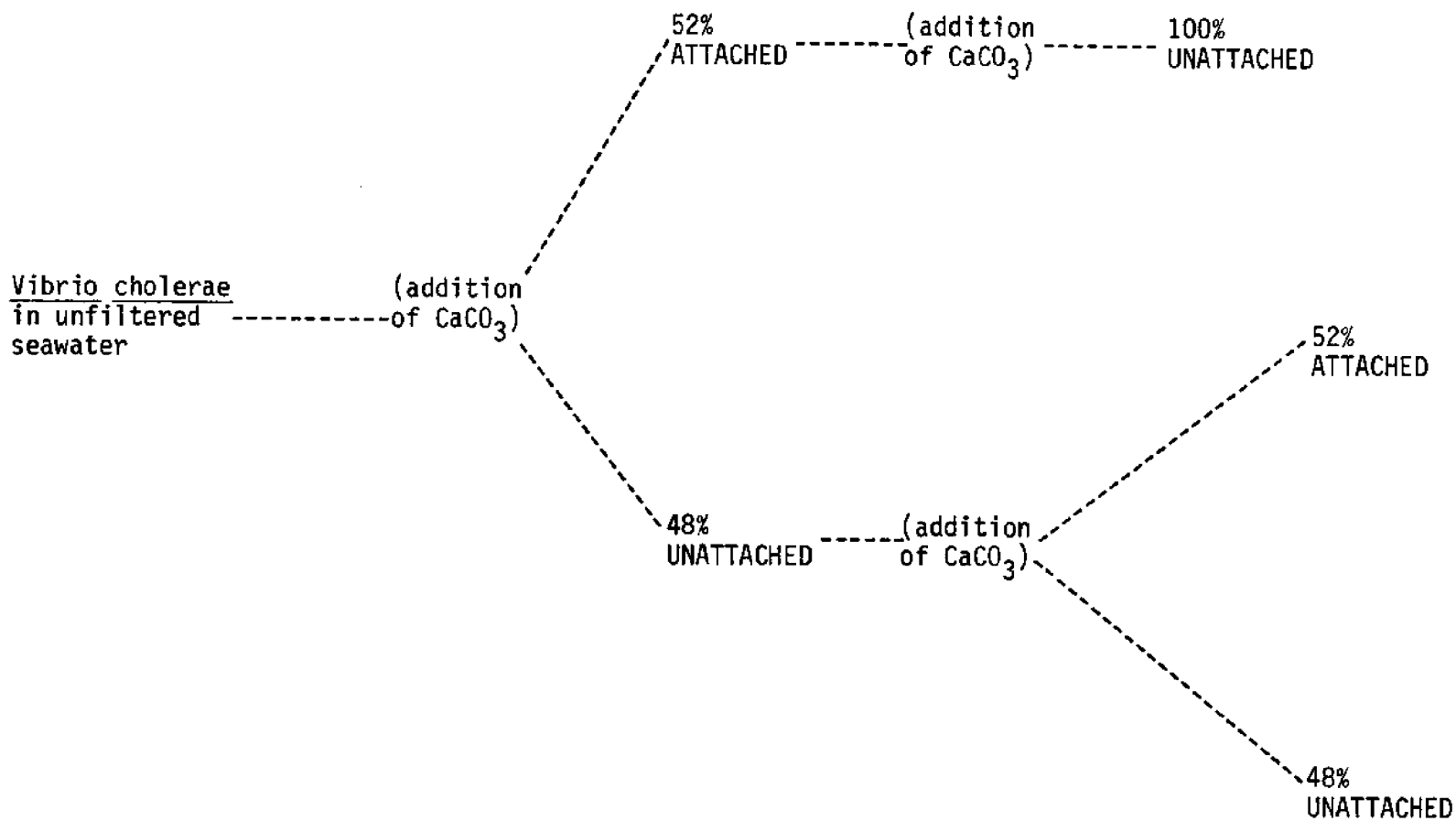

Figure 2. Influence of inoculum source on attachment capability of *V. cholerae* in seawater microcosms.

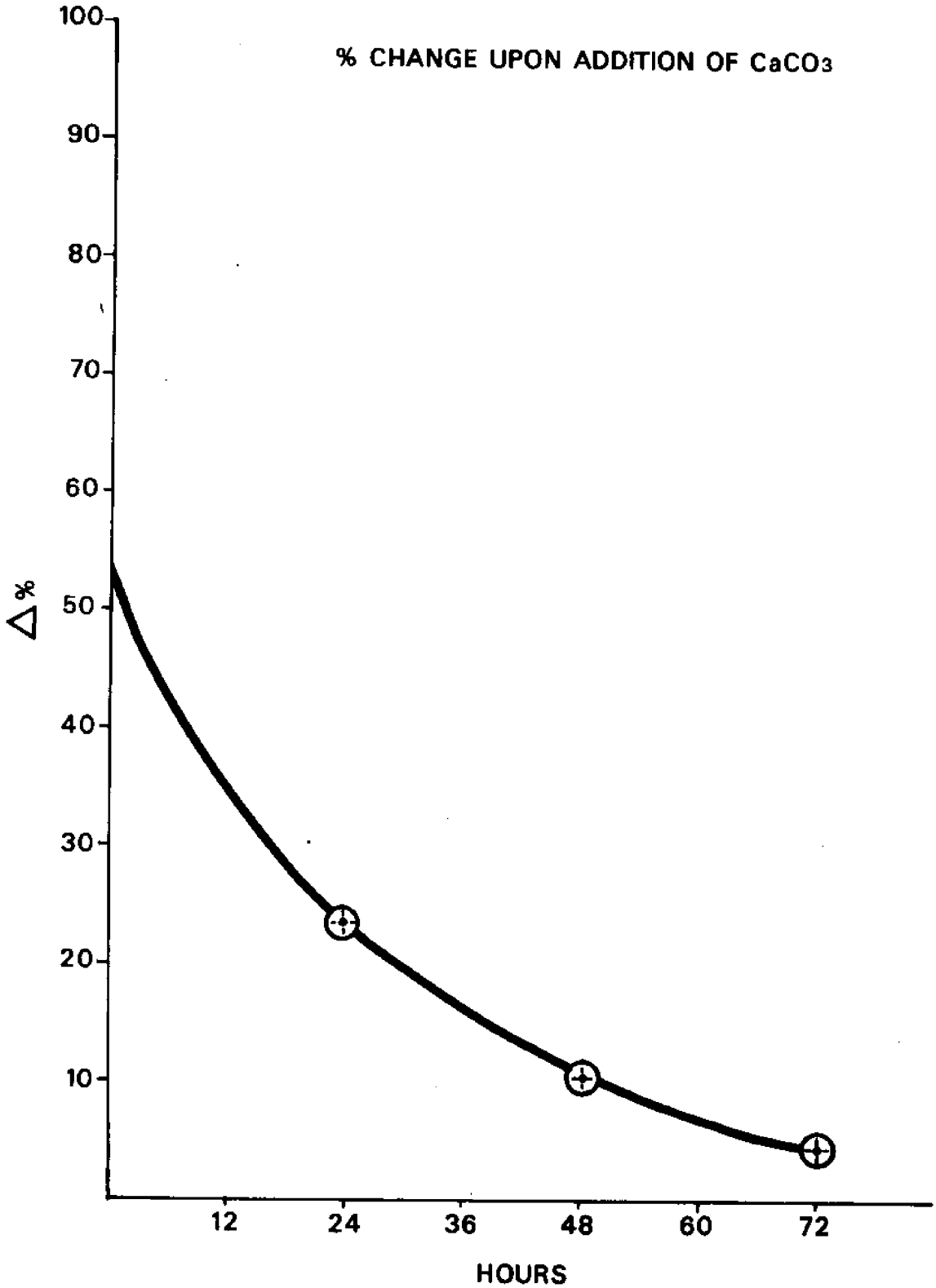

Figure 3. Decrease in surface association affinity with increasing time (increasing nutrient stress).

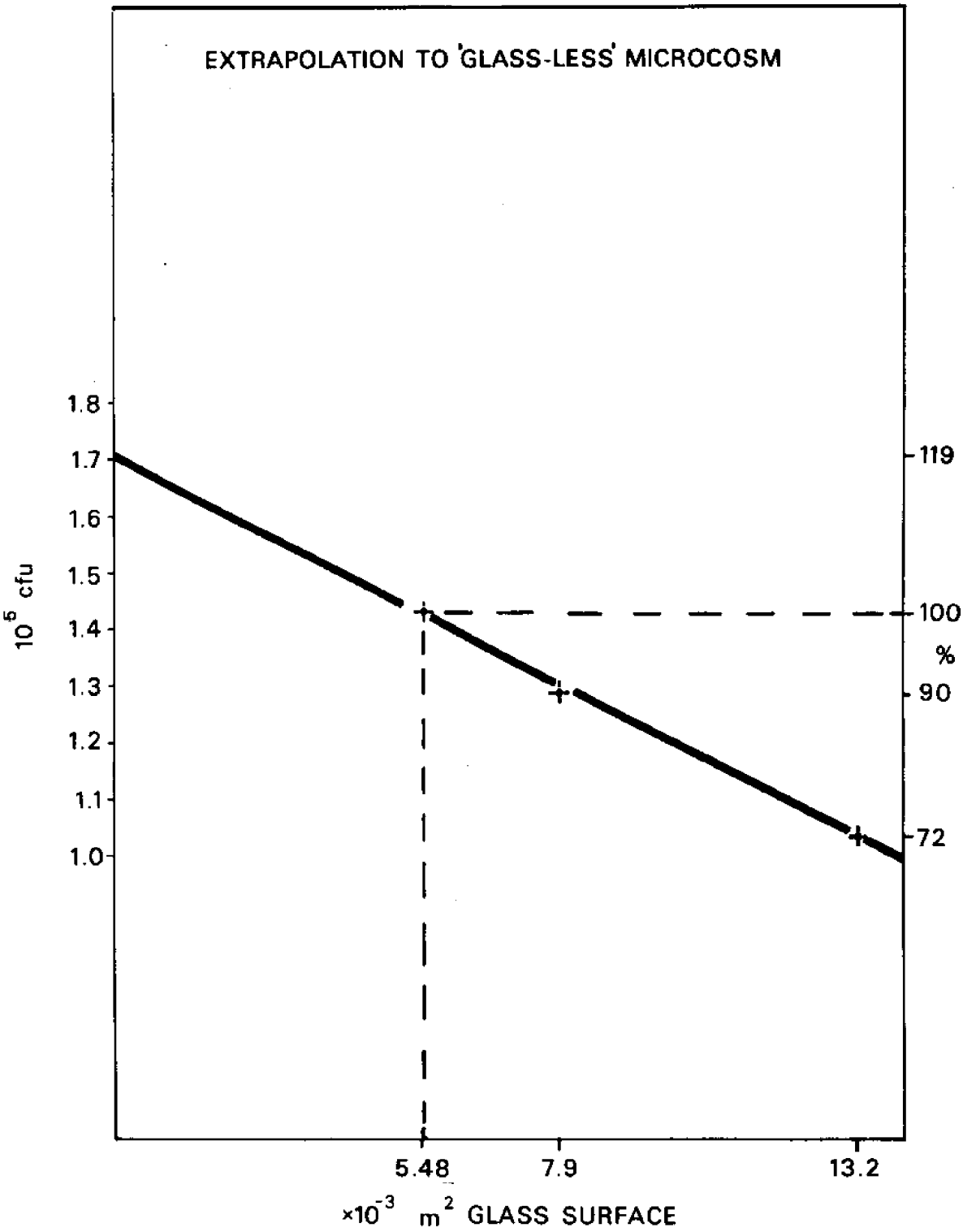

Figure 4. Effect of surface area on planktonic populations of *V. cholerae*.

demonstrated that no detectable attachment to chitin occurred (Table 3). However, when sterile $CaCO_3$ was subsequently added to this microcosm, a decrease in the planktonic population of approximately 50% was detected. However, when the reverse experiment was carried out, i.e. $CaCO_3$ added followed by chitin, although the $CaCO_3$ removed approximately 50% of the planktonic population, the cells remaining in the liquid phase did not attach to the chitin particles (Table 3). Similar results were obtained for all grades and kinds of chitin employed, as ball milled, grade B purified, and unpurified crude chitin fragments were tested. This experiment was then repeated employing non-stressed V. cholerae to determine if the physiological condition of the cells influenced the attachment phenomenon. The initial addition of either chitin or $CaCO_3$ resulted in a decrease of approximately 50% in the planktonic population with no detectable decrease occurring upon addition of the second particulates (Table 4).

The results of this study suggest that populations of non-stressed V. cholerae yield an approximately constant proportion of cells capable of association with particulates, i.e. attachment. This indicates that electrostatic attraction may be the driving force for this type of cell-surface interaction. In this case, the two-fold difference in cell numbers observed between the 0.2μm filtered, and the non-filtered seawater microcosms (fig. 1) may represent an association constant, suggesting the absence of cells with a phenotypic characteristic permitting or preventing attachment to a surface. This, however, does not appear to be the case, since we find that when a $CaCO_3$ particle (with associated cells) is used as the inoculum for a fresh microcosm, the 24 hr planktonic population yielded does not decrease with yet further addition of $CaCO_3$ particles (fig. 2). It is clear that the "daughter" cells developing from the attached cells which possessed the capacity to attach, remained associated with the particle. Only those cells demonstrating no affinity for attachment initially populated the liquid phase of the microcosm. Therefore, the addition of further $CaCO_3$ after 24 hr incubation had no detectable effect on the planktonic population. Since it has been demonstrated that surface association affinity decreases with stress (fig. 3), the attachment effect expected of the subsequent generations of planktonic cells is relatively small and presumably lost in the assay.

The attachment of V. cholerae and other Vibrio spp. to chitin particles has been shown to be influenced by physico-chemical parameters (1). Likewise, V. cholerae has been shown to selectively attach to copepods and, indeed, selectively attach to regions on the copepods -- around the mouth and the egg sac (6). However, these studies employed fresh, i.e. non-stressed, cultures of V. cholerae. In the present study, non-stressed V. cholerae were demonstrated to become associated with chitin and $CaCO_3$ (Table 4). However, when envirotolerant V. cholerae were employed no attachment to chitin was detected, although the proportion of the population capable of attachment to $CaCO_3$ remained unchanged.

Under certain conditions, V. cholerae can undergo alteration in its attachment capability and specificity. Affinity for the type of substrate to which it can or will become attached appears

Table 3. Attachment of Envirotolerant *Vibrio cholerae* to $CaCO_3$ and chitin particles in aquatic microcosms.

	CULTURABLE COUNT/ml	PERCENT CHANGE in culturable count
Initial inoculum size[a]	5.3×10^6	---
After addition of chitin	5.3×10^6	0%
After subsequent addition of $CaCO_3$	2.4×10^6	-55%
Initial inoculum size	6.9×10^6	---
After addition of $CaCO_3$	2.6×10^6	-62%
After subsequent addition of chitin	2.6×10^6	0%

[a]culturable count

Table 4. Effect of organic nutrient concentration on percent decrease in culturable counts of *Vibrio cholerae* of different physiological states resulting from attachment to chitin and $CaCO_3$ particles in aquatic microcosms

PHYSIOLOGICAL CONDITION	CHITIN		$CaCO_3$		CULTURABLE COUNTS
	UNSUPPLEMENTED*	SUPPLEMENTED**	UNSUPPLEMENTED*	SUPPLEMENTED**	
ENVIROTOLERANT	9×10^2	9×10^2	6×10^4	5.3×10^2	Pre-amendment
	0%	0%	53.2%	45.6%	
	9×10^2	9×10^2	3.2×10^4	2.4×10^2	Post-amendment
NON-STRESSED	3.6×10^3	3.5×10^3	4×10^3	3.7×10^3	Pre-amendment
	41.7%	51.4%	50.0%	35.2%	
	2.1×10^3	1.7×10^3	2×10^3	2.4×10^3	Post-amendment

* 24 $^o/_{oo}$ marine salts solution

** 24 $^o/_{oo}$ marine salts solution amended with 0.175% yeast extract

to be influenced by the physiological state of the cells. Although the envirotolerant V. cholerae attaches to $CaCO_3$, in this physiological state, the cells appear initially to lack the capability to attach to chitin. Such selectivity in attachment may indeed be related to survival mechanisms of V. cholerae.

CONCLUSION

The apparent selectivity in attachment of V. cholerae to particulates would also be supportive of the hypothesis of Colwell et al. (3) regarding the role of V. cholerae as an autochthonous member of the estuarine bacterial community. If, in estuarine systems, the V. cholerae population does, in part, exist associated with crustaceans, cells which, for whatever reason, become unattached and enter the water column would seemingly face two alternatives: (i) continue to grow unassociated, or (ii) reattach to a host orgamism in order to ensure survival. The results of Singleton et al. (10) however, indicate that, in the presence of optimum salinity, V. cholerae can exist for extended periods in the planktonic state. Under nutrient or salinity stress, survival of V. cholerae (as indicated by recovery) is decreased significantly. Furthermore, V. cholerae can assume an envirotolerant state characterized by morphological changes as the cells decrease in size (8) and metabolic activity (9). By selective association with inert particulates, i.e. $CaCO_3$ and glass (sand), the cells could be transported to the sediment where they would encounter a microenvironment greatly enriched in available nutrients and many, depending on prevailing conditions, undergo reversion to the nonstressed cellular form (8). Alternatively, once in the sediment, in which V. cholerae has been shown to survive for extended periods (5), the cells may be taken into organisms feeding on or in the sediment thereby providing the opportunity for reassociation of V. cholerae with higher trophic level organisms. Such an hypothesis is currently under investigation.

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SURVIVAL OF VIBRIO CHOLERAE 01 IN SEAFOOD DURING FROZEN AND REFRIGERATED STORAGE

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INTRODUCTION

The Consumer reaction to the cholera outbreak, which occurred in Louisiana, in 1978 (1), caused considerable economic harm to the seafood industry. The more recent outbreak, in October, 1981 (2), might serve as a reminder that cholera should be regarded as a continuing problem. Previous work in our department (5,6) has been directed toward the effects of cooking on *Vibrio cholerae* in seafood. The purpose of this study was to investigate the survival of *V. cholerae* in frozen and refrigerated seafoods. Miyaki *et al* (4) conducted basic investigations of the survival of El Tor vibrios, noting that previous workers had studied only the classical strains. More extensive studies of the El Tor biotypes are lacking, and no survival studies have been reported, concerning the Louisiana isolates of *V. cholerae*.

MATERIALS AND METHODS

Sterile seafood homogenates were inoculated with *V. cholerae* cells, and placed in frozen and refrigerated storage. The surviving microorganisms were enumerated periodically, to demonstrate the persistence of *V. cholerae* in the samples, without the influence of competing microflora. A flow diagram is depicted in Figure one, which outlines the experimental approach.

Cultivation of cell crops. Log phase cells of *V. cholerae* 01 (Louisiana strain 5875) were used to inoculate the various food samples. The organism was cultivated for 18 h at 35°C on two successive slants of trypticase soy broth (Difco) plus 1.5% agar (TSB+A). The cell crop was washed from the final slant into TSB, and the suspension was incubated at 35°C until the absorbance at 650 nm_8 reached 0.75. This procedure yields a culture containing approximately 10^8 organisms per milliliter (Angela Dicharry, unpublished data). The cells were harvested by centrifugation at 5000 x g and resuspended in physiological saline.

Preparation and storage of homogenates. Seafoods were purchased locally for preparation of the homogenates. Oysters were processed with the liquid in which they were packed; shrimp and crab meats were mixed with an equal mass of distilled water. Also, a specimen containing only distilled water was included. All of the preparations were autoclaved

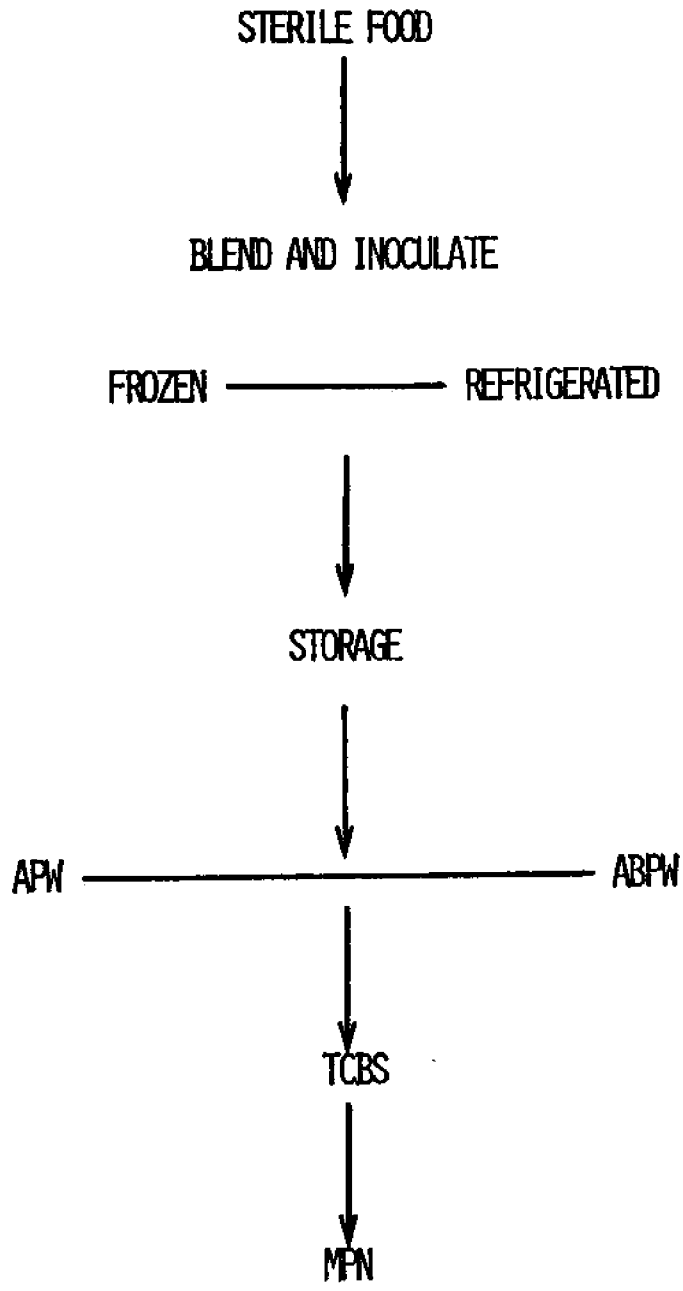

Fig. 1. Flow diagram of experimental procedures

at 121°C for 15 min, and were macerated by processing in a blender for one minute. V. cholerae cells, diluted in saline, were blended into the homogenates to a theoretical concentration of 10^6 cells per gram. Replicate aliquots of the inoculated homogenates were divided between frozen storage (-20°C) and refrigerated storage (8°C).

Enumeration of surviving organisms. The homogenates were assayed for V. cholerae, at day zero and periodically thereafter, by the three-tube most probable number (MPN) technique. Dilutions were prepared in saline peptone water [0.85% sodium chloride, 0.1% peptone, and 0.01% antifoam B (Sigma)]. Two enrichment media were compared in this experiment, both inoculated from common sets of dilutions: alkaline peptone water (APW) is the standard enrichment medium for V. cholerae (3); alkaline bile peptone water (ABPW) was recommended in a recent publication by Spira and Ahmed (7). Alkaline bile peptone water consists of alkaline peptone water, to which 0.5% sodium taurocholate has been added.

The enrichments were held for 6-8 h at 35°C and streaked onto plates of TCBS agar (Difco). The plates were incubated at 35°C for 18 - 24 h, and examined for the occurrence of typical V. cholerae colonies which indicated positive enrichment cultures.

RESULTS AND DISCUSSION

The number of viable V. cholerae cells in the frozen homogenates declined over time, as illustrated in Fig. 2. In this graph, the base ten logarithms of the alkaline peptone water MPN values are plotted as functions of storage time. V. cholerae cells were eliminated rapidly from distilled water, perhaps due to osmotic effects. A day zero count, before freezing, detected only 10^4 cells per gram, although the sample had been inoculated with approximately 10^6 organisms per gram. By day three, the MPN for frozen distilled water samples had decreased to less than three per gram. Some similarities seemed to appear between oyster and crab. In frozen samples of these homogenates, the counts had declined to low numbers of survivors by day three, which were no longer detected at day 21. V. cholerae seemed to acquire some protection in the frozen shrimp homogenate. The count decreased more gradually than in the other frozen homogenates, but was also less than three, by day 21.

Figure three depicts the survival of V. cholerae during refrigerated storage. The counts diminished very rapidly in distilled water, and were reduced to low numbers by day seven in the crab homogenate. The MPN in refrigerated shrimp homogenate decreased initially to an intermediate level, which was relatively stable through day 21. V. cholerae survived very well in refrigerated oyster homogenate, the numbers diminishing only after 14 days of storage.

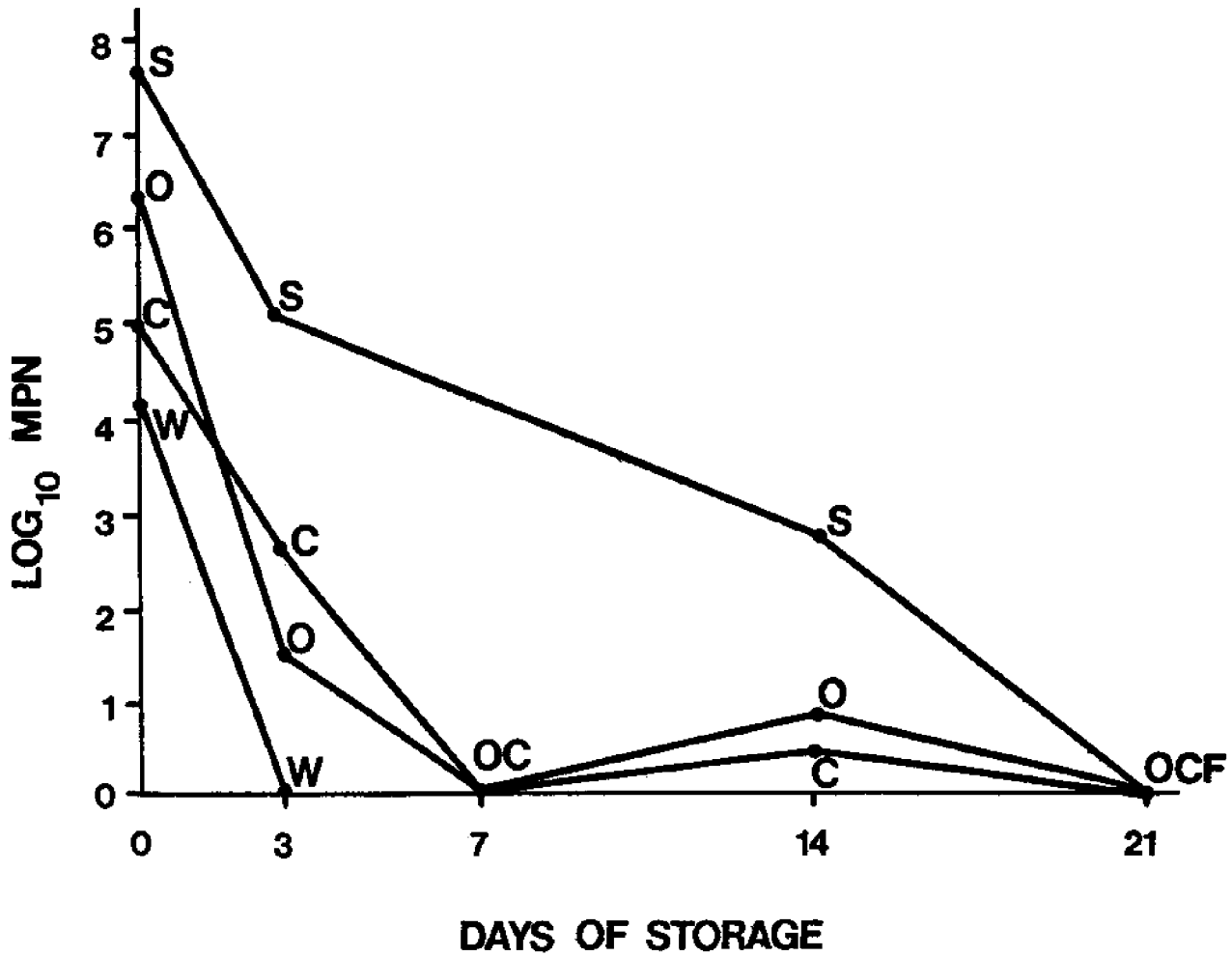

Fig. 2. Survival of V. cholerae 01 during frozen storage: W, distilled water; C, crabmeat homogenate; O, oyster homogenate; S, shrimp homogenate.

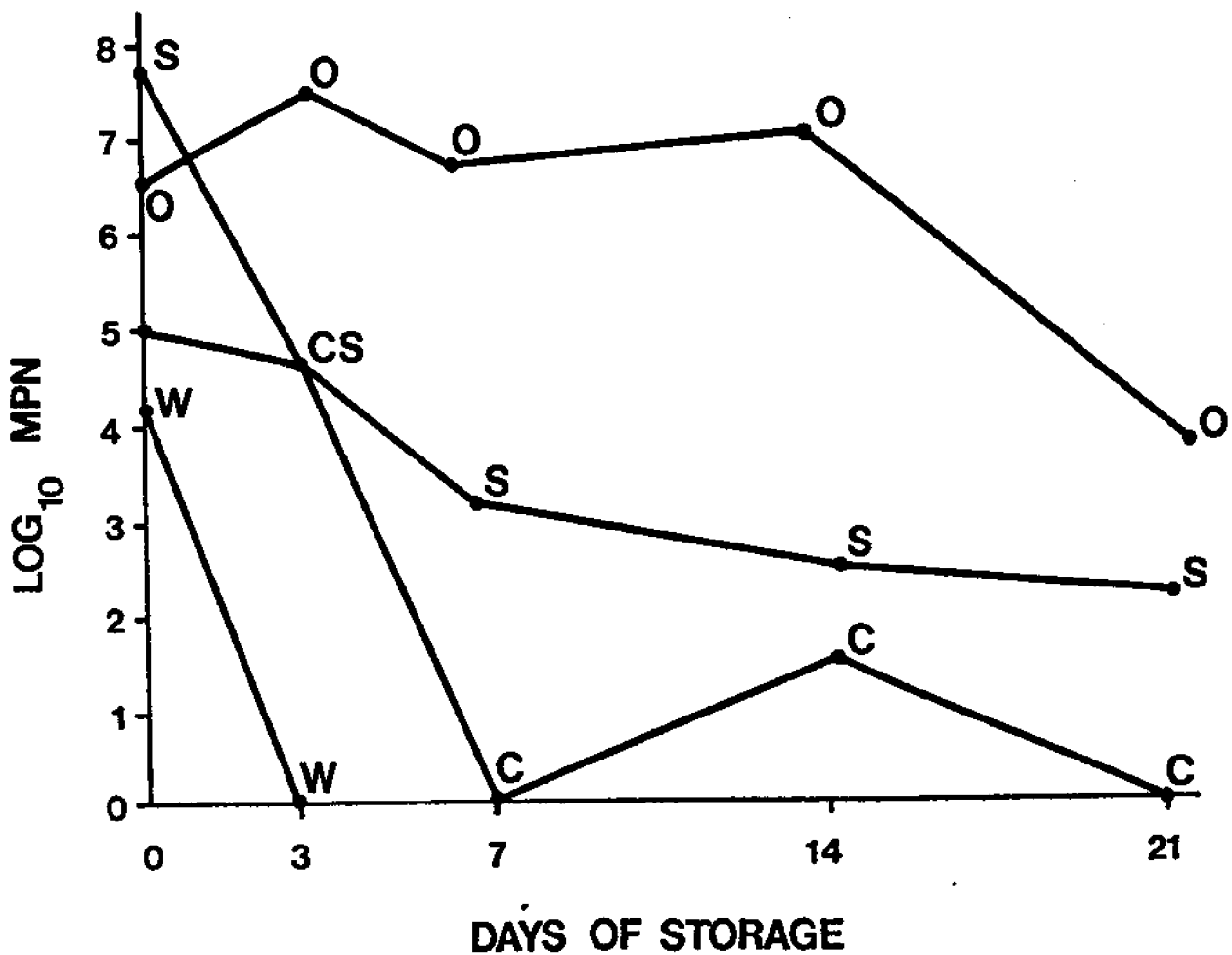

Fig. 3. Survival of V. cholerae 01 during refrigerated storage: W, distilled water; C, crabmeat homogenate; O, oyster homogenate; S, shrimp homogenate.

The survival of V. cholerae during refrigerated storage, is similar in some of the homogenates, to frozen storage. The organism disappeared so rapidly from both the frozen and the refrigerated samples of distilled water, that a variation could not be observed. The survival of V. cholerae in crabmeat homogenate was similar for both frozen and refrigerated storage (Fig. 4). There was also considerable similarity between the observations for frozen and refrigerated shrimp homogenates (Fig. 5).

The survival of V. cholerae in refrigerated oyster homogenate was much greater than in the frozen counterpart (Fig. 6). In refrigerated storage, the organisms persisted at the level of inoculation for more than 14 days, and more than 1,000 per gram remained viable after 21 days.

Through the course of this experiment, alkaline bile peptone water was compared to the standard enrichment medium, alkaline peptone water. Tables one and two list some typical counts, using the two media on refrigerated and frozen samples, respectively. The mean counts in refrigerated samples were 2.7×10^6, by the APW method and 7.8×10^6, by the ABPW method. In the frozen samples, the mean for APW was 7.0×10^3, and for ABPW was 1.2×10^3.

A further comparison of the APW and ABPW enumeration procedures was made by subjecting the method differences for samples to the t-test, again considering frozen samples and refrigerated samples separately. The null hypothesis, that the mean of the method differences equals zero, could not be rejected at the 95% level, so that a difference between the alkaline peptone water and alkaline bile peptone water procedures was not detected.

The next phase of this study will involve non-sterilized seafoods, inoculated with V. cholerae, and stored refrigerated and frozen, to determine the survival and recovery of the cholera organism under these conditions.

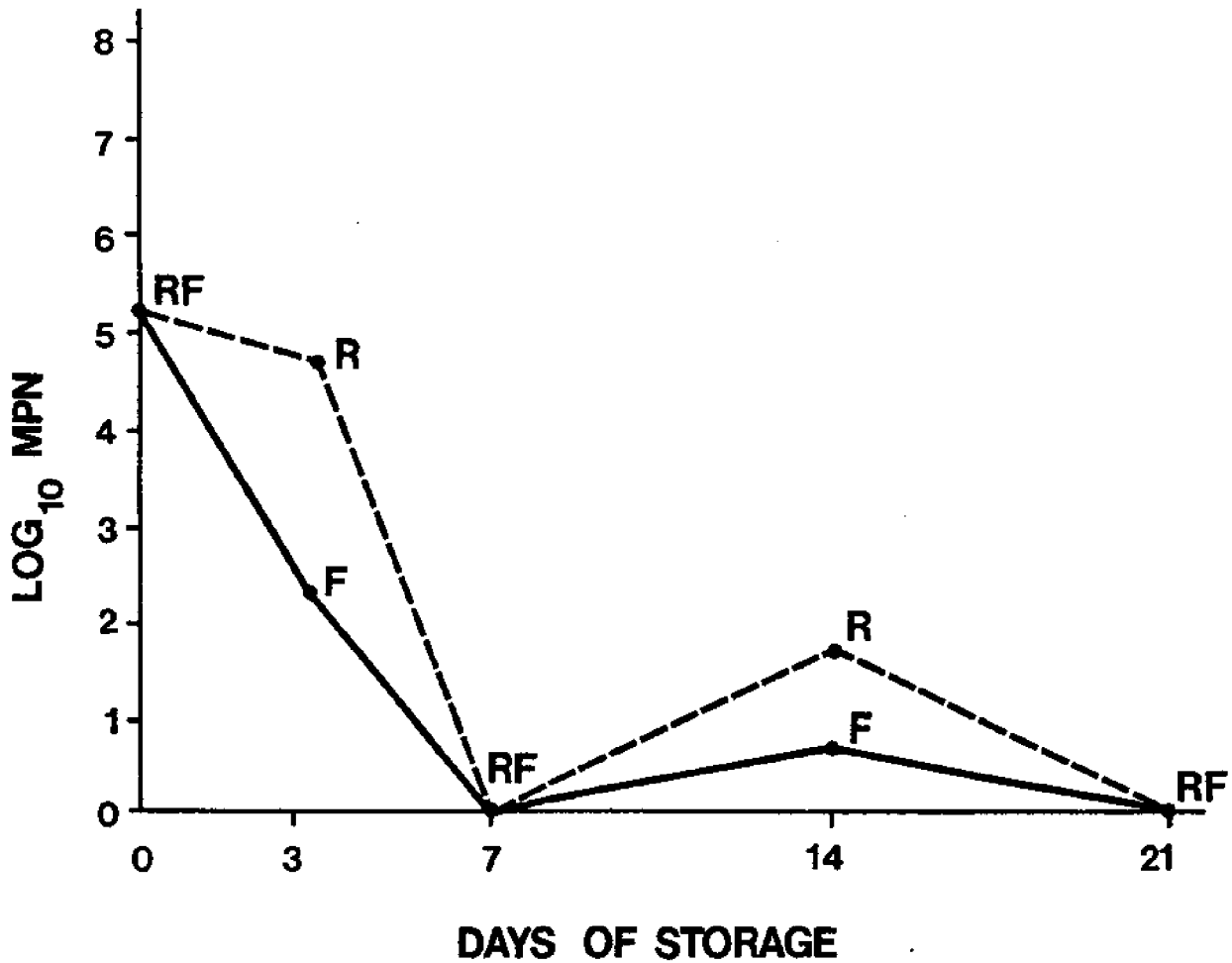

Fig. 4. Survival of V. cholerae 01 in crab meat homogenate: F, frozen storage; R, refrigerated storage.

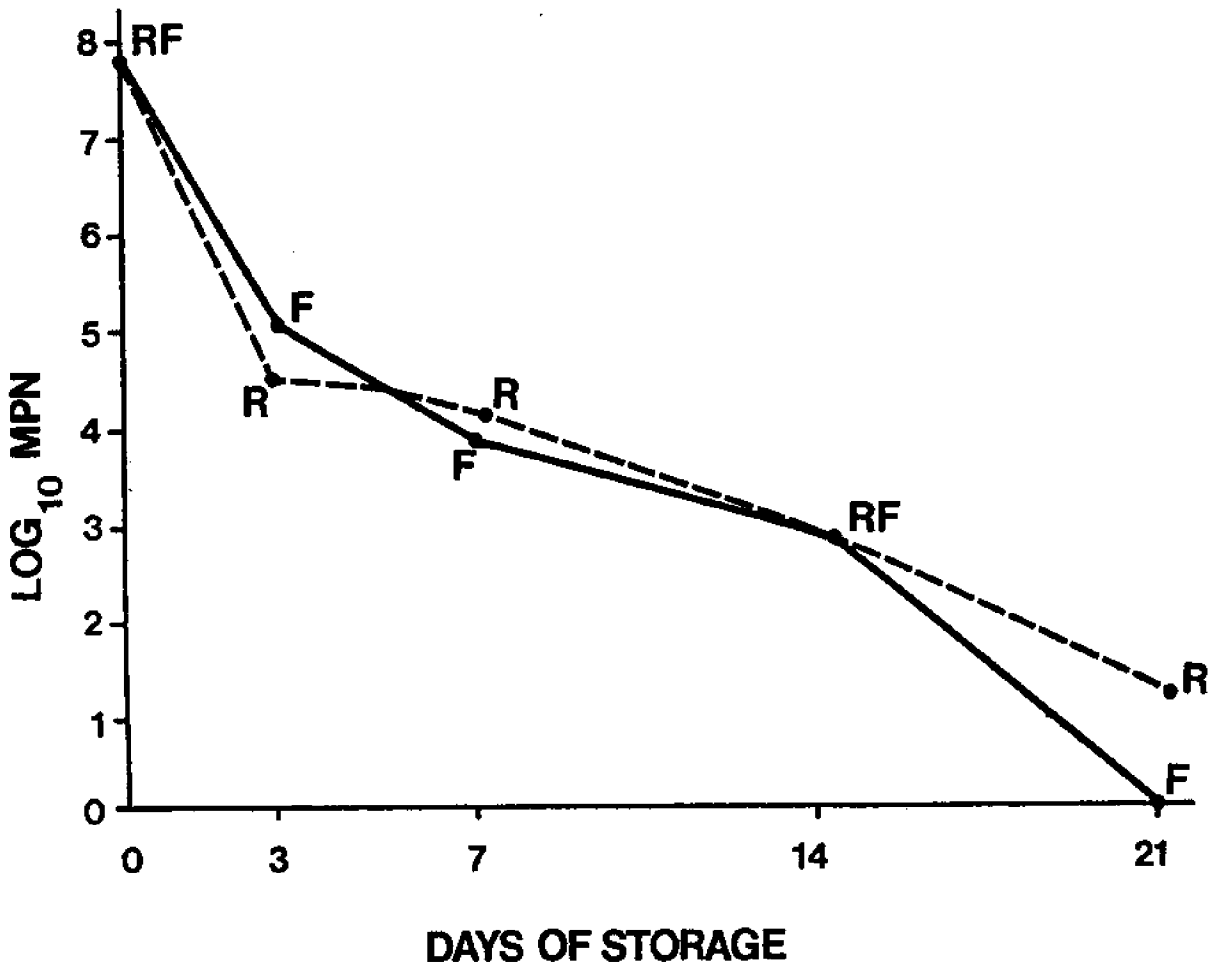

Fig. 5. Survival of V. cholerae 01 in shrimp homogenate: F, frozen storage; R, refrigerated storage.

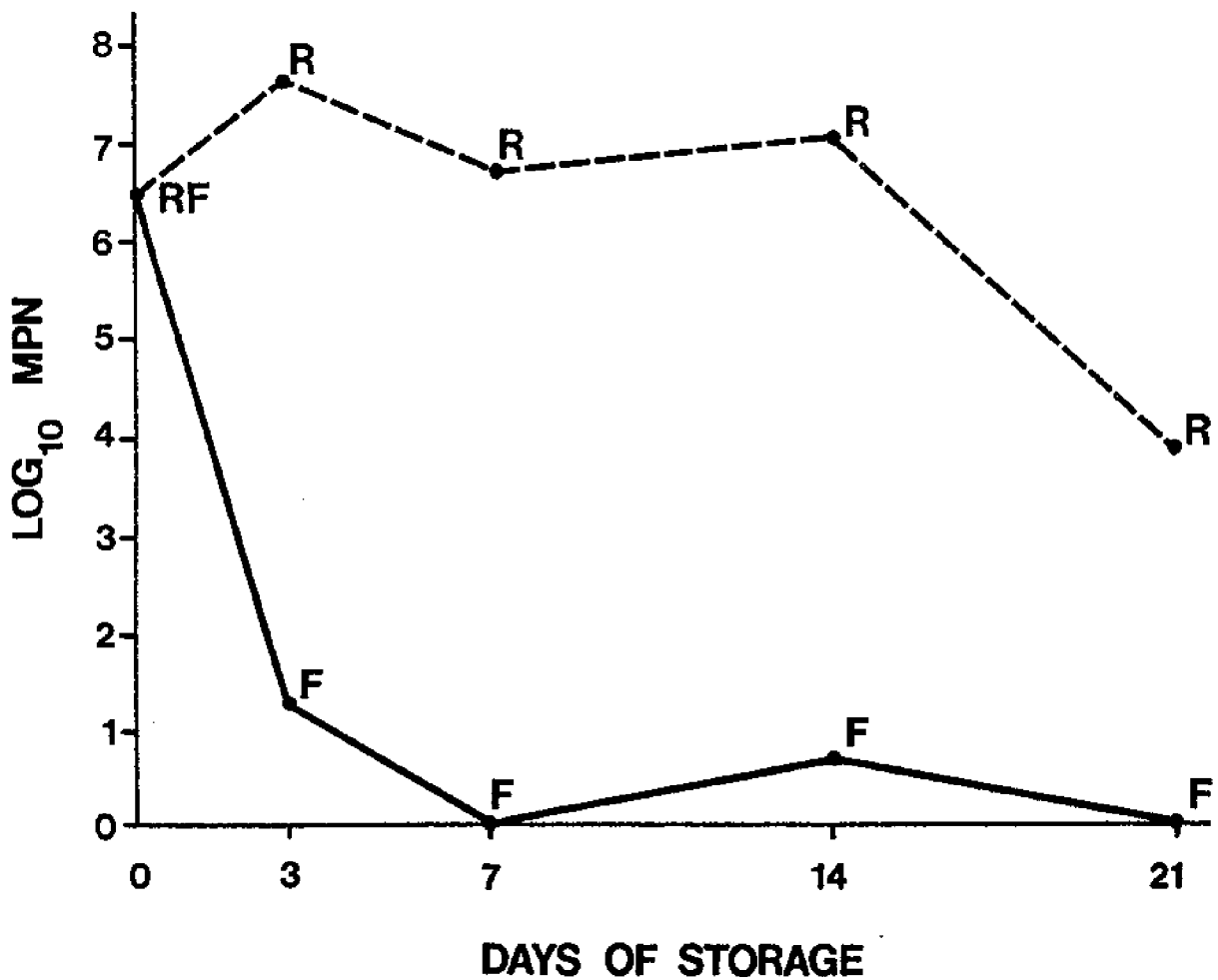

Fig. 6. Survival of V. cholerae 01 in oyster homogenate: F, frozen storage; R, refrigerated storage.

TABLE 1

Comparison of alkaline peptone water (APW) and alkaline bile peptone water (ABPW) enrichments: refrigerated storage.

Food	Day	Representative Counts, MPN/g	
		APW	ABPW
Oyster	3	2.4×10^7	7.5×10^6
Shrimp	7	1.5×10^3	2.3×10^3
Crab	14	43	4
Oyster	7	2.4×10^6	2.4×10^6
Crab	3	2.3×10^4	9.3×10^3
Shrimp	21	93	15

TABLE 2

Comparison of alkaline peptone water (APW) and alkaline bile peptone water (ABPW) enrichments: frozen storage.

Food	Day	Representative Counts, MPN/g	
		APW	ABPW
Oyster	3	2.1×10^1	9.3×10^1
Shrimp	7	4.3×10^3	9.3×10^3
Crab	14	4.0	9.0
Oyster	7	Less than 3	Less than 3
Shrimp	14	4.3×10^2	3.0
Crab	3	2.3×10^2	2.3×10^2

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DETERMINATION OF THERMAL DEATH TIME OF *VIBRIO CHOLERAE* IN OYSTER MEAT HOMOGENATES (*CRASSOSTREA VIRGINICA*)

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INTRODUCTION

There have been five outbreaks of non-laboratory acquired *Vibrio cholerae* infections in the United States since 1911 (5,13,15,16,24). Cases reported occurring in Texas, Alabama, and the Gulf Coast could not be traced or sources identified; however, the patient in Alabama had a previous history of eating large quantities of raw oysters. Crabs which were eaten after being improperly cooked and/or stored were implicated in the 11 cases of cholerae in Louisiana in 1978.

Since the oyster is a filter feeding shellfish, it can concentrate bacteria from the water passing through and over its gills, which results in natural contamination (6,19). The cultivation, processing, and packaging procedures for market oysters subjects them to other sources of contamination (7). The natural flora of market oysters is predominated by the psychotrophic gram negative asporogenous rods of the Pseudomonas/Vibrio group (6). Oysters and other shellfish have previously been implicated in cholerae outbreaks in Portugal (4) and in Italy (2,18).

V. cholerae is able to survive in salty, alkaline environments such as estuaries (14) and has been isolated from the Chesapeake Bay (6) and the Gulf Coast (cited in 20). The organism has also been isolated from shellfish and crusteaceans taken from the Chesapeake Bay.

It is generally recognized that *V. cholerae* is a very fragile organism (14,16). The disease can be controlled by employing proper sewage disposal and safe drinking water systems and avoiding hazardous dietary habits (e.g. eating raw seafoods) (2,16,24).

Relatively little information has been published on the thermal resistance of *Vibrio cholerae* 01 (Biotype El Tor, Serotype Inaba) Louisiana Strain #5875. This study was undertaken to determine the thermal resistance of the organism in packaged fresh oyster meat.

MATERIALS AND METHODS

Organism. The organism used in this study was *Vibrio cholerae* 01 (Biotype El Tor, Serotype Inaba) Louisiana Strain #5875 which was obtained from the Department of Microbiology at Louisiana State University. Stock cultures of the organism were stored at room temperature on peptone salt agar slants and transferred to fresh media at 4-6 week intervals.

Media. Peptone water (PW) with a final pH of 6.4 consisted of 1% Bacto-peptone (Difco) and 1% sodium chloride in distilled water. Alkaline

peptone water (APW) was prepared by adjusting the pH of PW to 8.6 with 1.0 normal sodium hydroxide. Peptone salt agar (PSA) was prepared by adding 1.5% Bacto-agar (Difco) to PW. Thiolsulfate citrate bile salts agar (TCBS, Difco) was prepared according to the manufacturer's directions. All media, except TCBS, were sterilized at 121°C for 15 minutes.

Preparation of the bacterial suspension. A loopful of the *V. cholerae* was transferred to 15 ml of nutrient broth. After incubation at 37°C for 20-24 hours, the culture was throughly mixed and transferred to a sterile spectrophotometer tube. The optical density (OD_{650}) of the suspension was adjusted to approximately 0.19 with sterile nutrient broth.

Enumeration of the bacteria. Decimal 1:10 dilutions of the bacterial suspension were made in PW, and 0.1 ml aliquots of the appropriate dilutions were spread-plated on PSA. Colonies were counted on a New Brunswick Scientific Co. colony counter after incubating the plates for 24-48 hours at 37°C.

The 3 tube Most Probable Number (MPN) technique was also used to enumerate bacteria in the cell suspension. One milliliter aliquots of the dilutions were pipetted into the MPN tubes containing 10 ml of APW. After incubation at 37°C for 6-8 hours, a loopful of the culture was removed from the pellicle of the MPN tubes and streaked on TCBS plates (24). Plates on which typical *V. cholerae* colonies were formed were considered positive.

Preparation and inoculation of the oyster homogenate. Packaged oysters were purchased from local supermarkets and stored at approximately 4°C until ready for use. Excess fluid was drained from the oysters before they were weighed. The oyster homogenate was formed by blending the oysters in a Waring blendor at high speed for two minutes. The pH of the homogenate was adjusted to 6.0 with 1.0 normal NaOH, if necessary.

A 1:10 dilution of the *V. cholerae* suspension was prepared in PW and added to the homogenate to give a final concentration of approximately 10^6 organisms/gram.

Preparation of thermal death-time tubes. Pyrex glass tubing with an outer diameter of 13 mm was cut into 15 cm lengths and heat sealed at one end with an oxygen-acetylene torch.

Four gram aliquots of the oyster homogenate were placed into the tubes by using a sterile animal force feeder equipped with a large needle. The tubes were heat sealed with the oxygen-acetylene torch in lengths of 10 cm.

Determination of the efficiency of the recovery system. The recovery procedure was evaluated to determine its efficiency for isolating *V. cholerae* organisms from the homogenate. This was determined by placing a previously determined number of cells into the homogenate which was exposed to all procedures for end point determination, except heat treatment. The number of organisms in the homogenate was then determined using the MPN technique. MPN determinations were also performed on the original inoculum and on a control sample which was not inoculated with *V. cholerae*. The percentage of viable organisms recovered was calculated by dividing the MPN of the inoculated oyster homogenate by the theoretical number of organisms introduced into the homogenate.

Determination of the come-up time. The come-up time of the homogenate at various temperatures was determined by filling three tubes with 4 grams of homogenate each. The tubes were connected to copper-constantan thermocouples and immersed in the water bath set at the required temperature. The amount of time required for the sample to go from room temperature to the treatment temperature was recorded on a multipoint recorder (Speedomax by Leeds & Northrup). The time required for the slowest heating tube to reach the treatment temperature was used as the come-up time for all other experiments at that temperature. The timing of heat treatments was begun after the end of the come-up period. The lethal effect of the heat during the come-up time was determined mathematically (1,22).

Endpoint determination. The endpoint was determined by immersing the sealed tubes containing the oyster homogenate into a water bath heated from 48.89°C (120°F) to 82.22°C (180°F) at 5.56°C (10°F) intervals. The endpoint was bracketed by heating inoculated samples at 5-10 minute intervals. One sample was removed at each interval and cooled in an ice water bath for 30 seconds. The samples were tested for the presence of *V. cholerae* by an APW enrichment and isolation on TCBS (see above).

Control samples consisted of both inoculated homogenate and uninoculated homogenate which underwent the same treatment as the experimental samples, except no heat treatment was applied.

The endpoint was determined by heating sets of 15 tubes at 1 minute intervals above and below the time which produced negative results in the preliminary one tube tests. The time period after which no organisms could be recovered from 15 tubes of heated homogenate was defined as the endpoint.

Inoculated pack studies. Whole packaged oysters were weighed and injected with a 24 hour *V. cholerae* culture grown at 37°C in nutrient broth. *V. cholerae* at 10^6/g were injected into the oysters with a syringe.

Fifty grams of the injected oysters were cooked using one of two methods. One group of the oysters was cooked in a liter of hot oil (190.5°C, 375°F) for one minute, and the other group was cooked in one liter of boiling water for one minute.

After cooking, a 1:10 dilution was prepared of 25 grams of the oysters in APW in a blendor jar. The mixture was blended for 3 minutes at high speed in a Waring blendor. The blendor jar containing the cooked oysters was incubated at 37°C for 8 hours. A loopful of the incubated suspension was removed from the surface and streaked in duplicates on TCBS agar. A control sample consisting of inoculated oysters was treated in the same manner as above, except the oysters were not cooked.

RESULTS AND DISCUSSION

Recovery of *V. cholerae* from the homogenate. This information was important since death of the organism was defined as the inability of the cells to form colonies on TCBS after an enrichment in APW. It was therefore necessary to be able to recover a large percentage of the live organisms form the homogenate if the results were to be accurate.

In 3 separate trials, relatively large percentages of the organisms were recovered (Table 1). Though only 44% of the organisms were isolated from

sample 1, 94% and 100% were isolated from sample 2 and 3, respectively. More organisms were isolated from the homogenate than were placed in sample 3. This was probably due to the inaccuracy of the MPN method used for enumeration (11).

TABLE 1. Efficiency of recovery of V. cholerae from oyster homogenate

	Sample 1	Sample 2	Sample 3
Organisms placed in oyster homogenate	9.78×10^5	9.78×10^5	1.25×10^6
Organisms recovered from oyster homogenate	4.30×10^5	9.30×10^5	1.50×10^6
Percentage organisms recovered	44%	94%	100%

Endpoints. No V. cholerae were recovered from any of 15 tubes containing the inoculated homogenate which were heated for 34 minutes or more after reaching 48.89°C (120°F) (Table 2). At this temperature 4.67 minutes were required for the temperature of the homogenate in the tubes to rise from room temperature to the processing temperature. The time required to reach the processing temperature of 48.89°C was equivalent to heating the homogenate at 48.89°C for 2.66 minutes (Table 2). So although the tubes containing the homogenate remained in the water bath for a total of 38.67 minutes this was actually equivalent to heating the tubes for 36.66 minutes at 48.89°C (Table 3).

No V. cholerae were recovered from the homogenate heated for 1 minute after reaching the temperatures from 54.44°C to 82.22°C 2.76 minutes at 54.44°C to 1.84 minutes at 82.22°C (Table 3). The minimal heating time used in these studies was one minute. However, no organisms could be recovered from any of the thermal death time tubes 1 minute after the homogenate reached the temperatures from 54.44 - 82.22°C. This meant that the true endpoints for these temperatures were less than one minute. Therefore the true corrected endpoints were less than those calculated, which would cause the calculated D values to be greater than the true D values.

D values. D values for each temperature were calculated using the corrected endpoints. The values ranged from 4.71 minutes at 48.89°C to less than 0.24 minutes at 82.22°F (Table 3).

TABLE 2. Endpoints for V. cholerae and come-up times in oyster homogenates

Temperature (°C)	Endpoint (min)	Come-up Time (min)	Lethality of Come-up Time (min)
48.89	34[a]	4.67	2.66
54.44	1	3.50	1.76
60.00	1	3.83	1.74
65.55	1	3.17	1.61
71.11	1	3.83	1.37
76.67	1	3.83	1.32
82.22	1	3.50	0.84

[a]Endpoint based on no recovery/growth from 15 tubes containing a total of 6.0×10^7 organisms.

[b]Values obtained at these temperatures are less than those given due to the minimal measuring time of one minute.

TABLE 3. Corrected endpoints and D values for V. cholerae in oyster homogenate

Temperature (°C)	Corrected Endpoints (min)	D values	7D values
48.89	36.66	4.71	32.98
54.44	2.76	0.35	2.48
60.00	2.74	0.35	2.46
65.55	2.61	0.33	2.35
71.11	2.37	0.30	2.13
76.67	2.32	0.29	2.09
82.22	1.84	0.24	1.65

Heat treatments which yield from 4 to 7 D values are generally considered adequate for pasteurization (20). The amount of time required to produce 7 log reduction of the V. cholerae population in oyster homogenate was calculated by multiplying the time required for 1 log reduction by 7 (Table 3).

V. cholerae is very heat sensitive. It has been reported that the organism is killed after 10 minutes at 55°C (21). Another study reported that the organism could tolerate temperatures up to 41°C (3). These studies were not done on the El Tor strain however, which is considered to be one of the most heat resistant strains of the organism (11).

In peptone water V. cholerae #5875 has a D value of 1.70 minutes at 48.89°C, and a D value of 8.15 minutes at the same temperature in crab meat homogenate (20). So although the oyster meat provided more protection for the organism than the peptone water, it provided less protection than the crab meat.

Many factors influence the thermal resistance of bacteria (12). The lower pH and the presence of some inhibitory substances in oyster meat (10) may be the cause of the lesser resistance of the organism in the oyster meat than in the crab meat. Acid and alkaline conditions decrease the heat resistance of bacteria. In this study the pH of the oyster homogenate was 6.0. Oyster with a pH between 5.9 and 6.2 are considered to be good (7), but the pH of fresh crab meat is between 7.2 and 7.4 (8). The viability of V. cholerae has been proven to be less in an environment with a pH of 5.5-6.2 than it is in an environment with a pH greater than 6.2 (17).

The presence of colloidal substances such as proteins and fats in both meat homogenates were possibly responsible for the greater resistance of the organism in the meat homogenates than in the peptone water (9).

Inoculated packs. It has been reported that cooking oysters in hot oil (190.5°C, 375°F) or in boiling water for one minute will destroy 4.0×10^5 Vibrio parahaemolyticus cells injected into the body of the oyster (19). In this study, the ability of these methods to destroy 10^6 V. cholerae organisms/gram in oysters was investigated.

The oysters cooked in hot oil weighed from 5.59 to 24.95 grams (Table 4). The weight of the oysters was important because it determined the rate of heat penetration to the cold point of the oysters. The internal temperature of an oyster weighing 6.26 grams is greater than 100°C (212°F) after 1 minute in the hot oil, while the temperature of an oyster weighing 19.67 grams is approximately 79°C (174°F) (19). No V. cholerae were recovered from any of the three samples of inoculated oysters cooked in the oil, but the organism was isolated from the uncooked inoculated sample.

The results obtained when inoculated oysters weighing 6.81 to 19.61 grams were cooked in boiling water are shown in Table 5. The internal temperature of an oyster weighing 6.26 grams is 88°C (190°F) after 1 minute in boiling water, and the internal temperature of an oyster weighing 19.67 grams is approximately 71°C (160°F) (19). the results are similar to those obtained when the inoculated oysters were cooked in the hot oil. Both methods proved to be effective for destroying 10^6 V. cholerae/gram of oyster meat. Raw oysters were implicated in the recent cholerae outbreak which occurred in Italy (18). By cooking the oyster properly, the risk of a V. cholerae infection is substantially lowered or completely removed, even though the oysters may have been contaminated with the organism.

TABLE 4. Survival of *V. cholerae* in oysters cooked in oil at 190.5°C (375°F) for one minute

Sample	Weight of oysters (grams)	Results
1	10.11-24.95	-[a]
2	5.59-13.96	-
3	6.89-14.35	-
uncooked	5.40-10.65	+[b]

[a] - = No *V. cholerae* recovered from the sample
[b] + = *V. cholerae* recovered from the sample

TABLE 5. Survival of *V. cholerae* in oysters cooked in water at 100°C (212°F) for one minute

Sample	Weight of oyster (grams)	Results
1	10.88-12.79	-[a]
2	6.81- 9.80	-
3	11.16-19.61	-
uncooked	5.40-10.65	+[b]

[a] - = No *V. cholerae* recovered from the sample
[b] + = *V. cholerae* recovered from the sample

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STUDIES ON THE ECOLOGY OF *VIBRIO CHOLERAE*

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INTRODUCTION

Vibrio cholerae, the causative organism of epidemic cholera, has been isolated from a variety of clinical and environmental samples (2,4,6,8,9,10,12,13,19,26). Although the majority of these isolates are non-01 serovars (19,27), some isolates are 01 serovars, even in regions with no known oubreaks of cholera (4). To account for the sporadic distribution of *V. cholerae* in the aquatic environment, Colwell et al. (10) have hypothesized that *V. cholerae* is a member of the autochthonous bacterial community of estuarine waters.

The influence of environmental parameters on *V. cholerae*, as well as a possible ecological role in the estuarine waters, has not been extensively investigated, due primarily to the fact that, previously, investigations of the biology of *V. cholerae* have been designed based on the hypothesis that this species is solely of clinical origin. However, in order to develop an understanding of the biology of this important bacterial species, it is necessary to consider the hypothesis of the status of *V. cholerae* as an estuarine denizen.

To evaluate accurately the influence of selected environmental parameters on a particular species, difficulties such as a constantly changing physico-chemical environment, replication of experimental conditions, and enumeration of the organisms must be overcome. Surveys of various environments allow for derivation of correlations between environmental parameters and the distribution of a species. However, such an approach does not demonstrate specific effects of a selected parameter on an organism or species.

Laboratory microecosystems, i.e., microcosms, allow for simulation, but not duplication, of *in situ* conditions. Microcosms have been employed by many investigators in an effort to circumvent many difficulties associated with studying the influence of selected environmental parameters on organisms. In addition to allowing control of environmental conditions,

microcosms also permit replication of the experimental conditions and units within and between experiments. Therefore, laboratory microcosms were employed in this study to evaluate the influence of salinity, nutrient concentration and temperature on the survival and growth of V. cholerae. In addition, the association of V. cholerae and zooplankton was studied in laboratory microcosms.

MATERIALS AND METHODS

Microcosms, designed for survival and growth studies, were prepared by adding 50 ml of a chemically-defined sea-salts solution (31), adjusted to appropriate salinities, to acid cleaned, 125 ml Erlenmeyer flasks. The sea-salts solution was amended with selected concentrations of an organic nutrient, tryptone, and autoclaved for 15 min at 121°C. After cooling to room temperature, microcosms were inoculated with a washed-cell suspension of test strains of V. cholerae and maintained on rotary shakers (125 revolutions/min) at selected incubation temperatures.

Microcosms used to study the association of V. cholerae with zooplankton, i.e., copepods, consisted of water collected from the Patuxent River, Sunderland, MD; Chesapeake Bay near Solomons Island, MD; and Buriganga River, Dacca, Bangladesh. Samples were collected from each site. The water samples were stored in sterile plastic containers and transported, at ambient temperature, to the laboratory. Both environmental and clinical strains of V. cholerae, O1 and non-O1 serovars, were included in the study.

Strains of V. cholerae employed in the study were obtained from the stock culture collection at the University of Maryland. Fresh cultures on tryptic soy agar slants were prepared, as needed, from stock cultures maintained under liquid nitrogen. Cells inoculated into microcosms were grown for 18 hr at 30°C in tryptic soy broth and collected by centrifugation. Following five washings in sterile 0.85% NaCl solution, cell number was determined by acridine orange direct count (AODC) (11,14,17), appropriate dilutions prepared in 0.85% NaCl solution, and microcosms inoculated to an initial concentration of approximately 4×10^2 cells/ml.

In experiments designed to study the ionic requirements of V. cholerae, the rinse and diluent consisted of a basal salts solution with no added NaCl (vide infra). For V. cholerae-copepod studies, the inoculum was prepared by culturing V. cholerae in alkaline peptone water at 30°C for 12 hr, then washed thrice in phosphate buffered saline before use.

Samples for bacterial enumeration were collected aseptically from microcosms, using sterile 1 ml pipets. Total viable bacteria, i.e., culturable, were enumerated by spread plating onto

Triptic Soy agar (Difco Laboratories, Detroit, MI). Samples for bacterial enumeration were diluted in sea-salts solution prepared to the same salinity as the microcosms being sampled. All culture plates were incubated for 7 days at 25°C, before counting colony-forming units. In order to estimate accurately the number of culturable bacteria, a statistically designed sampling regime was employed, based on a preliminary components of variance analysis (21). For the experiments reported herein, the sampling regime consisted of three microcosms per treatment, one sample per microcosm and three culture plates per sample. However, for total bacterial counts, i.e., AODC, a 1 ml sample was collected from each microcosm and processed accordingly (11,14,17).

To evaluate the survival of V. cholerae at low temperatures, a series of microcosms were prepared, with salinities adjusted to 5 °/oo and 25 o/oo, and inoculated with 4×10^2 cells/ml. These microcosms were incubated at 10°C and, after appropriate intervals, duplicate microcosms of each salinity were transferred to 25°C and incubated for an additional four-day period. Samples for culturable and total bacterial counts were collected immediately after inoculation, prior to transfer to 25°C, and after the four-day incubation period at 25°C.

The heterotrophic activity of V. cholerae populations in microcosms was measured by the method of Hobbie and Crawford (16). Sterile acid-cleaned serum vials, each containing 25 ml of sea-salt solution, with salinity or specific salt concentration adjusted to desired levels, were inoculated with 25 µl or 50 µl samples collected from microcosms following appropriate incubation. In some experiments, a washed-cell suspension of V. cholerae was used as the inoculum for heterotrophic activity assays. Each vial was amended with a ^{14}C-labelled amino acid mixture (57 milliCi/mg-Atom carbon; Amersham Corp., Arlington Heights, Il) to a final concentration of 50 µg/l or 100 µg/l. Under the experimental conditions employed, these concentrations of amino acids did not give a substrate saturation phenomenon.

After addition of amino acids, vials were sealed with serum stoppers fitted with a plastic cup containing a folded 1 cm^2 piece of filter paper (Whatman No. 1). All vials, including controls fixed with 0.2 ml of a solution of 30% (w/v) trichloroacetic acid and 0.05% (w/v) mercuric chloride in 40% (v/v) formalin, unless otherwise stated, were incubated at 25 ± 1°C for 2 hr before the reaction was terminated by injecting the trichloroacetic acid-mercuric chloride-formalin solution. The filter paper wicks were saturated with 0.2 ml phenethylamine to absorb $^{14}CO_2$ during an additional 1 hr incubation period. Following the second incubation period, the filter wicks were placed into scintillation vials and the sea-salts solution filtered through a 0.2 µm membrane filter (Gelman Scientific Inc., Ann Arbor, MI) and rinsed with 10 ml of sterile sea-salts solution. The membrane filters were air-dried and placed into scintillation vials. Ten ml of scintillation cocktail (ACS,

Amersham Corp.) were added to each vial and radioactive counts made using a Beckman Liquid Scintillation Spectrophotometer Model LS-3155T. Uptake of amino acids ($\mu g\ l^{-1}\ hr^{-1}$) was calculated according to the procedure of Hobbie and Crawford (16).

The influence of different concentrations of NaCl on growth and viability of V. cholerae was evaluated by employing microcosms prepared with a salt solution consisting of (g/l) $MgSO_4$, 2.86; KBr, 0.107; H_3BO_3, 0.019; $MgCl_2.6H_2O$, 7.74; $CaCl_2$, 0.82; $SrCl_2$, 0.017; K_2CO_3, 0.05; and amended with selected concentrations of NaCl. To minimize the change in total salinity, as well as conserve Cl^- and monovalent cation concentrations, molar equivalents of KCl or LiCl were substituted for NaCl, as the latter's concentration was decreased. Also, microcosms, thusly prepared, allowed determination of the extent to which Na^+ requirement, if any, could be spared by K^+ or Li^+ (15,22-24).

To investigate further any ion requirements of V. cholerae, a turbidimetric assay was employed to determine growth in the presence of different ion concentrations and substrates. Acid-cleaned culture tubes (10 mm) containing basal salts solution were supplemented with either 1% (w/v) tryptone (Difco), 1% (w/v) glucose (Sigma Chemical Co.), or 1% (w/v) glucose plus 0.05% yeast extract (Difco). The culture tubes were then amended with selected concentrations of NaCl, NaCl plus KCl, or NaCl plus LiCl. The culture tubes were inoculated with a washed-cell suspension of V. cholerae to an initial concentration of approximately 1×10^5 cells/ml and incubated at 25°C. After inoculation, the culture tubes were examined at 24 hr intervals, up to a maximum of 14 days, for growth, as indicated by visible turbidity. All tubes positive for growth were sampled to verify viability and purity of the culture by streaking onto Tryptic Soy agar and thiosulfate citrate bile salts sucrose agar (Oxoid, Columbia, MD).

To evaluate an association, as indicated by attachment, of V. cholerae with copepods, the copepods were collected aseptically, washed four times with water. Filter sterilized water, collected from the same site as the copepods, was used throughout the experiment. A number of flask combinations were prepared for the experiment: (i) live copepods, live bacteria and algae; (ii) live copepods, dead bacteria and algae. Dead copepods were obtained by freezing the copepods at -60°C for 30 min. In the experiments, ca. 500 washed copepods were placed into each of several two-liter flasks containing 500 ml of filter sterilized water. After inoculation, flasks were incubated at room temperature (23°-26°C). At each sampling time, five copepods were removed, along with two ml of water and homogenized in a teflon tipped tissue grinder.

For V. cholerae enumeration, Thiosulfate Citrate Bilesalt sucrose (TCBS) agar (Baltimore Biological Laboratory) plates were spread with 0.1 ml aliquots, after dilution with phosphate

buffered saline. Plates were incubated at 35°C for 18 hrs.

For scanning electron microscopy (SEM), five copepods were removed from each flask and fixed in Bouin's solution (3). Samples were washed in filter sterilized river or bay water, to remove all traces of fixative and were then dehydrated in isopropyl alcohol gradients and fixed with 1% oxmium tetroxide in saturated mercuric chloride (25) solution. Samples were freeze dried and coated with gold palladium alloy (5,7) in a Denton DV503 vacuum apparatus (Denton Ltd., Cherry Hill, NJ).

Prepared stubs were examined in an AMR 1000-A scanning electron microscope (Advanced Metals Research Corps., Bedford, MA) at an accelerating voltage of 20 KV. Micrographs were prepared using PN 55, black and white positive negative film (Polaroid Corp., Cambridge, MA).

RESULTS AND DISCUSSION

Initial studies were carried out to evaluate the influence of nutrient concentration and salinity on growth and survival of *V. cholerae*. For these studies, *V. cholerae* LA 4808, a toxigenic 01 strain, isolated from a patient who became ill following ingestion of improperly prepared seafood, was employed. Microcosms were prepared with salinities adjusted to 15 o/oo, 25 °/oo, 35 °/oo, and 45 °/oo, and amended with tryptone concentrations of 100 µg/l to 10,000 µg/l. Following a four-day incubation period the total (AODC) and culturable bacterial counts were compared (Fig. 1). A comparison of culturable bacterial counts with total cell counts demonstrated that a salinity effect was evident in the presence of low tryptone concentration, i.e., 500 µg/l or less. In microcosms with salinity adjusted to 15 °/oo or 45 °/oo, although cells were observed by epifluorescent microscopy, no culturable cells were detected. This was not true for microcosms with salinity adjusted to 25 °/oo or 35 °/oo, as the two enumeration techniques yielded similar results. Microcosms which were amended with a concentration of tryptone of 1000 µg or more did not demonstrate such a marked salinity effect. The largest total and culturable bacterial counts were consistently detected in the presence of 25 °/oo salinity, indicating this salinity to be near optimum for growth of *V. cholerae* LA 4808.

Since 25 /oo salinity was found to be optimum for growth of *V. cholerae* LA 4808 and 500 µg tryptone/l did not mask any salinity effect, growth of additional *V. cholerae* strains was compared in microcosms prepared to these standards. All isolates including clinical and environmental 01 serovars, grew to approximately the same cell concentration (Table 1). Using $\log_{10}$-transformed counts, culturable bacterial counts were compared by Duncan's multiple comparison test (28) and were found not to be significantly different ($p \leq 0.05$). Since all strains tested developed to approximately the same cell concentration,

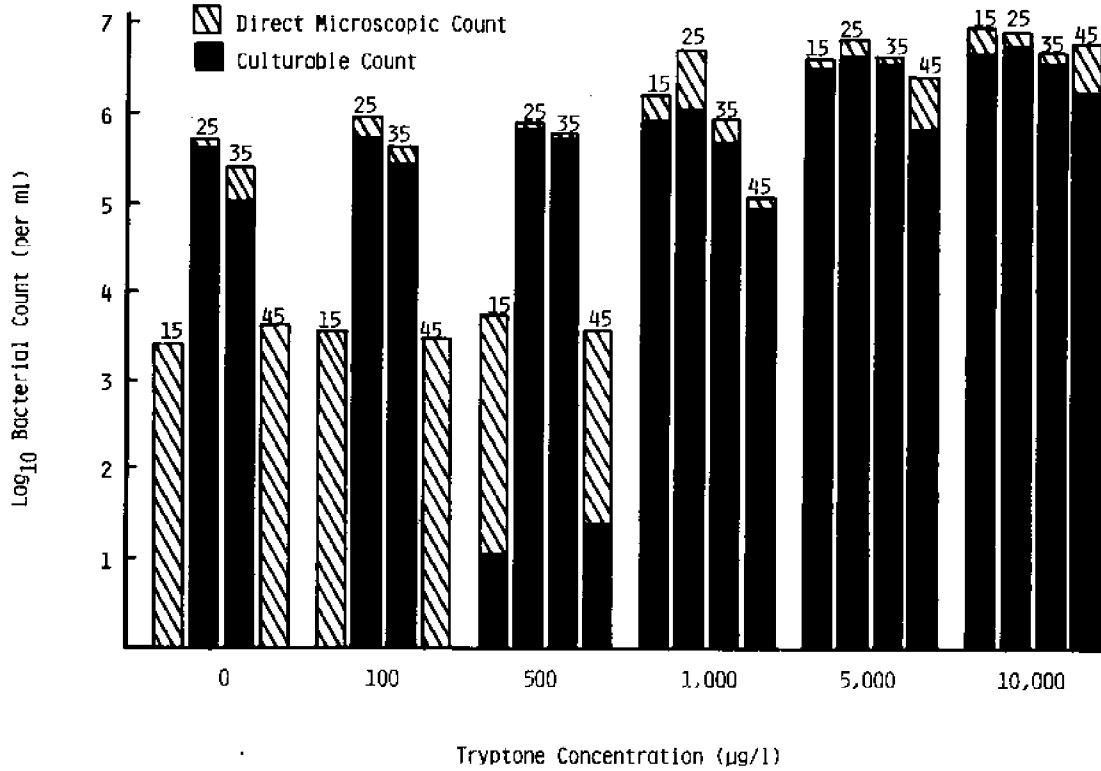

Figure 1. Comparison of direct microscopic and plate counts of *Vibrio cholerae* grown in a medium containing various concentrations of tryptone.

Table 1

COMPARISON OF POPULATION SIZES OF *V. CHOLERAE* STRAINS

IN MICROCOSMS

Strain	Source	Mean log Colony count/ml	Mean log Total cell count
v-69	Environmental	5.788 ± 0.055[b]	5.806 ± 0.054
N-19	Environmental	5.780 ± 0.029	5.908 ± 0.016
N-17	Environmental	5.730 ± 0.018	5.763 ± 0.055
N-1400	Environmental	5.845 ± 0.022	5.857 ± 0.130
N-1403	Environmental	5.769 ± 0.048	5.869 ± 0.063
N-33	Environmental	5.571 ± 0.060	5.716 ± 0.080
N-999	Environmental	5.685 ± 0.025	5.732 ± 0.110
SGN-7677[c]	Environmental	5.685 ± 0.034	5.748 ± 0.175
LA-4808[c]	Clinical	5.840 ± 0.010	5.954 ± 0.004
CA-401[c]	Clinical	5.746 ± 0.035	5.972 ± 0.051

[a]No significant difference between the mean colony counts was detected using Duncan's multiple comparison (24).

[b]Mean ± standard error

[c]01 serovar

strain LA 4808 was employed in survival and activity experiments.

Microcosms prepared to selected salinities and amended with 50 µg tryptone/l were incubated at temperatures of 10, 15, 20 and 25°C to evaluate the influence of temperature on V. cholerae. At 10°C a decrease in culturable bacterial counts was detected at all salinities tested (Fig. 2). There were, however, viable V. cholerae present in the microcosms throughout the 8-day test period, indicating extended survival at 10°C.

An incubation temperature of 15°C yielded similar results, except for microcosms for which the salinity was adjusted to 15 °/oo in which case, culturable cell counts increased (Fig. 2). At salinities of 5, 25, and 35 °/oo, an initial decrease in the number of culturable cells was noted, which continued in 5 °/oo and 35 °/oo microcosms. In the presence of 25 °/oo salinity, however, after the second day of incubation the culturable counts gradually increased throughout the experiment.

Development of V. cholerae populations at 20°C was influenced by salinity, indicated by the significant increase ($p \leq 0.05$) in culturable and total counts after two days incubation in microcosms with salinity adjusted to 15 °/oo but no significant change ($p \leq 0.05$) from the inoculum size in microcosms with salinities adjusted to 5 °/oo, 25 °/oo, and 35°/oo (Fig. 3). By the fourth day, however, populations of V. cholerae for all salinities demonstrated a significant increase ($p < 0.05$) above the inoculum size. After incubation for 8 days, the maximum culturable and total population sizes were detected in 25 o/oo salinity microcosms. Since the AODC and culturable cell counts were found to be approximately the same under the test conditions, only the culturable V. cholerae counts are reported for the survival and growth experiments.

Results obtained for microcosms incubated at 25°C were similar to those at 20°C, although no lag in development of V cholerae populations at any of the salinities tested, was noted (Fig. 3). Culturable bacterial counts increased to maxima by day 4. During the last four days of testing, although total counts remained stable at all salinities tested, culturable counts were constant only at 15 °/oo and 25 °/oo salinities, since significant decreases ($p \leq 0.05$) were detected at 5 °/oo and 35 °/oo salinities.

After the final sampling of microcosms for enumeration (day 8) samples were collected and inoculated into 25 ml of basal salts solution prepared to the same salinity as the microcosms sampled. The fresh basal salts solution was then amended with ^{14}C-labelled amino acid mixture, incubated at the same temperature as the original microcosm, and the heterotrophic activity of the V. cholerae populations measured. At 25°C and 20°C, populations of V. cholerae from microcosms for which the salinity was adjusted to 25 °/oo, utilized more amino acids

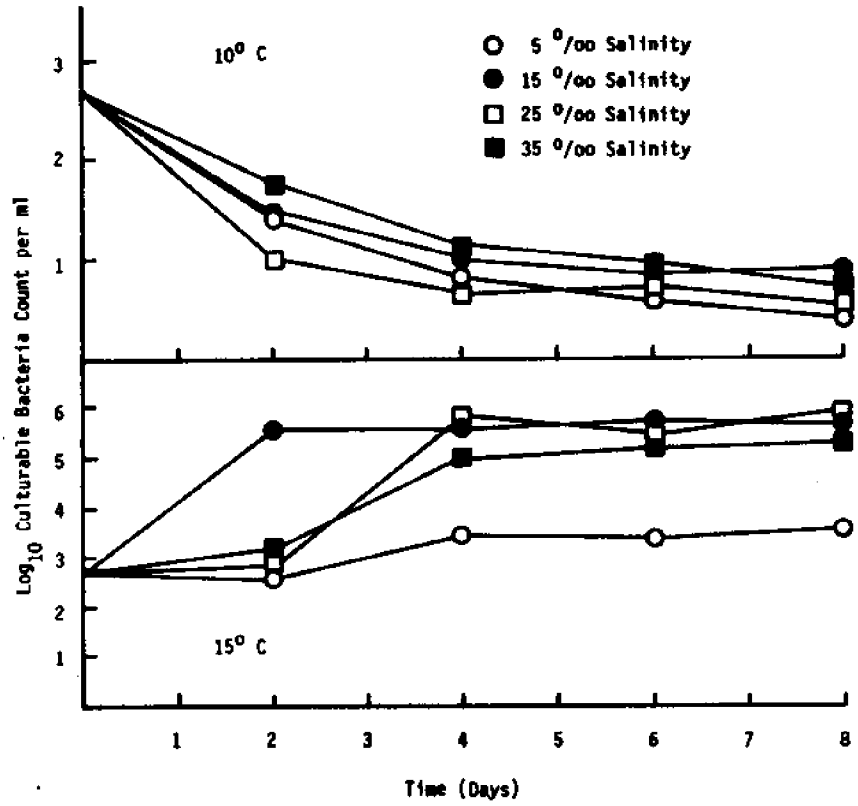

Figure 2. Comparison of counts of Vibrio cholerae grown at 10°C and 15°C in a medium of various salinities.

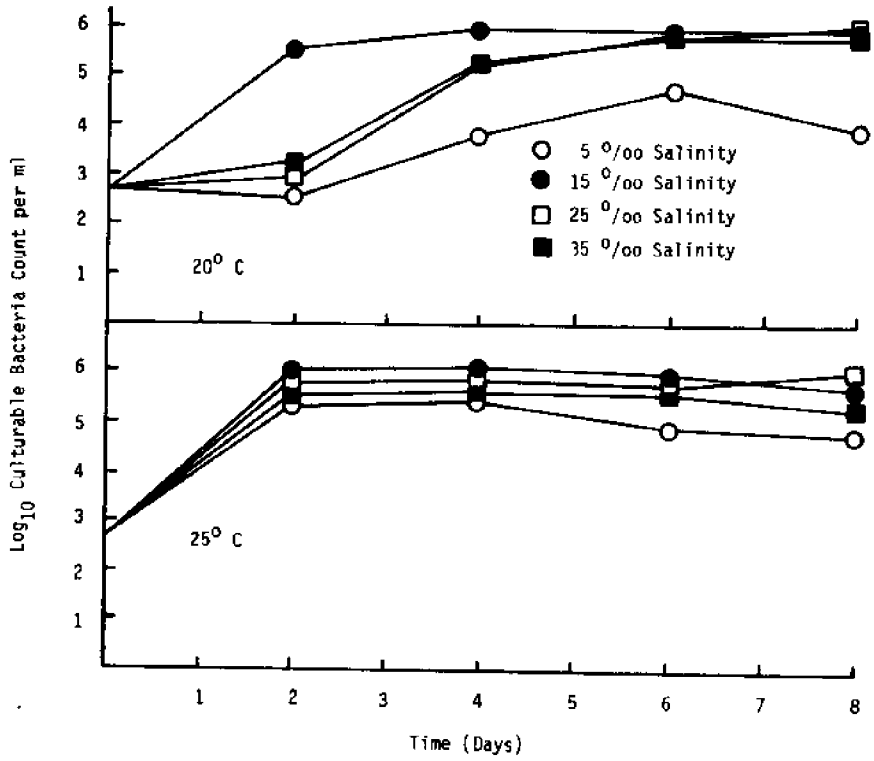

Figure 3. Comparison of plate counts of Vibrio cholerae grown at 20°C and 25°C in a medium with the salinity adjusted to 5, 15, 25 and 35 parts per thousand (°/oo).

than populations at the other salinities tested (Table 2). Lowest uptake values at 20°C and 25°C were obtained for populations in microcosms of 5 °/oo salinity.

Since the original microcosms contained different population sizes of V. cholerae, in order to obtain a more representative comparison of the influence of temperature and salinity on heterotrophic activity, uptake of ^{14}C- amino acids on a per culturable cell and per total cell number were calculated (Table 2). For microcosms incubated at 25°C, for the total population, the largest uptake value per cell was obtained for microcosms of 25 o/oo salinity. Uptake values for cells of all other salinities were approximately equivalent, but at a level approximately 50% less than that of 25 o/oo microcosms. This was not true, however when uptake values per culturable cell were compared as larger values were obtained for 5 °/oo and 35 °/oo microcosms. Similar results were obtained for microcosms incubated at 20°C, although the largest uptake per cell was for 5 °/oo microcosms.

To determine whether the observed effects of salinity on viability and survival of V. cholerae were due, in part, to a requirement for specific ions, a series of microcosms, containing a basal salts solution prepared with no added Na^+, was used. To determine whether a requirement for Na^+ by V. cholera, if any, could be satisfied by the presence of another monovalent cation, and in an effort to minimize change in salinity, molar equivalents of LiCl or KCl were added. As the NaCl concentration decreased from 0.30M to 0.10M and the KCl concentration increased to 0.20M, the V. cholerae population increased, although not significantly ($p \leq 0.05$), determined by Duncan's multiple comparison (Table 3). As the NaCl concentration decreased to 0.01M, the population size also decreased. When no NaCl was added, no culturable V. cholerae cells were detected. When LiCl was used to replace NaCl, no growth of V. cholerae occurred at LiCl concentrations tested.

Since no growth of V. cholerae occurred in microcosms containing Li^+, a washed-cell suspension of V. cholerae was employed to evaluate the influence of Na^+, K^+, and Li^+ on heterotrophic activity (Table 4). Uptake of ^{14}C-amino acids increased with decreasing Na^+ concentration and increasing Li^+ concentration until 0.20M NaCl and 0.10M LiCl were present. Similar results were obtained when KCl was added to compensate for decreasing NaCl concentration. However, maximum uptake occurred in the presence of 0.01M NaCl and 0.20M KCl. The smallest uptake of amino acids was detected in the absence of added NaCl.

To determine if, indeed, Na^+ was required for growth of V. cholerae, culture tubes containing basal salts amended with different substrates and salt concentrations were inoculated with a washed-cell suspension of V. cholerae. From the growth patterns obtained, it was evident that Na^+ is required for growth of V. cholerae (Table 5). In the presence of 1% (w/v) tryptone growth of V. cholerae occurred in the presence or absence of NaCl.

Table 2

HETEROTROPHIC UPTAKE OF ^{14}C-LABELED AMINO ACIDS BY *VIBRIO CHOLERAE* AT SELECTED SALINITIES AND TEMPERATURES

Temperature (°C)	Salinity (°/oo)	Uptake rate[a] (µgl/hr x 10^{-2})	Uptake rate/ culturable cell[b] (µg/lhr)	Uptake/ total cell number[c] (µg/l/hr)
25	5	2.43	6.93×10^{-7}	1.08×10^{-7}
	15	4.78	1.16×10^{-7}	8.68×10^{-8}
	25	14.40	1.75×10^{-7}	1.81×10^{-7}
	35	2.74	3.38×10^{-7}	8.64×10^{-8}
20	5	2.95	6.82×10^{-6}	3.58×10^{-6}
	15	7.36	9.97×10^{-8}	1.13×10^{-7}
	25	12.1	1.33×10^{-7}	1.66×10^{-7}
	35	4.94	1.14×10^{-7}	1.71×10^{-7}
15	5	N.D.[d]	-	-
	15	3.36	4.94×10^{-8}	6.87×10^{-8}
	25	N.D.	-	-
	35	N.D.	-	-
10	5	N.D.	-	-
	15	0.54	-	-
	25	N.D.	-	-
	35	N.D.	-	-

[a] Mean of duplicate flasks per treatment.

[b] Obtained from mean culturable colony count.

[c] Obtained from acridine orange direct microscopic count (AODC) (8,11,14).

[d] None detected, when compared with killed controls.

Table 3

VIBRIO CHOLERAE POPULATION SIZES IN MICROCOSMS AMENDED WITH SELECTED CONCENTRATIONS OF NaCl, KCl and LiCl

NaCl (Molar)	KCl (Molar)	LiCl (Molar)	Mean $\log_{10}$ Culturable count/ml	Mean $\log_{10}$ Total cell count/ml
0.30	0	0	5.550 ± 0.045[a]	5.821 ± 0.030
0.15	0.15	0	5.584 ± 0.028	5.947 ± 0.005
0.10	0.20	0	5.778 ± 0.035	6.000 ± 0.029
0.05	0.25	0	5.640 ± 0.041	5.808 ± 0.028
0.01	0.29	0	5.480 ± 0.056	5.687 ± 0.149
0	0.30	0	N.D.[b]	N.D.
0.15	0	0.15	N.D.	N.D.
0.10	0	0.20	N.D.	N.D.
0.05	0	0.75	N.D.	N.D.
0.01	0	0.29	N.D.	N.D.
0	0	0.30	N.D.	N.D.

[a]Mean ± standard error.

[b]None detected.

Table 4

EFFECT OF Na^+, Li^+ and K^+ ON UPTAKE OF ^{14}C-AMINO ACIDS BY VIBRIO CHOLERAE

Molar Quantity Added			Mean uptake Rate	Respiration
NaCl	LiCl	KCL	($\mu g l^{-1} hr^{-1} \times 10^{-1}$)	Incorporation
0.30	-	-	4.18	1:1.2
0.295	0.005	-	5.80	1:1.3
0.25	0.05	-	8.83	1:1.2
0.20	0.10	-	9.18	1:1.2
0.15	0.15	-	8.20	1:1.2
0	0.30	-	2.36	1:0.3
0.15	-	0.15	3.76	1:1.0
0.05	-	0.25	3.29	1:0.86
0.01	-	0.29	3.92	1:0.42
0.005	-	0.295	2.34	1:0.42
0.0005	-	0.2995	1.51	1:0.16
0	-	0.30	1.40	1:0.14

Table 5

GROWTH OF VIBRIO CHOLERAE IN BASAL SALTS SOLUTION AMENDED WITH SELECTED SUBSTRATES AND CONCENTRATIONS OF NaCl, KCL and LiCl

Added Salt(s) Concentration (Molar)	Incubation time (days before growth						
	1	2	4	8	10	12	14
NaCl:							
0.30	+						
0.20	+						
0.10	+						
0.075	+						
0.05	+						
0.025	+						
0.01	-	+					
0	-	-	-	-	-	-	-
NaCl + KCl:							
0.30 + 0	+						
0.20 + 0.10	+						
0.10 + 0.20	+						
0.075 + 0.225	+						
0.05 + 0.25	+						
0.025 + 0.275	-	+					
0.01 + 0.29	-	-	-	-	-	-	-
0 + 0.30	-	-	-	-	-	-	-
NaCl + LiCl:							
0.30 + 0	+						
0.20 + 0.10	+						
0.10 + 0.20	+						
0.075 + 0.225	+						
0.05 + 0.25	+						
0.025 + 0.275	+						
0.01 + 0.29	-	+[a]	+				
0 + 0.30	-	-	-	-	-	-	-

[a]slight

Whereas, basal salts amended with either 0.05% (w/v) yeast extract or 1% (w/v) glucose yielded no growth of V. cholerae. A combination of 0.05% (w/v) yeast extract and 1% glucose demonstrated the requirement of Na^+ by V. cholerae (Table 5). With the smaller NaCl concentrations, the only detectable influence on growth was the extended lag period before growth was observed. Results obtained also demonstrated that neither K^+ nor Li^+ can satisfy the Na^+ requirement.

Results of aforementioned studies demonstrate that V. cholerae has the ability to, not only survive, but grow under simulated estuarine conditions. Also, a definite requirement for Na^+ for growth of V. cholerae was demonstrated. Therefore, possible relationships between V. cholerae and other estuarine organisms were investigated.

From the copepod experiment it has been observed that V. cholerae increased in number, up to 100 fold, in the presence of live copepods, compared to cold-killed dead copepods. Fig. 4 shows the observed phenomenon when V. cholerae CA 401 was added to Patuxent River water. It was also observed the V. cholerae survived or remained culturable longer in the presence of live copepods.

SEM revealed that the washing of copepods with filter sterilized river or bay water effectively removed all surface bacteria prior to using them in the experiment. Examining the contaminated copepods, it was observed that V. cholerae attached onto the live copepods. No significant attachment was seen on the surfaces of the cold-killed copepods. Attachment onto the live copepods appeared to be selective since the heaviest concentration was observed on the oral region (Fig. 5) and on the egg sac (Figs. 6, 7) of the copepods. Other organisms used as control did not behave as did V. cholerae.

With V. cholerae an indigenous member of the bacterial community of estuarine systems, and having the capability to survive for extended periods under adverse conditions, many of the limited outbreaks of cholera, in regions of no known, or apparent, focus of infection can now be accounted for. Recent outbreaks of cholera in Louisiana (4) and Texas (8) have been associated with, or related to, consumption of shellfish. Thus, an environmental reservoir of V. cholerae can serve as a source of cholera if shellfish carry V. cholerae, either through a non-specific association or, as has been hypothesized (9), by interaction of V. cholerae in a commensal relationship with higher trophic level organisms whereby V. cholerae enterotoxin may fill a role in salt tolerance and/or osmoregulation, most probably in crustacea.

Another potential ecological role of V. cholerae toxin may be that of sequesting Na^+ for growth of the organism. When cholera toxin binds to the cell receptor of human intestinal epithelial

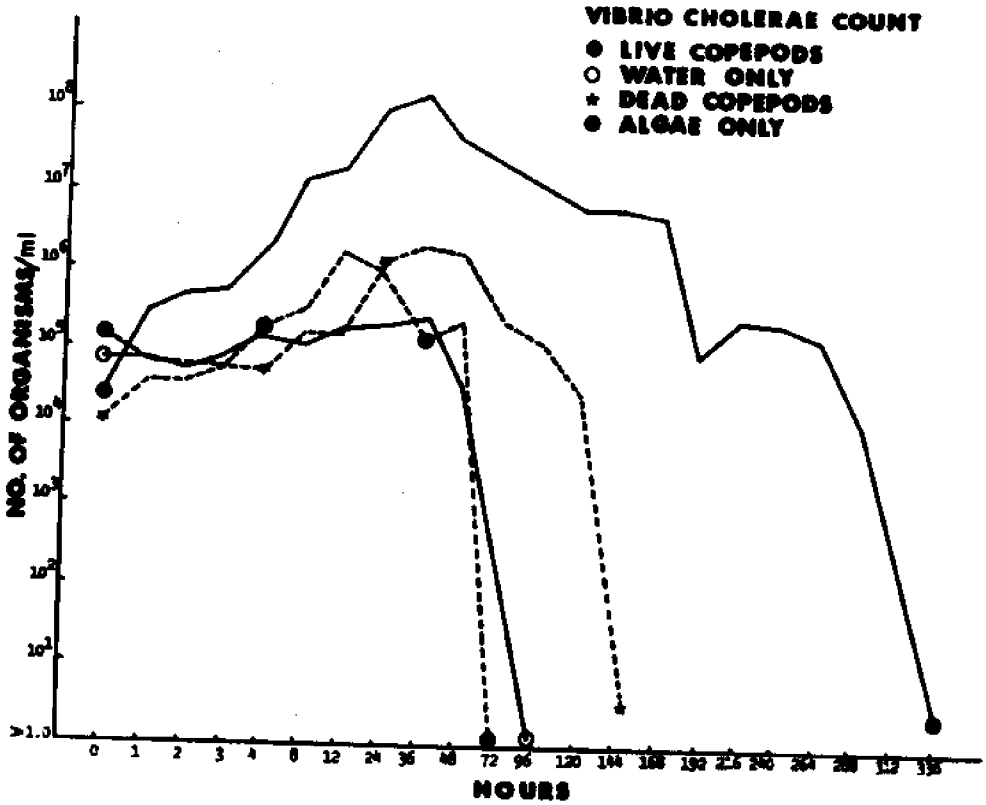

Figure 4. Comparison of culturable Vibrio cholerae counts in the presence and absence of copepods and algae.

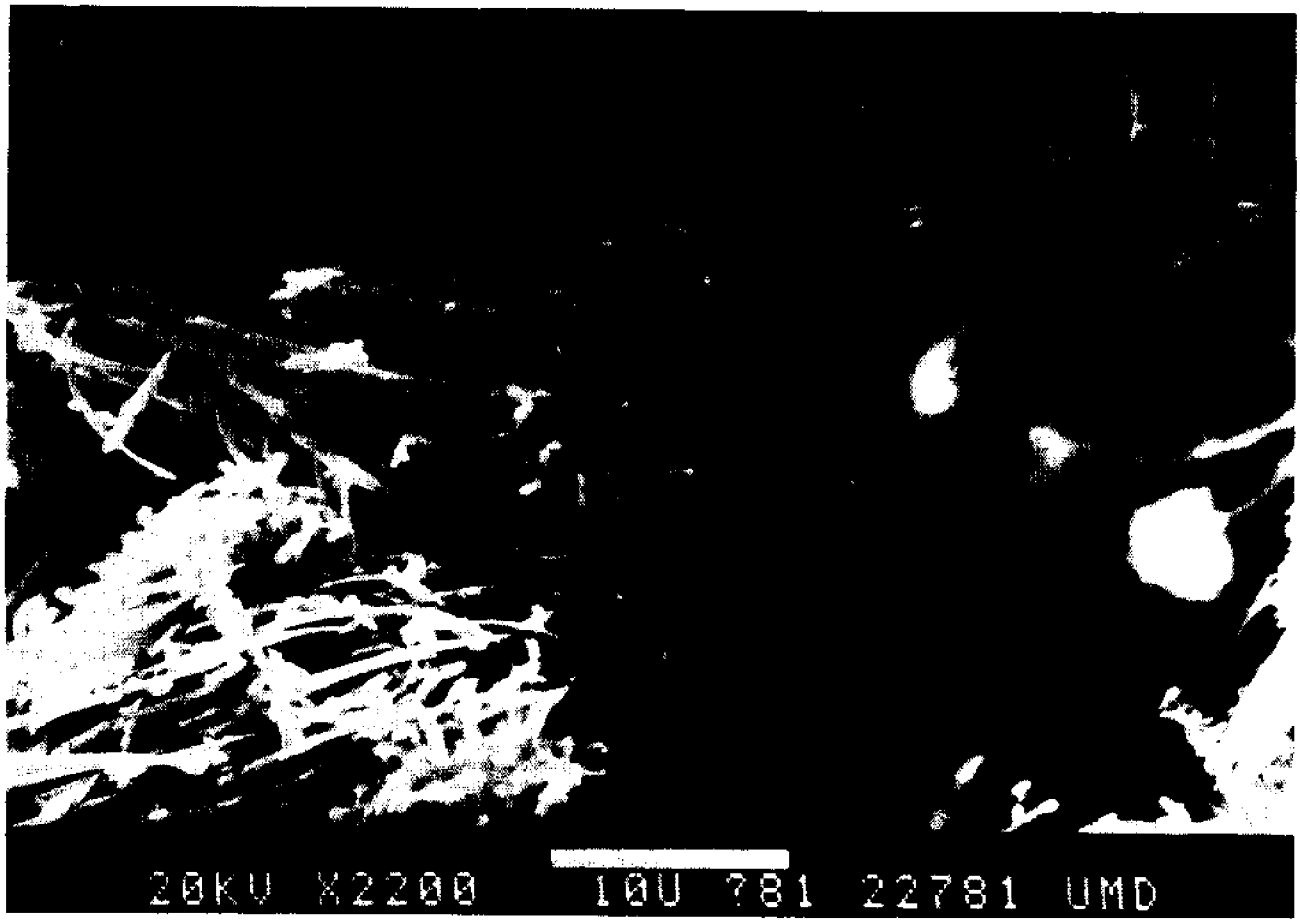

Figure 5. Attachment of *Vibrio cholerae* within the oral region of copepods.

Figure 6. Attachment of Vibrio cholerae to a copepod egg sac.

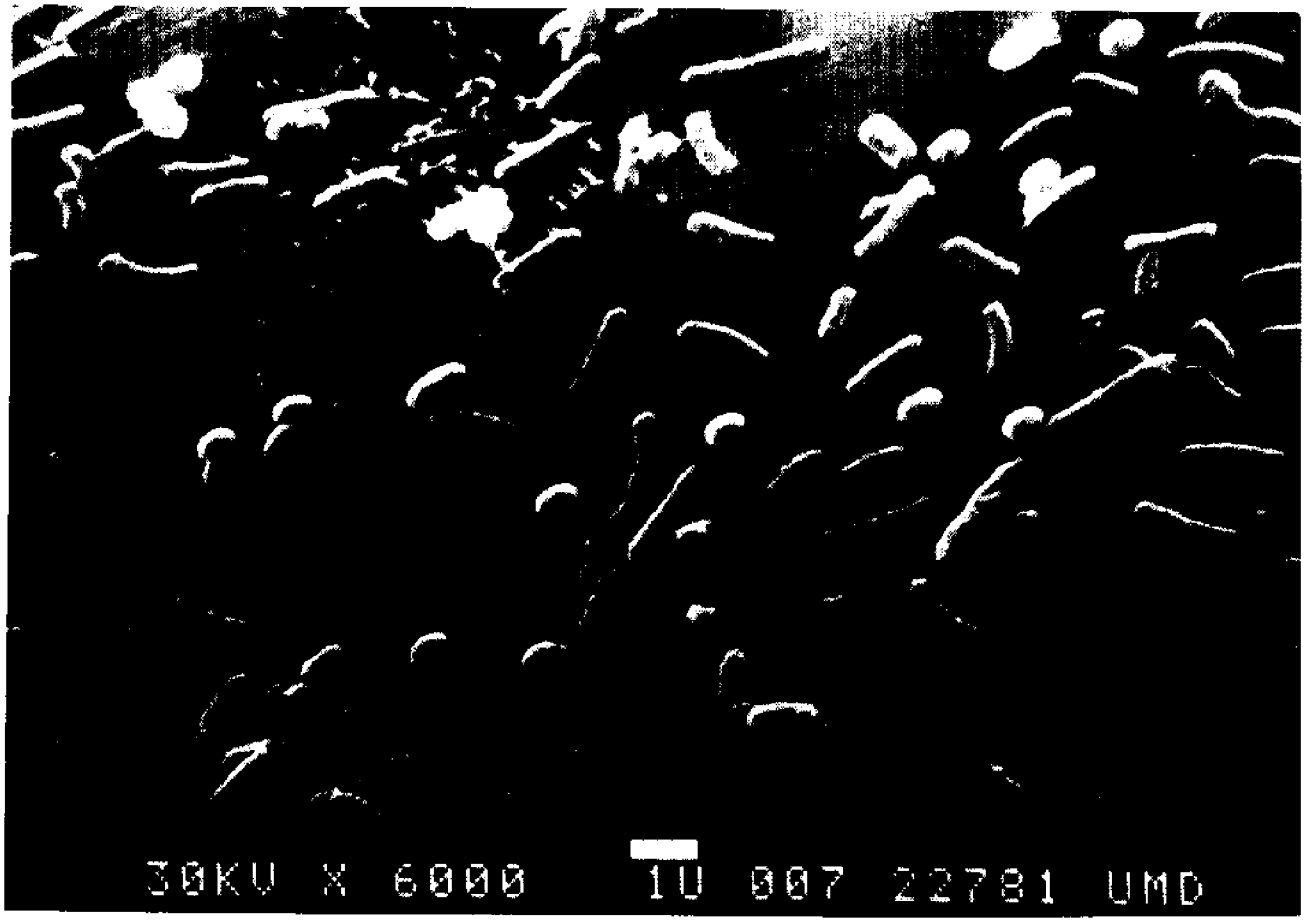

Figure 7. Attachment of Vibrio cholerae to the surface of a copepod egg sac.

cells, adenylate cyclase activity increases, which increases the level of cyclic AMP. The increase in cyclic AMP activity results in efflux of Na^+ from the cell, taking with it quantities of water, producing the symptoms associated with the disease in humans (18,20,29,30). Should cholera toxin affect epithelial cells of crustacea similarly, V. cholerae, in association with these organisms, would have a means of obtaining sufficient quantities of Na^+ from host organisms should the concentration in the environment fall below that required for survival and growth.

CONCLUSION

Based on the results reported herein, V. cholerae is concluded to be, as hypothesized by Colwell at al. (7), an indigenous member of the bacterial community of estuarine systems, not only from its requirement for Na^+ for growth, but also by its obvious preference for a salinity regime typical of a brackish water estuary. The distribution of V. cholerae would not be expected to be limited only to estuaries, since the need for an optimum salinity can be spared by a sufficiently high nutrient concentration (31). The results of this study demonstrate the need for new information from environmental surveys for V. cholerae to be combined with those obtained from laboratory-controlled microcosms to understand the role and function of V. cholerae in its natural environment.

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MICROBIAL QUALITY OF READY-TO-EAT SEAFOOD ITEMS AT RETAIL

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INTRODUCTION

Seafood products are known for the rapidity with which they undergo spoilage. Spoilage may be related to either tissue enzymes, microbial growth, or a combination of both (5). In addition to spoilage, seafood items may be involved in foodborne disease outbreaks associated with harvesting, processing or handling errors. In fact, about 9% of the foodborne disease outbreaks in the U.S. were due to consumption of shellfish and marine crustaceans (2).

Products sold as cooked or "ready-to-eat" are the most suspect regarding their potential for causing disease (4) and few published reports are available describing their microbial composition. The presence of low numbers of certain pathogens in products that will be cooked before eating are of less concern than if they were present in a ready-to-eat item. Therefore, in order to better understand the microbial quality of ready-to-eat seafood items, a survey was conducted in which 52 samples were analyzed for both indicator organisms and foodborne pathogens.

MATERIALS AND METHODS

Standard microbiological techniques were followed (1). Aerobic plate counts as well as yeast and mold counts were incubated at 25 C for 5 days. Salmonellae were determined by transferring a 25 g sample to lactose broth with incubation at 35 C for 24 hours. The sample was then selectively enriched in tetrathionate broth and transferred to plates of Bismuth Sulfite, *Salmonella-Shigella* and XLD agars for isolation. Samples were analyzed for *Vibrio parahaemolyticus* by innoculating 90 ml of Glucose Salt Teepol broth with 10 g of sample and incubating for 20 h at 25 C. Following enrichment, the sample was streaked to Thiosulfate Citrate Bile Salts medium. Sample pH was determined by preparing a 1:10 dilution in distilled water and obtaining the pH with an Orion Model 301 pH meter.

Both fresh and frozen samples were obtained at the retail level in Gainesville, Florida. Duplicate samples were obtained at time of purchase and analyzed separately. For presentation of data, values obtained from the two samples were averaged.

RESULTS AND DISCUSSION

All samples (Table 1) were negative for *Salmonella*, *V. parahaemolyticus* and *C. perfringens*. However, 46% of the samples were positive for *S. aureus*. While these counts are low, ranging from 4 to 40 per gram, the nature of these products and their intended use would dictate that they be free of *S. aureus*. A number of these products would be used in salads and spreads which

Table I. Microbial quality of 52 ready-to-eat seafood containing products.

Samples (duplicate)	pH	Yeast & Mold/ gram	Total Aerobic Plate Count/g	Coliform MPN/g	*S. aureus* MPN/g
Alaska Crab Meat & Shrimp	7.2	<10	40,000	<3	23
Alaska King Crab Legs	-	35	820	<3	6
Alaska King Crab Meat	7.2	<10	1620	<3	<3
Alaska Shrimp, Firm A	-	200	230,000	<3	4
Alaska Shrimp, Firm B	7.2	20	3200	<3	9
Caviar Spread	5.1	<10	765	<3	<3
Clam Dip:Sour Cream	4.7	<10	40	<3	<3
Cooked Shrimp	8.0	<10	1300	<3	4
Cream Cheese with Smoked Salmon	4.8	<10	150	<3	<3
Florida Lobster	-	62,000	750,000	1020	32
Herring in Cream Sauce	4.2	13	310	<3	<3
Herring in Wine Sauce	3.7	<10	30	<3	<3
Fresh Oysters (in can)	-	1010	780,000	480,000	20
Red Crab Meat	-	5	116,000	<3	<3
Sliced Smoked Salmon	6.4	8100	213,000	<3	<3
Shrimp Cocktail	-	<10	3430	<3	<3
Sliced Lunch Herring, Firm C	-	130	150	<3	<3
Sliced Lunch Herring, Firm D	4.2	<10	100	<3	<3
Smoked Conch	8.5	70	950,000	<3	40
Smoked Mackerel	-	<10	68,000	<3	<3
Smoked Mullet	6.1	650	4000	<3	<3
Snow Crab Claws	6.9	4100	59,000	9	9
Oysters in shell	6.7	900	635,000	150	9
Crab Meat, pasteurized	7.6	<10	30,500	<3	7
Smoked Mullet Spread	5.2	<10	450,000	2400	<3
Whipped Cream Cheese & Lox Smoked Salmon	4.7	30	75	<3	4

could be mishandled during and after preparation, permitting growth and possible toxin production. Several regionally produced items contained S. aureus which would indicate a need for improved plant sanitation and handling procedures.

Yeast and mold counts in most products were low; however, when they were elevated, the aerobic plate counts were also generally high. It would seem that a yeast and mold count of 62,000 org/g in cooked frozen Florida lobster is excessive under any conditions.

Aerobic plate counts in cooked products should be low (less than 10,000/g) regardless of existing recommended limits (3). However, many of the samples contained rather high numbers of organisms. Almost 30% of the samples exceeded 100,000 org/g and approximately 46% exceeded 10,000 org/g.

Dips, cocktails and pickled herring products distributed nationally were microbially of the best quality. One of the samples contained S. aureus and none contained coliforms. The reduced pH of these samples probably accounts for the low levels of microorganisms recovered in these samples. However, in two of the samples (caviar and mullet spread), the pH was above 5, increasing the potential for microbial growth.

Results from the analyses of raw oysters were not unexpected. Being an extremely perishable product kept on ice, both coliforms and aerobic plate counts were high. The high coliform counts can probably be attributed to growth of psychrotrophic species originally present in the sample.

The smoked fish samples were of reasonable quality; only one contained S. aureus and coliforms. Since information concerning the storage conditions of the smoked conch was not available, it is not possible to suggest a reason for the high aerobic plate count.

Of the products tested, it would appear that those of regional origin, e.g. Florida lobster, oysters, and mullet spread, were of the poorest microbial quality. Whether this is a reflection of poor in-plant sanitation or excessive display time cannot be determined. However, it does point out the need for increased regulation and educational programs for both producers and retail managers. The very nature and use of several of these ready-to-eat seafood items places them in a potentially hazardous situation if mishandled by the consumer (4).

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ISOLATION OF ORGANISMS OF PUBLIC HEALTH SIGNIFICANCE FROM CLAMS AND OYSTERS

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INTRODUCTION

"Salmonella is widespread in the natural environment as a result of contamination by faeces. In many areas salmonellae are common in animal and human communities and sewage entering seawater may contain large numbers of these organisms. Thus, mollusks and crustacean shellfish taken from polluted coastal waters may contain Salmonella." (5)

The above statement accurately describes the Salmonella problem as it is faced by the seafood industry today: an ever wider distribution of the organism, increased monitoring of the environment and an apparent increase in the incidence of salmonellosis. Salmonellae have been isolated from oysters (1,4) and are known to cause salmonellosis in humans following ingestion of contaminated shellstock. However, it is not clear as to the incidence of salmonellae in mollusks from approved areas of harvest. In order to gain additional insight into the salmonellae problem as it relates to mollusks harvested from approved areas in Florida, a study was undertaken involving two shellfish harvesting areas that were known to contain both clams and oysters in close proximity. The East Coast location was in the Intercoastal Waterway at Cresent Beach and the West Coast location was at the mouth of the Suwannee River.

MATERIALS AND METHODS

About forty to fifty shellfish were harvested at each sampling time. They were placed in a sanitized and insulated cooler, transported to the laboratory within three hours, and the analyses begun. No attempt was made to cool the samples because of the short duration from time of harvest to the initiation of the analyses. Thirty individual mollusks were analyzed for salmonellae from each location, while two composite samples (three or more) were prepared for aerobic plate count, coliforms, fecal coliforms, and Vibrio parahaemolyticus. Because of their large size, West coast clams were not composited. All analyses, except those for Salmonella, were performed in duplicate.

For Salmonella analyses, the samples were pre-enriched in lactose broth at 35C for 24 ± 2 hours with subsequent selective enrichment in both selenite cystine and tetrathionate broths. Bismuth sulfite, brilliant green, and XLD agars were used for isolations followed by transfer of typical colonies to TSI and LIA slants. Following isolation, all identification procedures followed those described in the Bacteriological Analytical Manual (2).

Aerobic plate counts were incubated at 25 C for five days using plate count agar. Analyses for Vibrio parahaemolyticus and coliforms followed the procedures outlined in the Bacteriological Analytical Manual (2). Identification procedures followed those found in Bergey's Manual (3).

Serological identification of selected Salmonella isolates was performed by the Florida Department of Health Laboratories in Jacksonville, FL.

RESULTS AND DISCUSSION

The isolation of Salmonella from 19 of the 120 clams and oysters was unexpected (Table 1). However, in reviewing the study, four factors were considered to be relevant to the outcome of this work: the samples were not refrigerated, they were analyzed within three hours of harvest, the aerobic plate counts were low, and whole individual samples were analyzed.

TABLE 1

Microbial profiles of clams and oysters harvested from two sites in Florida

	east coast		west coast	
	clams	oysters	clams	oysters
Salmonella	4/30	2/30	13/30	0/30
V. parahaemolyticus	+	+	+	+
Edwardsiella tarda	0	0	0	7/30
coliforms (per gram)	2	2	4	160
fecal coliforms (per gram)	2	2	0	4
aerobic plate count (per gram)	1100	800	220	5700

Refrigeration is known to have a deleterious effect on microorganisms, and because of this, no attempt was made to cool the samples. The time between harvest and processing was quite short (less than 3 hr.) with little opportunity for the samples to warm up, maintaining the total number of organisms low. A noninhibitory pre-enrichment technique was used with individual mollusks, allowing for resuscitation and outgrowth of any salmonellae present in the sample. This, coupled with very low levels of competing organisms, may have contributed to the high recovery rate. In addition, thirty individual animals were analyzed at one sampling period and it is possible, as in the case of the clams, that about 2400 grams of sample were analyzed at one time.

It is not uncommon during a Salmonella analysis to recover Edwardsiella tarda. E. tarda is an opportunistic pathogen that has been implicated in gastroenteritis in humans (3). Its isolation from the West coast oysters during the Salmonella analysis was attributed to contamination of the harvest area by large numbers of water birds that feed in the area during low tide.

Vibrio parahaemolyticus is widely distributed in the marine environment and its isolation from these samples was expected. Both duplicate samples analyzed from each group of clams and oysters yielded this organism.

Aerobic plate counts were low, indicating that pollution of the harvest waters was probably minimal, however West coast oyster meat samples contained excessive coliforms according to accepted quality standards (5). Interestingly, these were the only samples which did not contain Salmonella (Table 1).

The isolation of a number of uncommon salmonellae serotypes (Table 2) from four groups of samples would indicate that a single source of pollution was not involved at each sampling site. The highest level of contamination was found in the West coast clams harvested from an area relatively unaffected by development. The contamination was probably due to waterbirds and animals abounding in the area. Regardless of the source, these results clearly point out the wide distribution of salmonellae in the estuarine environment.

TABLE 2

Salmonella serotypes isolated from Florida mollusks

S. java

S. tallahassee

S. braenderup

S. muenchen

S. anatum

S. bareilly

The actual level of contamination may be quite low. Possibly one organism per 100-200 gram of sample, a level which may not be infectious under most conditions and easily missed when analyzing a composite sample. It would appear that until a number of questions regarding the possible source of contamination, methods of harvesting and handling as well as analytical procedures are answered, these results should be interpreted cautiously.

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PRELIMINARY REPORT ON A RAPID METHOD FOR THE DETECTION AND CONFIRMATION OF FECAL COLIFORMS (E.COLI) FROM SEAFOODS

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INTRODUCTION

The importance of Escherichia coli as an indicator of fecal pollution has long been recognized. E. coli is often preferred over the coliform group as an indicator because it is more specific and more reliably reflects fecal origin. Current detection assays for E. coli are based on the properties of acid or gas production from lactose. Some of the accepted methods include the membrane filter method with mFC medium, the Most-Probable-Number method involving a presumptive test followed by a confirmed test and the pour plate method using Violet Red Bile (VRB) agar. All these tests are laborious and time consuming, some requiring 48 - 96 h to complete.

The detection assays that we have developed for immediate confirmation of E. coli are based on the presence of B-D-glucuronidase activity. Previously, Kilia and Bulow (10) formulated an agar medium to detect the production of glucuronidase by E. coli by using a chromogenic substrate 4-nitrophenyl B-D-glucopyranosiduronic acid. Others used sodium phenophthalein glucuronide as the substrate (2,6, 11). The use of the fluorogenic substrate, 4-methyl umbelliferone B-D-glucuronide (MUG), to detect glucuronidase was first reported in 1955 by Mead et al. (13). In 1973, Dahlen and Linde (5) incorporated MUG into a non-selective medium to screen for bacterial glucuronidase. When the substrate is hydrolyzed, 4-methyl umbelliferone was liberated; this product is stable and fluoresces under long-wave UV light.

Production of glucuronidase by bacteria appears to be fairly specific. Kilian and Bulow (9) tested 633 Enterobacteriaceae; 97% of the E. coli and 50% of the Shigella tested were positive for glucuronidase; all other Enterobacteriaceae were negative. LeMinor (12) reported that of 4000 strains of Salmonella tested, 30% possessed glucuronidase. We tested 75 cultures of E. coli and found that 96.5% were positive, correlating well with the results of Kilian and Bulow (9). A fair percentage of Salmonella (17%) and Shigella (40%) tested also produced glucuronidase. In addition, several strains of Enterobacter, Citrobacter, Klebsiella, Proteus, and others were also tested; none of these produced glucuronidase(7). Although the production of

glucuronidase was not absolutely specific for E. coli, the fact that Salmonella and Shigella also produce this enzyme would not significantly alter the sensitivity or the purpose of the assay. Recently, Feng and Hartman (7) reported that most of the strains they tested produced a B-D-glucuronidase that will cleave 4-methylumbelliferone-B-D-glucuronide to produce a fluorogenic end product. Pure or mixed cultures containing E. coli produced fluorescence under long-wave UV light, after incubation for 4 hrs (most strains) to 24 hrs (a few weakly positive strains).

The possibility of using 4-methylumbelliferone-B-D-glucuronide (MUG) directly in existing coliform detection methodologies using seafood samples was investigated.

MATERIALS AND METHODS

Samples. Seafood samples were obtained from local retail seafood markets in the Madison, Wisconsin area. Fresh finfish, fresh shellfish, frozen fish, frozen shellfish and breaded seafoods were bought and transported to the laboratory. Samples were tested the same day of purchase.

Media. Lactose Broth (LB), Violet Red Bile Agar (VRB), M-Endo Broth with and without 100 μg/ml of 4-methylumbelliferone-B-D-glucuronide (MUG) (Sigma Chemical Co., St. Louis, MO), EC Broth, Eosin Methylene Blue (EMB) and Standard Methods Agar (SMA) were used. All the media were manufactured by GIBCO Laboratories (Madison, WI).

Analyses of Seafood Samples. Figure 1 shows a schematic diagram of the methodology used in testing the coliform content of the seafood samples. Samples were blended in a Waring Blender at low speed for two minutes. Appropriate dilutions were made using Buttersfield Phosphate Buffer. Aerobic plate counts were done using Standard Methods Agar with 0.5% NaCl added. Plates were incubated at 20 C for 5 days. MUG was dissolved in water, filter sterilized and added to the media at a final concentration of 100 μg/ml. With the membrane filter M-Endo Broth technique, MUG was added directly to the broth. Following incubation at 35 C, E. coli appeared as fluorescent colonies on the filter. With the VRB method, MUG was incorporated into the VRB overlay. E. coli colonies were readily distinguished from non-E. coli colonies by the appearance of a fluorescent halo around the colonies. This enabled both actual coliform count and a specific E. coli count to be made by using the same plate in a one-step procedure. In the most-probable-number (MPN) method, MUG was added to Lactose Broth tubes. Here the presence of E. coli was reflected by the appearance of fluorescence throughout the tube. Thus, the LB- MUG provided a presumptive coliform enumeration based on gas formation, then provided an immediate confirmation for E. coli based on fluorescence. Lactose Broth without MUG incubated at 35 C for 24 hrs, Brilliant Green Bile Broth (BGLB), EC Broth were used as control. Positive EC Broth tubes were streaked onto Eosin Methylene Blue (EMB) plates for confirmation of E. coli.

All analyses were done twice in duplicate.

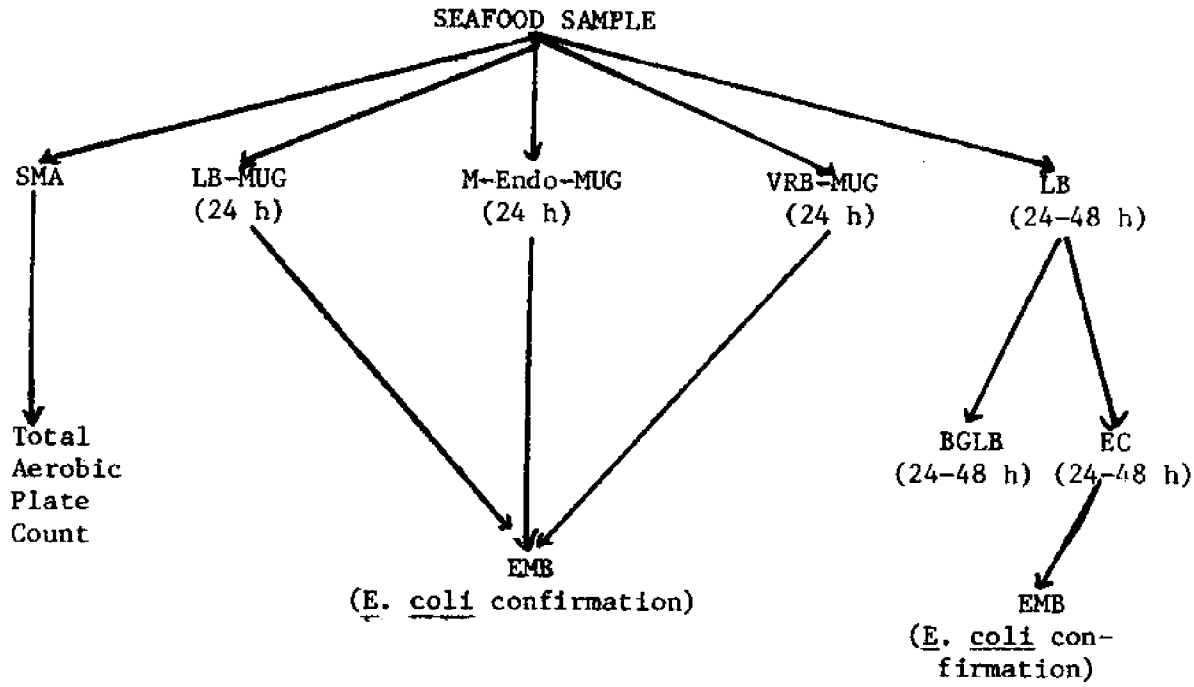

FIGURE 1. Methodology used for testing the coliform content of selected seafood samples.

RESULTS AND DISCUSSION

For many years, the coliform group was the mainstay of the sanitarian's tools for detecting the presence of fecal contamination in the aquatic environment. The high specificity of E. coli as an index of the presence of fecal contamination and the preliminary epidemiological evidence that it may be the best bacterial indicator for assessing health hazard risk in foods and water.

In order to determine the sensitivity of the LB-MUG assay, cell counts were correlated with time of appearance of fluorescence. With an inoculum of approximately 1×10^4 organisms/ml, fluorescence was observed in 4 h. Detectable gas evolution at this same inoculum level did not occur until approximately 9 h. Also, if one viable cell was present initially, fluorescence was detected within 20 h. Therefore, the sensitivity of LB-MUG was good, and the results were available much faster than when checking for gas evolution.

Using the LB-MUG method, 5 water samples were examined. Each sample was enumerated for total coliforms (gas), fecal coliforms (fluorescence), and a comparative fecal coliform count was also done by using the membrane filter-mFC method with incubation at 45.5 C. Table 1 shows the average counts of the 5 water samples. Based on fluorescence, 5% of the total coliform count detected was E. coli. The mFC method was only able to detect 2.3%; therefore, the efficiency of LB-MUG was twice that of the membrane filter-mFC method.

Five different types of seafood categories were examined by using the LB-MUG, VRB-MUG and M-Endo-MUG method. Tables 2-4 show the average counts of four replicates for each sample, and the percentage of E. coli present based on fluorescence. The confirmatory test results for the presence of E. coli based on fluorescence are currently being done. All three assays enumerated the E. coli's present in the seafood samples examined are within the ranges shown by Jay (8).

One of the problems commonly encountered during seafood analysis is the presence of stressed or injured cells. Factors such as heating, freezing, drying, or the presence of toxic chemicals such as salt or chlorine may injure cells (1,3,4,14). Stressed cells have the ability to recuperate and grow on nonselective media, but not on selective media. The ability of LB-MUG to detect heat-stressed cells was examined by Feng and Hartman (7). A culture of E. coli was heated until 99% were injured: these grew on Trypticase Soy Agar (TSA), but not on VRB agar. The LB-MUG system (fluorescence) was far superior to the VRB-2 method in detecting heat-injured cells. This was expected, since LB is a relatively nonselective medium. Perhaps this is one of the reasons why the LB-MUG resulted in higher recoveries of E. coli from the seafood samples tested. The effect of freezing and drying should be further studied.

It appears that the LB-MUG, VRB-MUG and M-Endo MUG assays work relatively well in both water and seafood analysis, providing a total coliform count, and also a reasonably reliable E. coli count. These assays are sensitive--able to detect the presence of one viable cell, are fairly efficient in detecting heat-injured cells, can detect the

LB-MUG					
GAS	(%)	FLUORESCENCE	(%)	mFC	(%)
2.0×10^5	(100)	1.0×10^4	(5.0)	4.5×10^3	(2.3)

Table 1. Enumeration and percentage recovery of fecal coliforms (fluorescence) from water by the LB-MUG assay and the mFC method. (Average of 5 water samples).

SAMPLES	NUMBER	LB-MUG GAS	LB-MUG FLUORESCENCE	%
Fresh finfish	5	4.5×10^2 (100%)	8.0×10^1	(17.7)
Fresh shellfish	4	5.8×10^2 (100%)	1.6×10^1	(2.7)
Frozen fish	7	2.5×10^2 (100%)	2.6×10^1	(10.4)
Frozen shellfish	5	3.5×10^2 (100%)	1.0×10^1	(2.8)
Breaded seafoods	9	3.9×10^3 (100%)	1.0×10^3	(25.6)

[a]Average of four replicates/sample

Table 2. Enumeration of total coliforms (gas) and fecal coliforms (fluorescence) and the percentage of *E. coli* present in seafood samples using the LB-MUG assay.[a]

SAMPLES	NUMBER	VRB-MUG		
		TOTAL	FLUORESCENT HALO	%
Fresh finfish	5	4.5×10^2	2.0×10^1	(7.1)
Fresh shellfish	4	4.6×10^2	2.8×10^1	(6.1)
Frozen fish	7	1.0×10^2	1.0×10^1	(1.0)
Frozen shellfish	5	2.8×10^2	1.2×10^1	(4.3)
Breaded seafoods	9	2.8×10^3	3.5×10^2	(12.5)

[a]Average of four replicates/sample

Table 3. Enumeration of total coliforms (gas) and fecal coliforms (fluorescence) and the percentage of E. coli present in seafood samples using the VRB-MUG assay.[a]

SAMPLES	NUMBER	M-Endo-MUG TOTAL COLONIES	M-Endo-MUG FLUORESCENT COLONIES	%
Fresh finfish	5	2.5×10^2	1.5×10^1	(6.0)
Fresh shellfish	4	4.0×10^2	1.5×10^1	(3.8)
Frozen fish	7	1.0×10^2	0.9×10^1	(0.9)
Frozen shellfish	5	2.5×10^2	1.0×10^1	(4.0)
Breaded seafoods	9	2.2×10^3	2.8×10^1	(12.7)

[a]Average of four replicates/sample

Table 4. Enumeration of total coliforms (gas) and fecal coliforms (fluorescence) and the percentage of E. coli present in seafood samples using the M-Endo-MUG assay.[a]

presence of non-gas producing strains of E. coli, and can probably detect E. coli in the presence of large numbers of competing bacteria. Any of the assays described in this study can be routinely used to discriminate seafood samples with large numbers of E. coli (fecal contamination) present.

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EVALUATION OF VARIOUS MEDIA FOR THE RECOVERY OF ENTEROCOCCI IN FRESH AND FROZEN SEAFOOD FROM THE GULF OF MEXICO

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INTRODUCTION

The fecal streptococci in foods are usually enumerated as an aid to the estimation of the sanitation practices in food factories, particularly regarding inadequate cleansing and disinfection of factory surfaces, to support other indications of fecal contamination, and as an index of general microbiological quality. Their capacity to cause food-poisoning is still in doubt. There is also continuing doubt about the significance of the pathological and ecological differences between the several species.

Fecal streptococci are defined as those streptococci which belong to Lancefield serological group D. These organisms are consistently present in significantly high numbers in fresh fecal excreta and with the exception of S. bovis and S. equinus, usually satisfy the "Sherman criteria" for the so-called "enterococci" (growth at 10 C, 45 C, at pH 9.6 and in 6.5% NaCl, reduction of 0.1% methylene blue in milk and survival 60 C for 30 minutes). Table 1, as presented by Hartman et al., (2) summarizes the various species of this group.

Various reports have appeared in the literature concerning methods and media for the isolation and enumeration of enterococci. Up-to-date, probably over seventy-five media are known to us for the selective recovery of these organisms. In 1972 Pavlova et. al.(6) published a list illustrating the complexity of selective enumeration of this group from a heterogeneous microbial population. The more important inhibiting substances which have been used in selective media for these organisms include: sodium azide, thallium salts, potassium tellurite, crystal violet, citrate, antibiotics, sodium taurocholate, bile salts, tween 80, selenite, tetrathionate, sodium chloride and phynethyl alcohol. Most of the selective media depend on the incorporation of either sodium azide, thallium salts or potassium tellurite. To increase the selectivity, these substrates may be used in conjunction with dyes (particularly crystal violet), antibiotics, incubation at 45 C, esculin, 2,3,5 triphenyltetrozolium chloride (TTC), tween 80, citrate, sodium taurocholate, sodium chloride, or others as shown by Pavlova et.al.(6).

FECAL STREPTOCOCCI		
Enterococcus Group	*S. fecalis*	Group D
	S. fecalis var. *liquefacians*	
	S. fecalis var. *zymogenes*	
	S. faecium	
	S. faecium var. *durans*	
Viridans Group	*S. bovis*	
	S. equinus	
	S. mitis	
	S. salivarus	

[1]Hartman et. al. (2).

Table 1. Definitions of the terms "enterococci", "group D streptococci" and "fecal streptococci" based on *Streptococcus* spp. belonging to each group.[1]

Two major studies, both finished over seven years ago, looked at the recovery of streptococci using various media(6,7). Both studies were probably a result of previous works and suggestions by Hartman et.al.(2). In a comprehensive review on the effects of various media ingredients on the recovery and growth of streptococci from foods, Hartman and co-workers concluded that further work in this area should be directed toward a comparison of media and methods using the same samples. Pavlova et.al. (6) reported on the selectivity of five media containing different classes of inhibitory agents to isolate fecal streptococci from feces, sewage, and foods. The lowest incidence of fecal streptococci was observed with m-Enterococcus and Azidesorbitol agar, while KF and Pfizer Selective Enterococcus agar gave the highest recoveries. Most foods examined yielded low numbers (approximately 10^2/g) of fecal streptococci. Two years later, Oblinger(7) used nine liquid and solid media to isolate and/or enumerate streptococci using 30 food samples. Streptococci were recovered from all of the samples surveyed. Azide Blood agar gave the highest recovery of streptococci and also allowed the observation of hemolytic reactions. Streptosel, KF streptococcus, and m-Enterococcus agars were used, with the latter giving the lowest recovery among the agars. Azide Dextrose, KF, and Streptosel broths gave similar recoveries from most samples.

Many changes in media formulation and composition have transpired in the last seven years. In addition, new media have been developed. A new medium, gentamicin-thallous-carbonate (GTC), was recently described by Donnely and Hartman(1) and Thian and Hartman(9). GTC was found superior to three other commonly used media for the determination of fecal streptococci in food, feces and water.

Although the role of enterococci in food poisoning is still somewhat questionable, researchers study this group because of their resistant nature and their presence is used to estimate the effectiveness of sanitation procedures and as an index of general microbiological quality. The purpose of this present study was to evaluate the selectivity of several commercially available media (most of them not previously evaluated) for the isolation and enumeration of enterococci from seafoods from the Gulf of Mexico. The criteria used in the appraisal of the selected media were: a) numbers of enterococci recovered; b) selectivity; c) incubation period required; and d) ease of differentiation and interpretation.

MATERIALS AND METHODS

Seafood Samples. Twenty-four seafood samples were obtained at seafood retail markets in Madison, Wisconsin. Fresh and frozen fish and shellfish obtained were all originally flown from seafood plants in the Gulf of Mexico (communication with seafood stores owners). In addition, breaded seafoods were also obtained from local markets.

Media. The following media were used: KF streptococcus Agar, Gentamicin-Thallous-Carbonate (GTC), CEM Agar, Selective Streptococci and Mf-Enterococcus with Mf-PYC enrichment. All the media used were obtained from GIBCO Laboratories, Madison, Wisconsin. Table 2 shows the typical ingredients of the media used in this study.

KF-Streptococcus Agar		GTC		CEM	
Peptone 180	10.0	Trypticase Soy Agar	40.0	Columbia CNA Agar	44.0
Yeast Extract	10.0	KH_2PO_4	5.0	Ferric Ammoniate Citrate	1.0
Sodium Chloride	5.0	$NaHCO_3$	2.0	Esculin	1.5
Sodium Glycerophosphate	10.0	Glucose	1.0	Mannitol	10.0
Maltose	20.0	Esculin	1.0	Phenol Red	0.034
Lactose	1.0	Thallous Acetate	0.5		
Sodium Azide	0.4	Ferric Citrate	0.5		
Bromcresol Purple	0.015	Tween 80	0.75ml		
Agar	20.0	Gentamicin Sulfate	25mg		
Aseptically- 10 ml 1% TTC					
Selective Strep		**Mf-Enterococcus**		**Mf-PYC**	
Peptone 140	10.0	Peptone 180	20.0	Nutrient Broth	4.0
Beef Extract	6.7	Yeast Extract	5.0	Peptone 140	12.0
Maltose	0.25	Dextrose	2.0	Peptone 100	10.0
Sodium chloride	5.0	Sodium Azide	0.4	Yeast Extract	6.0
Agar	15.0	Agar	10.0		
Nucleic Acid	6.0	Di-Potassium Phosphate	4.0		
Polymixin B Sulfate	200,000 Units	TTC	0.1		
Neomycin Sulfate	0.002g				

[a]The above formulations are typical, each production lot is adjusted to compensate for variables in and/or availability of raw materials.

Table 2. Typical formulas (ingredients[a]/liter) of the media used in this study.

Sample Preparation and Methodologies. Twenty-five gram samples of the seafoods were placed in sterile blender jars and diluted with 250 g of Butterfield's Phosphate buffer and blended at low speed for 2 min. Serial dilutions were prepared by transferring 11 ml aliquots into 99 ml of phosphate buffer dilution blanks. Plates were poured in duplicate and incubated at 35 C for the specified periods. For the membrane filtered samples, a 1/1000 dilution was filtered. Aerobic plate counts were done using Standard Methods Agar with incubation at 20 C for 5 days.

Figure 1 shows a schematic diagram of the methodology used in this study. KF and the membrane filtration media were incubated at 35 C for 48 hrs. All other media were incubated for 18-24 hrs. Representative number of colonies ($\sqrt{n}$, n = number of colonies on plates with 30-300 colonies) were selected from the various media and subjected to further identification using API 20S Streptococcus Kit (Analytal Products, Plainview, NY). All analyses were done twice in duplicate.

RESULTS AND DISCUSSION

Comparative Growth of Stock Cultures. To compare the ability of the selected selective media to recover specific species of *Streptococci*, six laboratory cultures were plated on five media. The results of a typical experiment are shown in Table 3. Except for the inability of *S. bovis* and *S. equinus* strains to proliferate or grow at all in most media except GTC, approximately the same numbers of colonies were recovered on each medium.

Seafood Sample Tests. *Streptococci* were recovered from all samples surveyed in this study. Overall, the presumptive enterococci counts obtained on CEM, GTC, KF and Selective Streptococci was not significantly different (α = 0.1 level). GTC gave the highest presumptive count in fresh fish, pre-cooked frozen shellfish, breaded fish and breaded shellfish samples. CEM gave the highest count for the fresh frozen fish samples and KF for the fresh shellfish samples. Table 4 summarizes the results of this section. Overall, the levels of enterococci found in the seafood samples were within the levels found by previous researchers(6,7).

Table 5 shows the specificity of the various media for the isolation of fecal streptococci. CEM and Selective Streptococci recovered more enterococci than the other media. GTC recovered more *S. bovis* and *S. equinus*. Donnely and Hartman and Thian and Hartman(1,9) reported the ability of GTC to recover non-enterococci than the other media. CEM proved to be a good selective medium for these groups of organisms. The colistin and nalidixic acid in the CEM formulation inhibit the growth of Gram-negative bacteria. Enterococci will grow on this medium and hydrolize the esculin to form a dark brown to black colored zone surrounding each colony.

The membrane filtration technique was cumbersome for some of the seafood samples and two media were required for the isolation of the enterococci. In addition, non-fecal streptococci were isolated at a relatively high rate (24%).

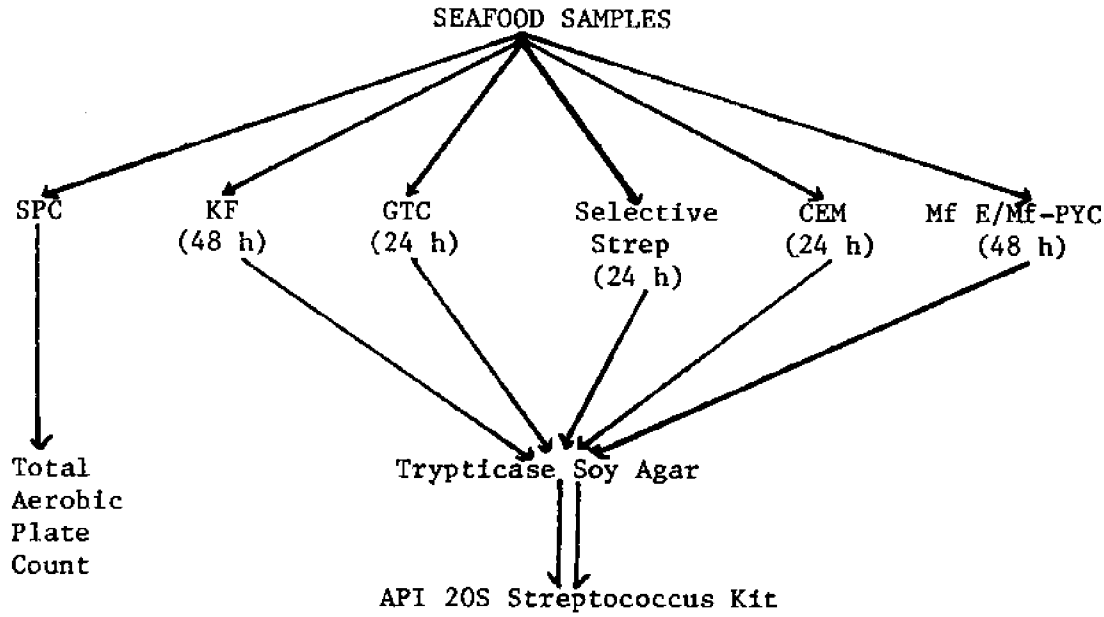

FIGURE I. Methodology for recovery of enterococci from seafood samples.

Medium	S. faecalis 63-1	S. faecalis WS	S. faecium 1	S. durans WLH	S. bovis 47	S. equinus 4-1
KF	304[a]	328	325	470	---[b]	---
GTC	298	312	370	408	210	180
CEM	410	276	470	500	26	15
Mf-E/MPYC	380	270	350	410	38	22
Sel Strep	372	350	350	340	---	---

[a]Mean counts ($x10^2$) were calculated from the average number of colonies on these plates of the appropriate dilution.
[b]Counts were less than 10^2/ml, and colonies were too small to count.

Table 3. Mean counts ($x10^2$) of control organisms per milliliter recovered on five selective media.

		Presumptive fecal streptococci per gram of seafood				
Samples	Number	CFM	KF	GTC	SelStrep	MfE/MfPYC
Fresh fish	4	$4x2x10^3$	$5.6x10^3$	$9.5x10^3$	$3.8x10^3$	$9.6x10^2$
Pre-Cooked Frozen Shellfish	5	$9.0x10^3$	$9.6x10^3$	$1.6x10^4$	$8.5x10^3$	$2.0x10^3$
Fresh Frozen Fish	4	$4.8x10^2$	$2.5x10^2$	$3.0x10^2$	$3.5x10^2$	$1.0x10^2$
Fresh Shellfish	4	$1.6x10^3$	$1.8x10^3$	$1.2x10^3$	$1.5x10^3$	$7.2x10^1$
Breaded Fish	3	$1.3x10^3$	$2.0x10^3$	$1.8x10^3$	$1.5x10^3$	$9.6x10^2$
Breaded Shellfish	4	$1.8x10^4$	$1.7x10^4$	$2.5x10^4$	$2.1x10^4$	$8.6x10^3$

Table 4. Comparison of presumptive fecal streptococci numbers per gram of various seafood samples on five selective media.

SELECTIVE MEDIA	Fecal streptococci		Non-Fecal Strep	Incubation Time (hr)
	Enterococci	Other		
CEM	56[a]	24	20	24
KF	45	18	37	48
GTC	45	38	17	24
Sel Strep	58	24	18	24
Mf-E/Mf-PYC	50	26	24	48

[a]Expressed as a percent of all isolates from the specific medium.

Table 5. Specificity of various media for the isolation of fecal streptococci.

Kenner et. al.(3) previously reported that KF-streptococcus agar yielded a high recovery of fecal and a low percent of non-fecal streptococci. However, Mossel et. al.(5), Mossel (4) and Raiband(8) reported that KF lacked selectivity. Results presented on this paper showed that KF agar was the least selective medium used. However, it still recovered a considerable number of enterococci.

Selective streptococci has never been used previously in the literature to recover enterococci. The medium proved to be very selective and allowed for the observation of hemolytic reactions without further subculturing. Selective Strep gave the highest enterococci count. Oblinger(7) reported that Azide Blood Agar allowed for the observation of hemolytic reactions and also gave the highest recovery of streptococci.

GTC agar allowed for an easy differentiation between fecal streptococci and other colonies by the presence of dark halos and by the colonial morphology. Donnely and Hartman(1) suggested that confirmation of enterococci colonies would be necessary with some types of samples. The results presented in this study show that with seafood samples, 83% of the colonies isolated were fecal streptococci.

Overall, the levels of enterococci present in the samples analyzed were within limits previously observed in the literature. Advantages of using GTC, CEM or Selective Streptococci for the recovery of fecal streptococci from seafoods include short incubation time (18-24 hrs), rapid enumeration and confirmation of isolates.

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VALIDITY OF BACTERIOLOGICAL STANDARDS FOR SHELLFISH HARVESTING WATERS

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ABSTRACT

Over 2.2 million acres are available for shellfish harvesting in Florida, but only 22% are in approved waters. Classification is based upon bacteriological standards using the number of fecal coliform bacteria present in the water. This research was undertaken to gain a better understanding of the ecology of Vibrios in Apalachicola Bay, in northwest Florida and to determine the relationship between Vibrios and fecal coliform standards in seawater, oysters, and sediments from both approved and prohibited shellfishing areas. Results suggest that the four major groups of *Vibrio* are a part of the normal ecosystem and not of human fecal origin. Non-01 *Vibrio cholerae* occurred at both approved and prohibited stations and showed little correlation with fecal coliform levels.

INTRODUCTION

The National Shellfish Sanitation Program was initiated to prevent the transmission of disease by edible shellfish. Through the cooperation of both state and federal governments, diseases transmitted by contaminated shellfish have been greatly reduced. Shellfish harvesting waters are approved if the fecal coliform bacteria most probable number (MPN) in the water does not exceed 14 per 100 ml of seawater in more than 10% of the samples tested. Fresh and frozen shellfish meats are approved for consumption if the fecal coliform MPN does not exceed 2.3 per gram of tissue and the 35 C aerobic plate count is no more than 500,000 per gram (7). Recently, however, some reports (1, 3) have suggested that some potentially pathogenic Vibrios may be endemic to the estuarine ecosystem and not necessarily of recent human fecal origin.

Currently in the state of Florida over 2.2 million acres are recognized as potential shellfish areas. Only 22% of this is approved for harvesting, 5% is conditionally approved, 13% is prohibited, and 60% is unclassified. Moreover, 88% of all Florida oysters are harvested from Franklin County which includes Apal-

achicola Bay.

There are four species of *Vibrio* which have been isolated from shellfish - or estuaries and which cause gastrointestinal and wound infections (Table 1). The most well known of these, *Vibrio cholerae*, has received widespread public attention since cholera in some countries occasionally has reached epidemic proportions. Since 1979 over 13 cases of oyster-associated cholera have occurred in Florida. For these reasons, this study was undertaken to gain a better understanding of the ecology of Vibrios in Apalachicola Bay, and to determine the relationship between Vibrios and fecal coliform standards in seawater, oysters, and sediments from both approved and prohibited shellfish harvesting areas.

MATERIAL AND METHODS

Eight stations located in approved, conditionally approved or prohibited shellfish harvesting areas in Apalachicola Bay (Fig. 1) were sampled eight times during 1980-81 for seawater, oysters, and sediment. Additional data were collected on salinity, temperature, dissolved oxygen, and total suspended load.

Samples for bacteriological analyses were dry-cooled to 8-12° C and processed within 6-8 hours of collection. The enumeration of fecal coliforms in seawater and oyster samples followed the mothods of Hunt and Springer (8) using A-1 broth.

The isolation and enumeration of *Vibrio parahaemolyticus*, *V. cholerae*, *V. vulnificus* and *V. alginolyticus* in seawater and oyster samples followed a modification of the Food and Drug Administration method (6). Modifications included aliquots of composite (3-5 individuals) oyster homogenates serially diluted tenfold from 10^{-1} to 10^{-6} using 1% peptone water, pH 7. One ml aliquots were then inoculated into alkaline peptone broth, pH 8.14 and incubated 8-12 hours at 35° C. Culture tubes slowing turbid growth were then streaked onto thiosulfate citrate bile salt sucrose agar (TCBS, Difco Laboratories) and incubated for approximately 20 hours at 35° C. Both green and yellow colonies were selected and screened for the presence of cytochrome oxidase. All colonies giving a positive cytochrome oxidase test were then tested biochemically using an API 20E system (Analytab Products, Plainview, N. Y.). All positives and negatives for *Vibrio sp.* were scored and an MPN calculated using a 3-tube series reference table.

RESULTS

Levels of fecal coliform bacteria in the water column at the 8 stations in Apalachicola Bay ranged from < 0.03 to 240 per 100 ml of seawater. Non-01 *Vibrio cholerae* ranged from 0 to 1400 per 100 ml. Station 29 in the totally prohibited area exhibited the highest levels of both fecal coliforms and non-01 *Vibrio cholerae*. Over 100 isolates of *Vibrio cholerae* were serotyped as non-01; *Vibrio cholerae* 01 was not isolated from any of the samples.

Organism Type	Principal Diseases	Principal Environments
Vibrio cholerae (non-01)	Gastroenteritis	man, estuaries & shellfish
Vibrio cholerae (01)		
classical biotype	Gastroenteritis	man, shellfish (?)
El Tor biotype	Gastroenteritis	man, shellfish
Vibrio parahaemolyticus	Gastroenteritis wound infection	estuaries & shellfish
Vibrio vulnificus	wound infection septicemia	estuaries & shellfish
Vibrio alginolyticus	wound infection	estuaries

Table 1. Major types of Vibrios and their associated diseases.

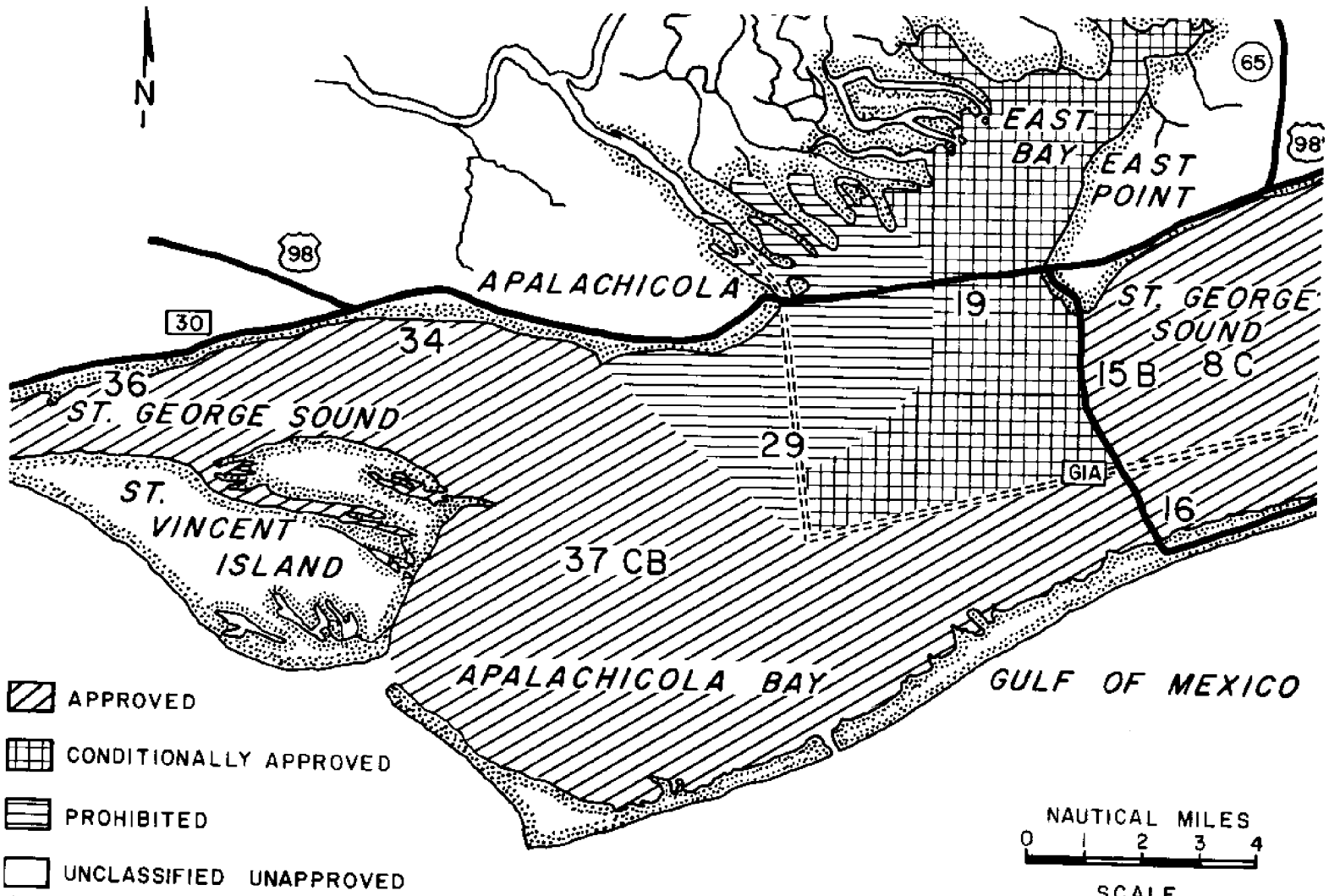

Fig. 1. Station numbers and locations in Apalachicola Bay.

Oysters from the eight stations contained fecal coliform bacteria which ranged from <0.03 to 4300 per g of oyster homogenate. The highest level of fecal coliforms occurred in one sample from station 29; however the oysters at both station 29 and 19 displayed consistently higher (> 45) levels than the oysters at the six approved stations.

All four species of *Vibrio* were found at some time during the study at both approved and prohibited stations. Table 2 shows the percentage of seawater samples from both approved and prohibited stations that contained non-01 *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio alginolyticus,* and *Vibrio vulnificus*. The number of occurrences of non-01 *Vibrio cholerae* at approved stations was only 2.4% higher than at prohibited stations. In contrast, the remaining three species of *Vibrio* occurred considerably more frequently at approved stations than prohibited stations.

The occurrence of these Vibrios showed no significant correlation with any of the water column data taken. Water temperatures ranged from 3.1 C to 29.5 C and salinity ranged from 0 $^{o}/oo$ to 32 $^{o}/oo$. The lowest salinities occurred at the prohibited stations which are greatly influenced by river flow. Salinity and temperature stratification were rarely observed since the water depth at all stations was less than 2 m and the bay is generally well mixed. Total suspended load varied seasonally from a low in December of less than 5 mg/l to a high in July of 116 mg/l. Dissolved oxygen levels varied seasonally but always remained close to saturation.

Levels of fecal coliform bacteria in oyster samples from the eight stations varied from < 0.03 to over 4300 per g of homogenate. Non-01 *Vibrio cholerae* ranged from < 0.03 to 280 per g of oyster homogenate. Although greater numbers of fecal coliforms occurred at prohibited stations, greater numbers of non-01 *Vibrio cholerae* were isolated from oysters collected from approved stations, particularly 15B, 8C and 16.

Levels of fecal coliform bacteria and *Vibrio sp.* in sediments were lower than, but were more closely correlated with, numbers in the oysters than with the numbers in the water column.

Vibrios were present in over 20% of the oyster samples analyzed from both approved and prohibited stations (Table 3). The percentage of occurrence for the four species was greater, however, at the approved stations than at the prohibited stations. Non-01 *Vibrio cholerae* was isolated from 13% more of the oysters from approved stations than from prohibited stations and *Vibrio vulnificus* was over 33% more common at approved stations.

DISCUSSION

In the present study, the presence of *Vibrio cholerae* (non-01), *V. parahaemolyticus, V. alginolyticus,* and *V. vulnificus*

	Approved	Prohibited
Vibrio cholerae	31.8	29.4
Vibrio parahaemolyticus	69.0	49.8
Vibrio alginolyticus	31.6	20.5
Vibrio vulnificus	54.7	20.5

Table 2. Percentage of seawater samples from approved and prohibited shellfishing areas in Apalachicola Bay that contained *Vibrio sp.*

	Approved	Prohibited
Vibrio cholerae	35.0	22.0
Vibrio parahaemolyticus	71.8	56.0
Vibrio alginolyticus	48.8	44.2
Vibrio vulnificus	55.5	22.0

Table 3. Percentage of oyster samples from approved and prohibited shellfishing areas in Apalachicola Bay that contained *Vibrio sp.*

showed little correlation with the standard fecal coliform MPN values for seawater (14 per 100 ml) and for oysters (2.3 per g). Current federal and state guidelines utilize fecal coliform counts as an indicator of fecal contamination and for the potential existence of human pathogens. For the most part, these standards have contributed greatly to the prevention of hepatitis, typhoid fever, and some forms of gastroenteritis. The results of this study in Apalachicola Bay, however, indicate that the standards are not valid for predicting the occurrence Vibrios.

Although much is known about the epidemiology of cholera (10), the ecology of Vibrios in general has only recently been investigated (9). The results of this study, as well as those of others (1, 3), suggest that non-01 *Vibrio cholerae* is endemic to the normal environment and is not a result of human fecal contamination. Like *Vibrio parahaemolyticus*, *Vibrio cholerae*, at least the non-01 serotype, may reside as an indigenous bacteria and is likely associated with plankton (4). The relatively large numbers of non-01 *Vibrio cholerae* observed (up to 1400 per 100 ml) in samples from Apalachicola Bay, in comparison to most samples from colder waters, may be indicative of the environmental preferences of the species.

Properly cooked seafoods pose minimal threat to causing a *Vibrio* gastrointestinal infection even if the bacteria are present in the uncooked meats. In 1978, *Vibrio cholerae* El Tor was isolated from 11 people who displayed signs of infection and who had eaten boiled and steamed crabs (2), but only non-01 *Vibrio cholerae* was isolated from the environment (5); contamination of the shellfish with the *Vibrio cholerae* 01 through handling and processing may have occurred. In Apalachicola Bay, only non-01 *Vibrio cholerae* was isolated and the reported gastrointestinal infections all resulted from non-01 organisms (R. Gunn, pers. comm.).

The prevention of *Vibrio* wound infections is more difficult. The majority of cases occur during occupational or recreational exposures to seawater, especially when the seawater is warm. Fortunately *Vibrio* wound infections do not occur very often and are readily treated with antibiotics.

It is possible that the incidence of *Vibrio* infections as a cause of morbidity and mortality in the United States is increasing only because the screening procedures used for isolating *Vibrios* are becoming more routine and physicians are becoming more cognizant of a potential problem. This research further demonstrates that the occurrence of *Vibrios* in the marine environment shows little relationship to the activities of man and that, unless *Vibrio cholerae* 01 which may be related to human feces is shown to be present, the bacteriological standards used to certify shellfish harvesting areas are not applicable to *Vibrio*-related diseases.

This does not in any way lessen the importance of present bacteriological standards for limiting the transmission of many pathogenic organisms by shellfish. However, the shellfish indus-

try has been and will continue to be in jeopardy as long as "cholera scares" are not put into their proper perspective. Further research is required on the ecology of Vibrios, particularly on non-01 and 01 *Vibrio cholerae*, in relation to the potential environmental influences on toxigenicity.

ACKNOWLEDGMENTS

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HYDROCARBON ANALYSIS OF SHRIMP FROM OIL POLLUTED WATERS

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INTRODUCTION

Due to the demand for petroleum products in the West, there has been a continuous increase in the transoceanic shipment of crude oil, as well as increased offshore exploratory drilling. This demand has led to several major spills such as the wreck of the "Torrey Canyon" off the coast of England which released 100,000 tons of crude oil (Blumer, 1969), the wreck of the "Amoco Cadiz" off the coast of France which released 200,000 tons of crude (Atlas, 1980) and the blowout of the offshore well in the Santa Barbara channel off the coast of California which released nearly 10,000 tons of crude oil (Blumer, 1969). In 1979 the Caribbean was hit by two major oil spills releasing almost 600,000 tons of crude oil. These spills were the blowout of the Ixtoc I well which released 500,000 tons (Atlas, 1980) and the double tanker collision between the "Atlantic Express" and the "Aegean Captain" which released 90,000 tons (Anon., 1979b). These spills plus uncountable smaller ones account for the increasing concern toward oil pollution of the oceans.

Ixtoc I, an offshore exploratory well in the Bahia de Campeche, blew out and caught fire on June 3, 1979 (Anon., 1979a). The well, located off the coast of Mexico, spewed out oil and gas for 295 days before it was finally capped. During the first two months the well lost an estimated 30,000 barrels of oil per day, then 10,000 barrels per day for three months and approximately 2,000 barrels per day until it was capped (Anon., 1980a). The world's greatest oil spill was finally capped on March 24, 1980 after spilling over 140 million gallons of oil into the Gulf of Mexico, most of which is unaccounted for (Anon., 1980b).

According to Blumer (1971), the marketing and eating of oil contaminated fish and shellfish, at the very least, increases the bodies burden of carcinogenic chemicals and may constitute a public health hazard. He also stated that oil and oil products must be recognized as poisons that damage marine ecology. Ehrhardt and Blumer (1972) estimated that from 5-10 million tons of petroleum hydrocarbons enter the oceans each year. Hydrocarbons enter the oceans from a variety of sources, including decaying phyto- and zooplankton, routine tanker and shipping operations, terrestrial runoff, atmospheric fallout, natural seepage, and from shipping and offshore well disasters (Ahearn, 1974).

In 1979, approximately 128 million pounds of shrimp worth $378 million were harvested in the Gulf of Mexico. This figure represents 61% of the catch for the entire United States. Of this total, Texas shrimpers landed 43.5 million pounds worth $157 million, making shrimp Texas' most valuable commercial fishery. Therefore, any interruption in the harvest of Gulf shrimp could have a severe impact on the local as well as national economy. One such potential interruption could be caused by oil pollution of the water in which shrimp are harvested.

The major problem is that all Gulf shrimp are harvested using a bottom trawl. Tar, or weathered oil, collects in the "cod end" of the trawl along with the shrimp. These tar balls then stick to the shrimp and can penetrate the shell causing off-flavor and off-odors that are unacceptable to the consumer. Hydrocarbons from the crude oil may also enter the food chain and accumulate in the lipid fraction of the shrimp (Teal, 1977).

Although work has been done on other marine species, very little has been done concerning shrimp. The general objective of this study was to fill the gap that exists regarding the levels of hydrocarbons in shrimp harvested from oil polluted waters. The specific objectives were (i) to determine, using gas-liquid chromatography (GLC), the level of hydrocarbons in shrimp harvested from oil polluted waters, and (ii) to determine, using GLC, the components of crude oil and it's derivatives; and compare this to the hydrocarbon components of shrimp harvested from oil polluted waters.

PROCEDURE

Hydrocarbon analysis of shrimp

Brown shrimp (Penaeus aztecus) were obtained from shrimp boats on the docks in the Corpus Christi, Texas area. The shrimp were spiked on a weight/weight basis with 1 PPM and 10 PPM crude oil, obtained from the Environmental Engineering Section of the Texas A&M campus. The shrimp were also spiked with 1 PPM commercial diesel oil and 1 PPM lubricating oil in order to reproduce possible bilge contamination.

The lipid and hydrocarbon fractions of the shrimp samples (300 gms shrimp/sample) were extracted using the method described by Bligh and Dyer (1959). This method uses a mixture of chloroform and methanol and was chosen due to its speed and quantitative aspects. The extract was then concentrated using a rotary evaporator. The hydrocarbon fraction was separated from the lipid fraction by column chromatography using florisil as the support medium and heptane as the solvent. This method was chosen due to its simplicity and effectiveness in separating the two fractions (Carroll, 1961). The eluate was concentrated using a rotary evaporator and dried with $Na_2(SO4)$.

The composition of the hydrocarbon fraction was determined using a Tracor 560 gas chromatograph with dual flame ionization detectors. Components were separated using a 6 foot, 1/8 inch OD glass column packed with 15% OV-1 and chromosorb W. The temperature was programmed starting at 90°C for 10 minutes, followed by a 4°C increase per minute until a final temperature of 225°C was reached. The flow rate for the nitrogen carrier gas was 16 ml per minute. Individual components were identified using standard hydrocarbon mixtures (C11, C12, C13 & C14 and also C12, C14, C16 & C18) obtained from AllTech Associates. By assuming crude oil contained paraffin hydrocarbons C11-C22, we identified peaks C15, C17, C19, C20, C21 and C22 from the crude oil "fingerprint" retention times.

Hydrocarbon analysis of oil and oil products

Diesel oil and lubricating oil were steam distilled in order to remove heavy molecules which may hang on the surface of the column. After steam distillation, they were concentrated on a rotary evaporator and injected

into the G.C. as described above. A large amount of low molecular weight compounds (<C10) were found in the steam distillate of crude oil. These compounds masked the n-paraffins from C11-C22. Therefore crude oil was dissolved directly into n-heptane and injected into the G.C. column. Since this was the last sample tested, it has no injurious effect on the column.

RESULTS AND DISCUSSION

The overall flow chart for the study can be seen in Figure 1. Due to difficulty encountered during the extraction phase using chloroform-methanol, it was not possible to quantitate any of the individual components in the samples. The problem was due to soluble protein that carried over during filtration of the extract. This protenaceous material would not allow complete separation of the chloroform phase (containing the lipids and hydrocarbons) from the methanol-water phase, resulting in the partial loss of the lipid-hydrocarbon fraction. In some samples the lipid volumn was only half what it should have been. Incomplete washing of the florisil column prior to chromatography resulted in minor contamination of the samples.

The hydrocarbon "fingerprint" for crude oil is found in Figure 2. This distinctly shows the n-paraffins from C11 to C22. All these peaks were found to be approximately the same size from the integration data (not shown) except for C14 and C17. This agrees with Koons (1965) who states that most petroleum oils show a smooth carbon number distribution among the n-paraffins (n-alkanes). The large peak at C17 could be attributed to the C19-isoprenoid pristane found in young (immature) oil and is usually resolved in the chromatogram with n-heptadecane (Ehrhardt and Blumer, 1972). The compound phytane, which is resolved in the n-octadecane peak was not found in this oil. A large peak was found proceeding C13, and smaller peaks were found preceeding C15 and C17. These peaks were not identified due to the limited equipment available.

The major peaks found in diesel oil (Figure 3) are C11, C12, C13 and C14, with lesser amounts of C15, C16 and C17. Figure 4 shows the components of lubricating oil. The major peaks were C14, C15, C16, C17, C18 and C19, with minor peaks of C11, C12, C13 and C20. Both of these figures show the peaks preceeding C13, C15 and C17 that were found in the crude oil "fingerprint". These peaks could be useful in determining whether a shrimp sample is contaminated. Neither of these two samples contained pristane nor phytane.

Figures 5 and 6 show the hydrocarbon "fingerprints" of frozen shrimp and frozen shrimp spiked with 10 PPM crude oil. It was assumed that the frozen sample was uncontaminated. Both samples showed the n-paraffins from C11-C20. The shrimp sample had major peaks at C11, C15, C17, C18 and C19. This agrees with the literature in that most biogenic hydrocarbons show an odd chain predominance. The peak at C11 could be a system contaminant, since this showed up in the control. The major peaks at C15 and C17 could be due to food chain magnification since Youngbood et al. (1971) found that C15 predominates in brown algae and C17 predominates in red algae.

The shrimp sample does not contain the peaks preceeding C13 and C15 that were found above, yet the spiked sample does. The shrimp sample also contains the C17-pristane and C18-phytane pairs. The spiked sample contains peaks at C21 and C22 that the other sample did not contain. From the integration data, there were major increases in the spiked sample over the frozen sample of C12, C13, C14, C18, C19 and C20; whereas C11, C15, C16 and

Figure 1. FLOW CHART

Shrimp Sample

↓

EXTRACTION

↓

Hydrocarbons and Lipids

↓

COLUMN CHROMATOGRAPHY

↓

Hydrocarbons

↓

GAS CHROMATOGRAPHY

↓

Hydrocarbon Fingerprints

Figure 2. CRUDE OIL

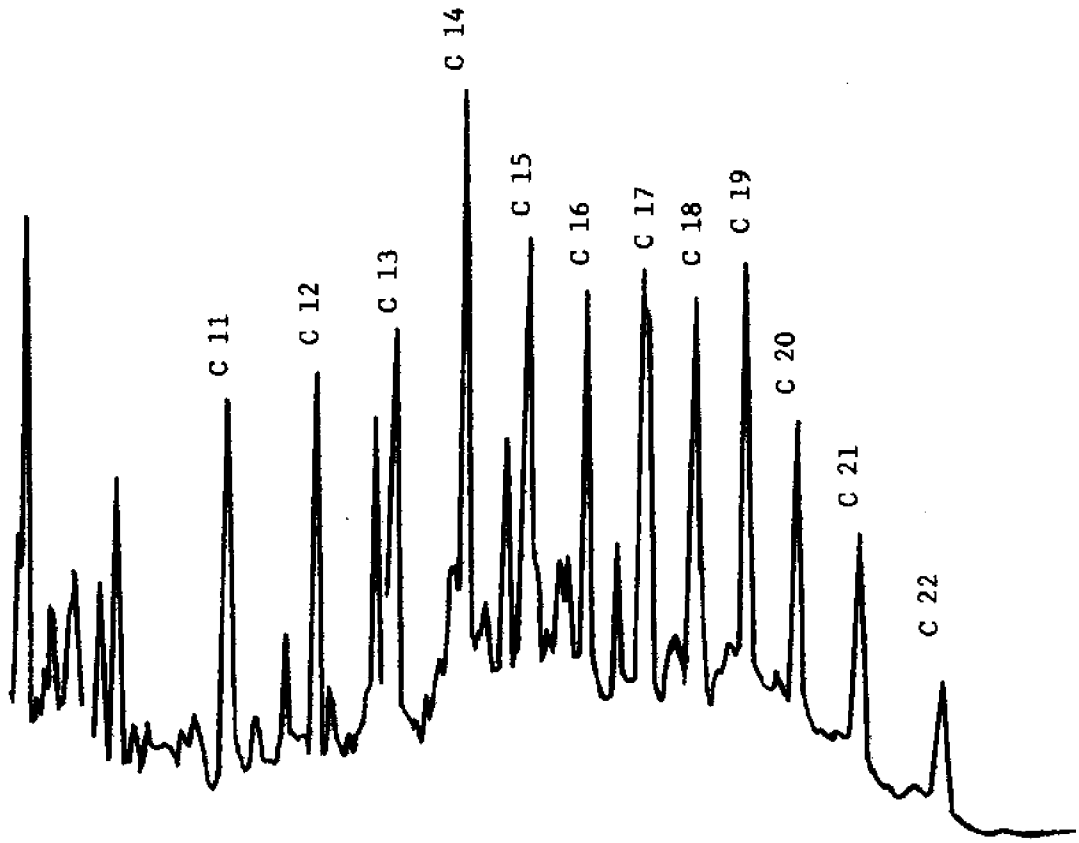


Figure 3. DIESEL OIL

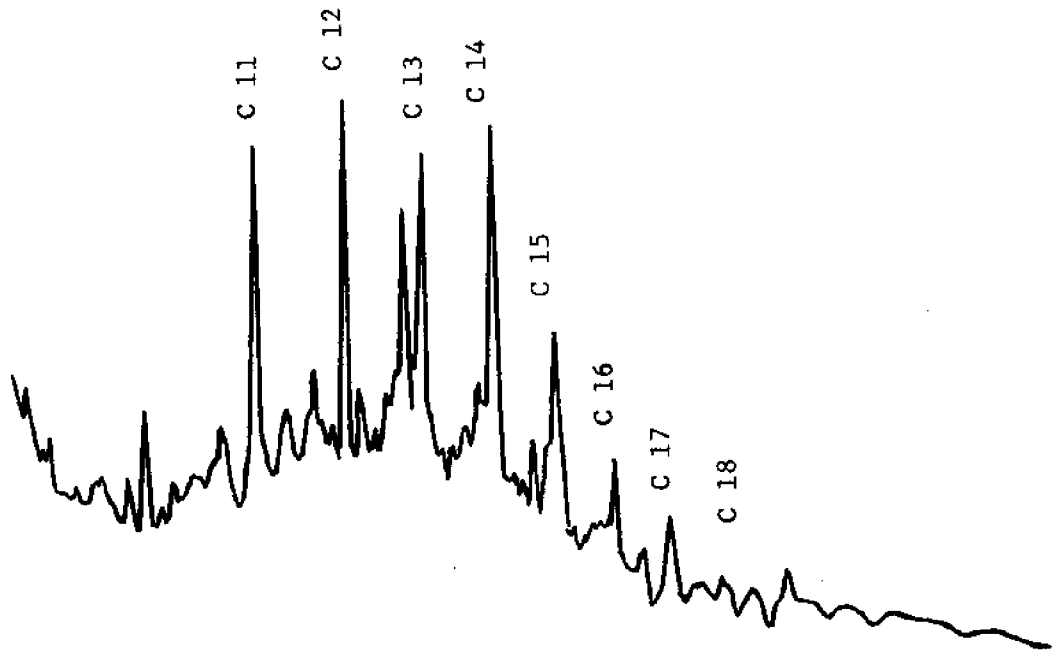


Figure 4. LUBRICATING OIL

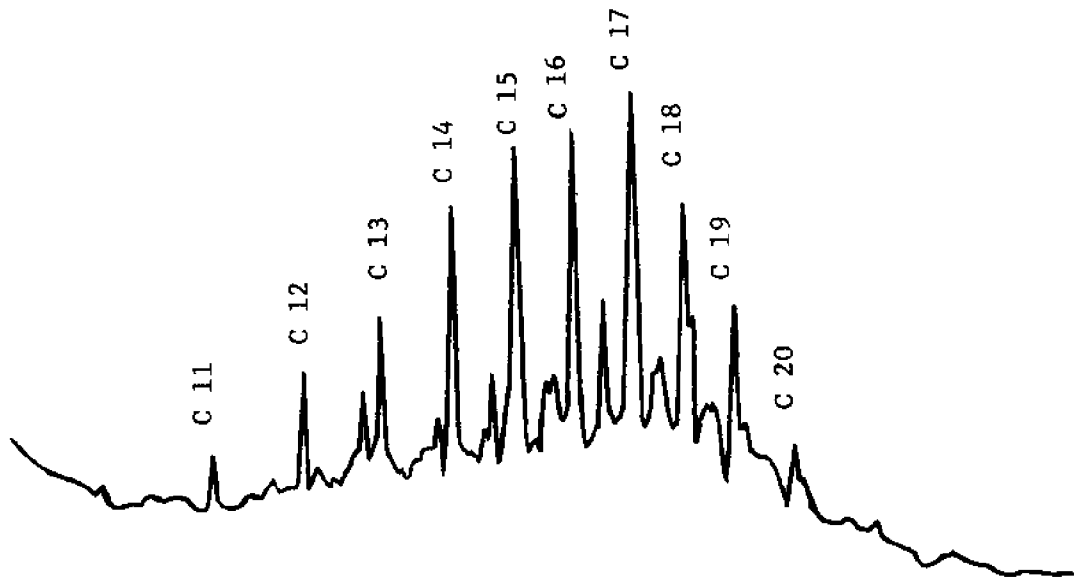


Figure 5. FROZEN SHRIMP

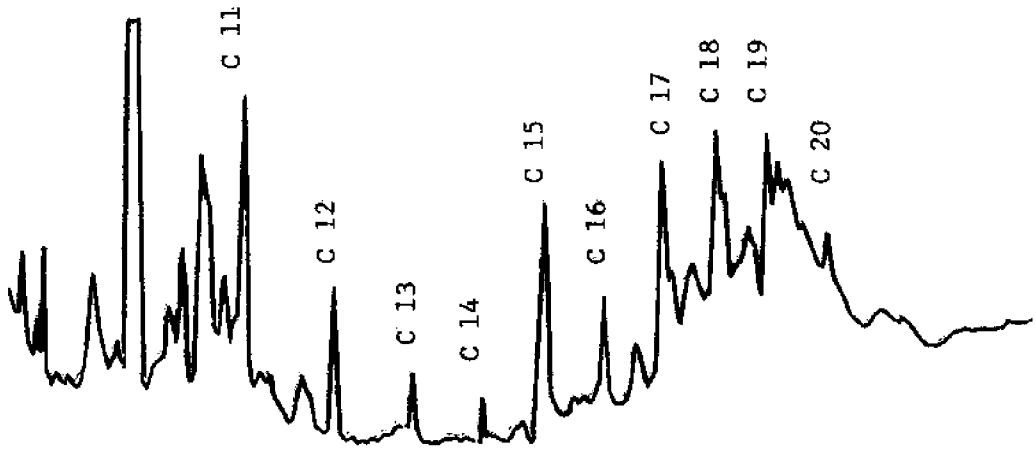
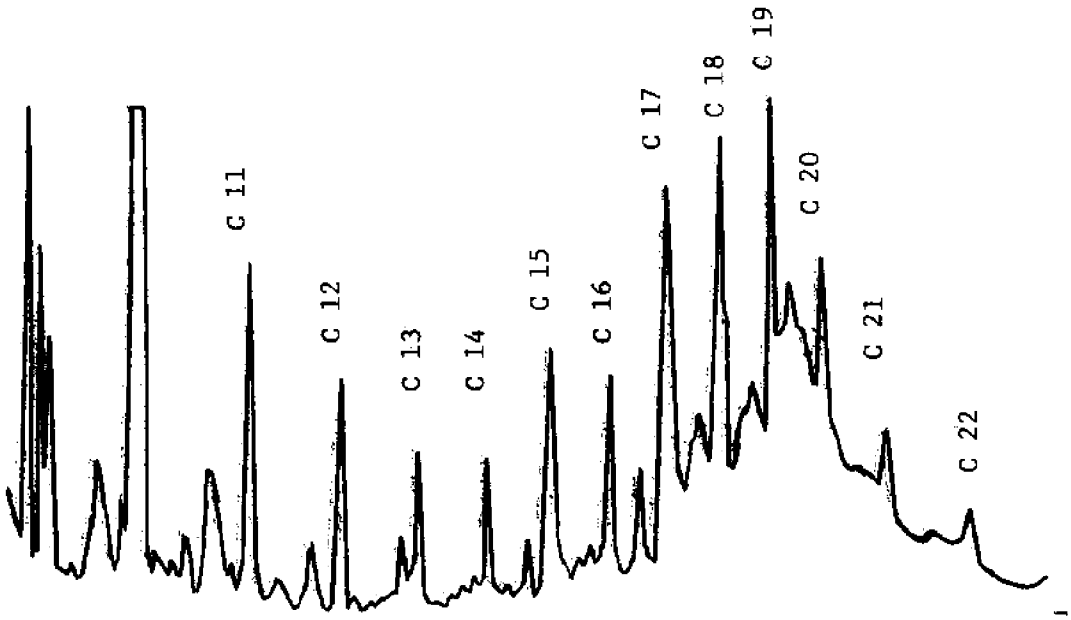


Figure 6. FROZEN SHRIMP SPIKED WITH 10 PPM CRUDE OIL



C17 did not show an increase. Since all the peaks from C11-C20 should have shown an increase, the two samples probably differed in the amounts of C11, C15, C16 and C17 that was originally present.

This paper describes a rapid and simple method for recovery of hydrocarbons from shrimp tissue, however it is impossible to differentiate between biogenic hydrocarbons and those from oil contamination. This could possibly be done by obtaining more data on weathered oil or tar balls since the crude oil used in this project was not weathered. Also more baseline data on shrimp biogenic hydrocarbons is needed. This would require shrimp that had not been exposed to any crude oil.

CONCLUSION

An acceptable hydrocarbon "fingerprint" of shrimp tissue can be obtained by using chloroform-methanol extraction, florisil chromatography for separation of the hydrocarbons from the lipids and gas chromatography. This method can be useful if good data is available to distinguish biogenic hydrocarbons from petroleum hydrocarbons.

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PROPERTIES OF PHENOLOXIDASE ISOLATED FROM GULF SHRIMP

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INTRODUCTION

Polyphenoloxidases are enzymes which catalyze the oxidation of phenolic compounds through diphenols and quinones to melanin a dark pigmented polymeric material. In shrimp, these enzymes also serve in the synthesis of compounds necessary for hardening of the cuticle after molting. The presence of these enzymes in shrimp also create problems for the industry. When shrimp tails are held on ice, black discolorations known as black spots are common and even though not related to eating quality it does affect the appearance and thus consumer acceptance. The objectives of this study were to purify the enzymes involved and to test potential inhibitors.

PROCEDURE

The purification procedure used was that described in the Proceedings of the Sixth Tropical and Subtropical Fisheries Conference. Liquid pressed from the shrimp heads was precipitated with cold acetone. Extracts form the precipitate were then cleaned and concentrated by ultrafiltration. Samples of this concentrate were eluted through a 2.6 x 36 cm Sephacryl S 200 column. After the gel filtration, fractions showing enzyme activity were pooled and introduced into a 5 x 30 mm diethylaminoethyl (DEAE) celulose column. The column was eluted stepwise by first lowering the pH to 6.2 and then increasing the buffer concentration in 0.05M increments) to 0.25M. At each step during the purification, samples of the enzyme preparation were analyzed by gel electrophoresis. Compounds with potential inhibitory activity were tested by measuring the effect of different combinations of substrate and inhibitor on the rate of reaction. Double reciprocal plots were prepared for each compound tested, and the inhibition mechanisms were determined on the basis of the reciprocal plots.

RESULTS AND DISCUSSION

Enzyme Purification

Figure 1 shows the pattern of protein elutions from brown shrimp extracts. The dotted line indicates the elution of the enzyme activity. Fractions with activity were pooled and further cleaned using the ion-exchange procedure. Table 1 summarizes the results of the purification, and Figure 2 shows diagrams of the gels prepared from samples of the enzyme preparations at each step on the purification procedure. Gels were stained for protein using Amido black 10B, while those stained for enzyme activity were stained by incubating in 5 mm DOPA (pH 6.2) until a definite dark band appeared at the position of the enzyme.

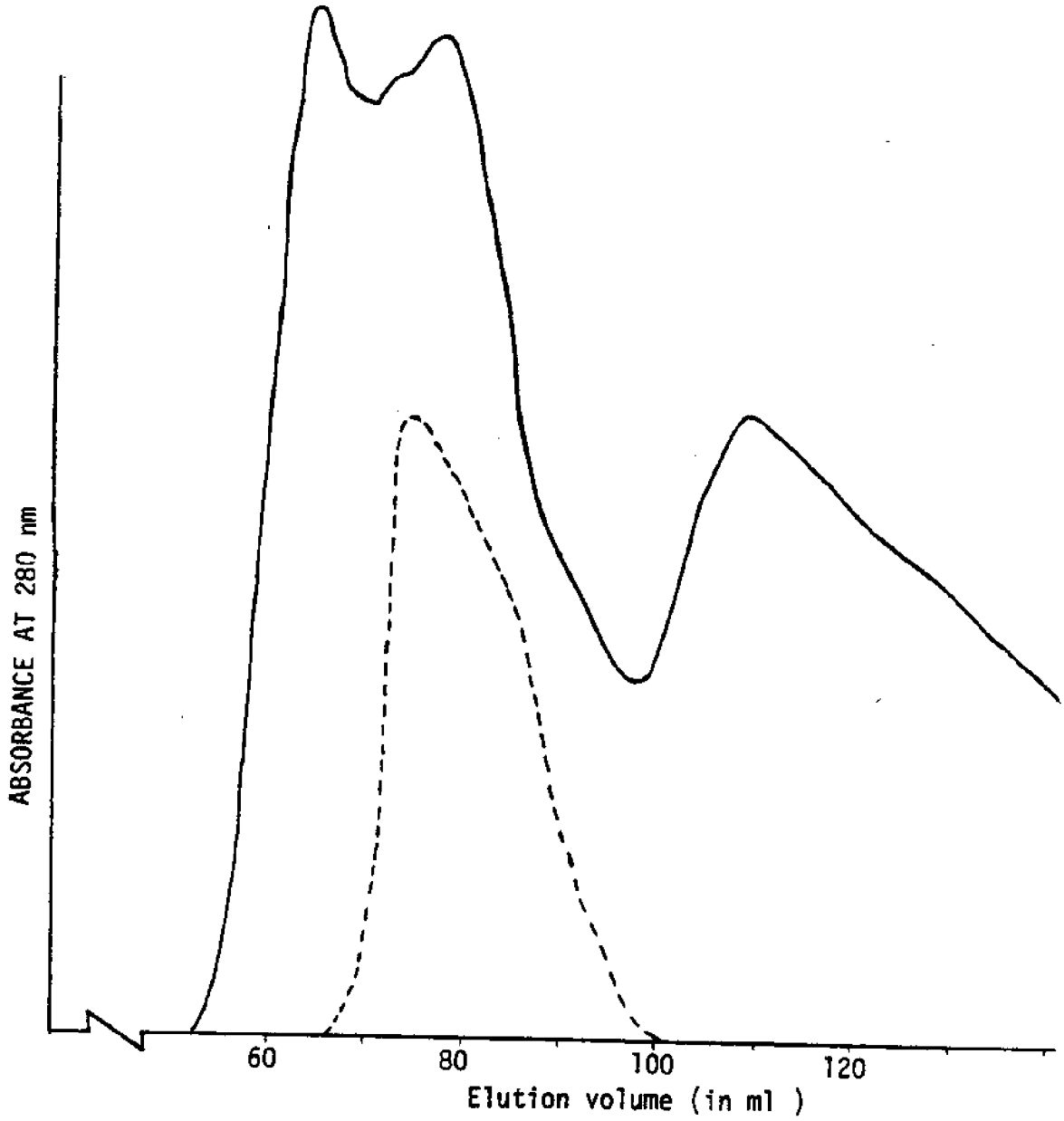

Figure 1.- Elution profile of proteins from brown shrimp.

Table 1. - Results of Purification Procedure

	VOLUME (IN ML)	ENZYME UNITS PER ML	TOTAL ENZYME UNITS	PROTEIN IN MG/ML	SPECIFIC ACTIVITY UNITS/MG	ACTIVITY RECOVERY	PURIFICATION (NO. OF TIMES)
ACETONE EXTRACT	13.6		9.05				
CONCENTRATE	(5.25)	0.666	(3.49)	27.3	0.024	100%	1
ACTIVE SEPHACRYL	81.0		4.45				
FRACTIONS	(56.0)	0.055	(3.08)	0.53	0.1038	127%	4.3
ACTIVE DEAE							
FRACTIONS							
0.15M	25	0.260	0.65	0.275	0.0945		3.9
0.20M	12.5	0.0304	0.38	0.165	0.1842	56%	7.62
0.25M	14.0	0.0239	0.334	0.092	0.2598		10.8

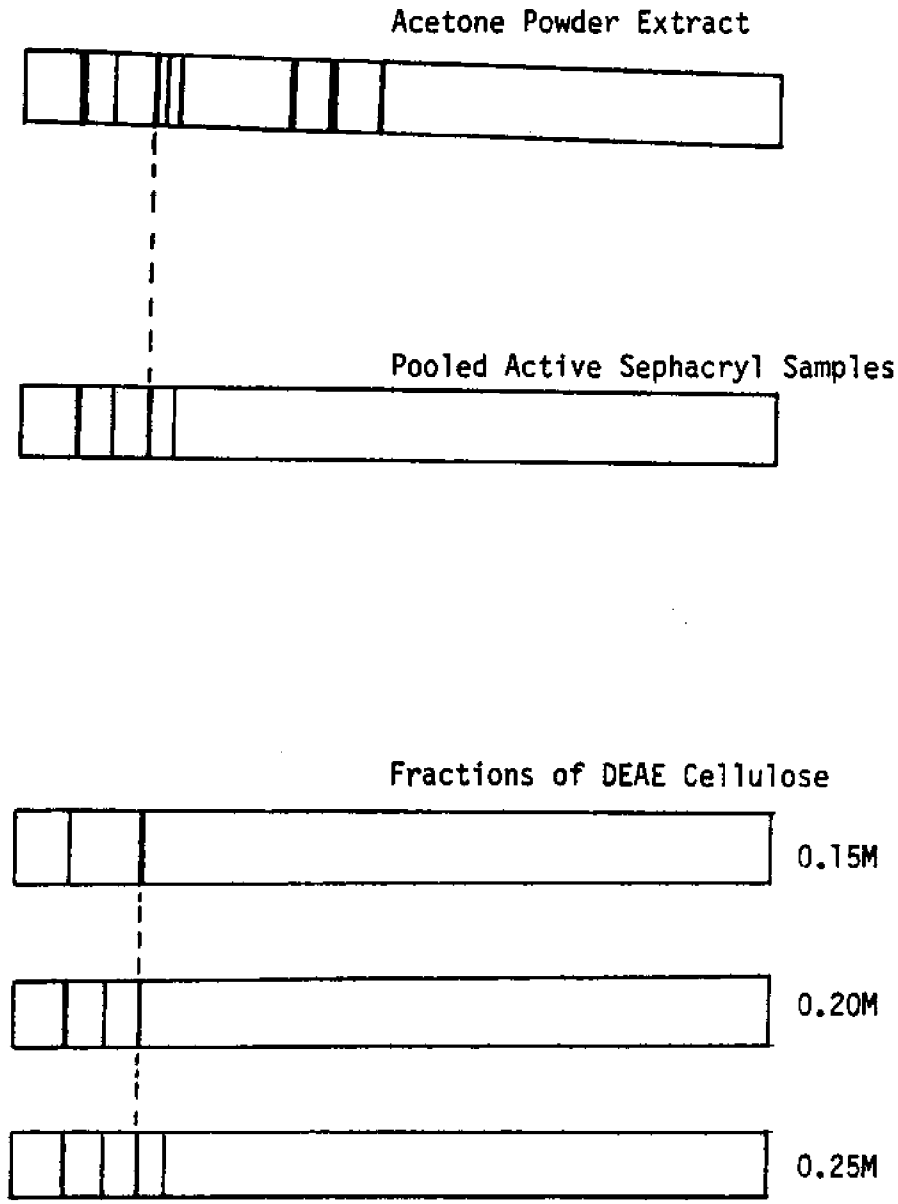

Figure 2.- Gel electrophoresis of samples at each stage of purification. The dotted line indicates the position of the enzyme.

Using the 2.6 x 36 cm Sephacryl S 200 column, a calibration curve was prepared by running four proteins of known molecular weight and then plotting Kav (a function of the elution volume) against the log molecular weight. The peak of enzyme activity (and thus elution volume of the enzyme) could easily be determined, and it gave an estimated molecular weight for the enzyme of 210,000 daltons.

Optimal pH and thermostability

Purified enzyme preparations were reacted with 5 mM DOPA at pH's from 3.5 to 8.5. DOPA solutions darken extremely fast at alkaline pH's. Below pH 5 the enzyme lost activity and precipitated. Enzyme precipitates could be separated by centrifugation and could be redissolved by adjusting the pH to 7. According to figure 3, the enzyme had a pH optimum between 6 and 6.5. Above this pH the enzyme gradually loses activity, but melanin formation is accelerated by autooxidation. Below pH 6, both enzymatic oxidation and autooxidation are reduced rapidly. Since the pH at the surface of the shrimp may go as high as 7.5 or 8 during ice storage due to the production of nitrogen bases, non-enzymatic melanin formations may be an important factor in black spot formation over prolonged ice storage. Bacterial growth could thus affect black spot formation in two ways, by keeping the environment reduced, preventing phenols from being oxydized, and by increasing the pH at the surface of the shrimp, which would promote non-enzymatic melanin formation.

For the thermostability test, purified enzyme preparations were kept in a constant temperature bath for 30 minutes. The activity of the preparations was measured before and after the heat treatment, and the results expressed in percent remaining activity, as shown in Figure 4. Phenoloxidase appears to be very stable under normal temperatures, but above 40°C the enzyme quickly loses its activity.

Inhibitor test

The appearance of black spot is the result of enzymatic oxidation of phenolic compounds, and most phenoloxidases studied to date have been shown to contain copper. The possibilities for inhibiting the reaction are therefore; using chemicals to chelate or bind to copper, using chemicals to affect the enzyme or the reaction, or altering the optimal temperature or pH of the reactions. As shrimp is sold fresh, there are limited possibilities to inhibit the reaction by pH modification. Reducing the temperature is a very effective form of delaying black spot, especially with the washing action of melting ice.

The copper binding or chelating compounds tested in this study were phenylthiourea, cysteine, sodium acid pyrophosphate (SAPP), sodium tripolyphosphate (STP) and sodium hexamethaphosphate (SHP). All of these compounds are approved as food additives, except for phenylthiourea, which was only used to ascertain if copper was involved in the reaction. Figure 5 shows the result of the inhibition by phenylthiourea. As phenylthiourea is very insoluble in aqueous solvents, its exact concentration was not known. The different concentrations tested were obtained by diluting the stock solution and the numbers given on Figure 5 are only to indicate the relative concentrations of the solution used. The graph shows it to be an uncompetitive inhibitor, and indicates that copper is needed in the reaction. Cysteine was tried but was found to work only as a reducing agent, delaying the start of the reaction depending on its concentration. Cysteine is known to combine with quinones,

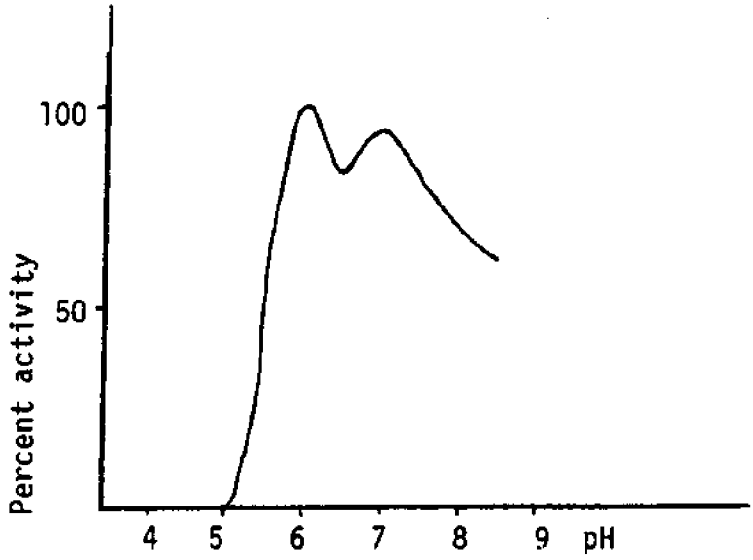

Figure 3.- Activity of the enzyme versus pH

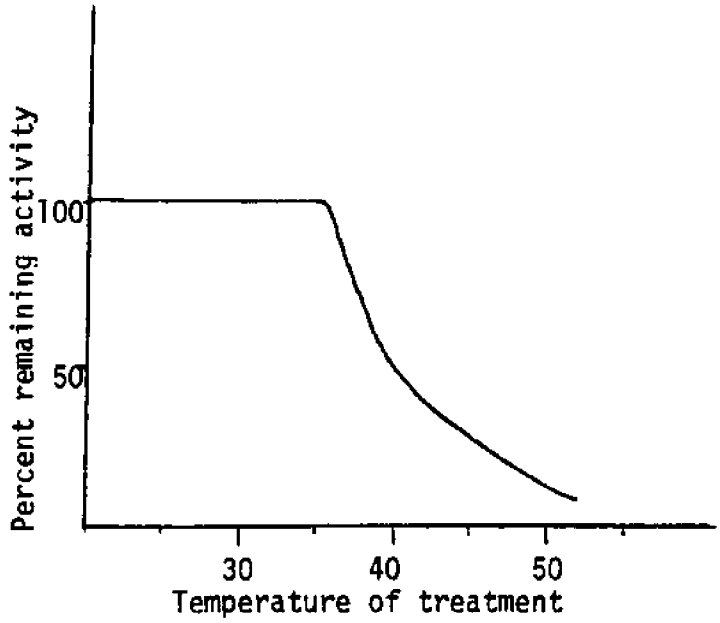

Figure 4.- Activity of the enzyme remaining after a 30 min heat treatment.

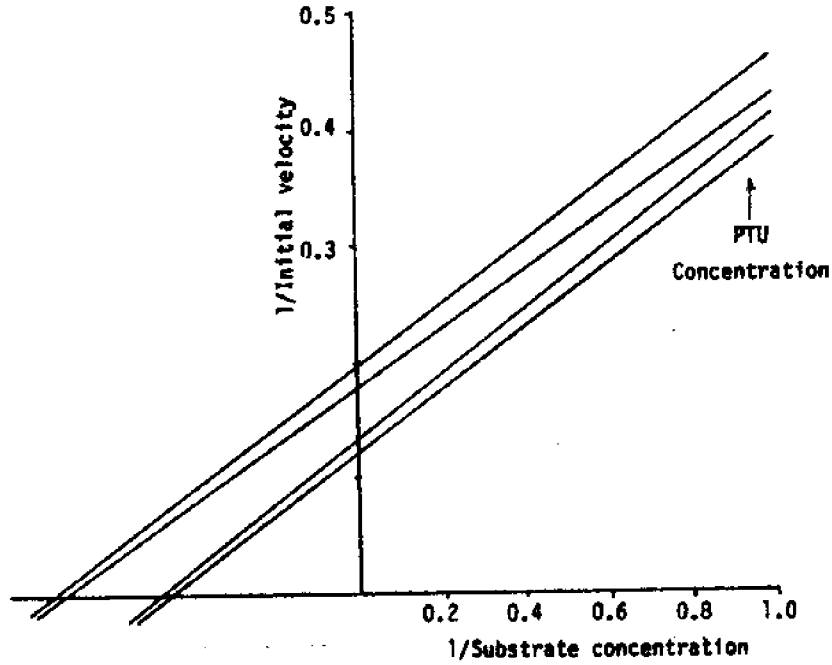

Figure 5.- Phenylthiourea Inhibition Test

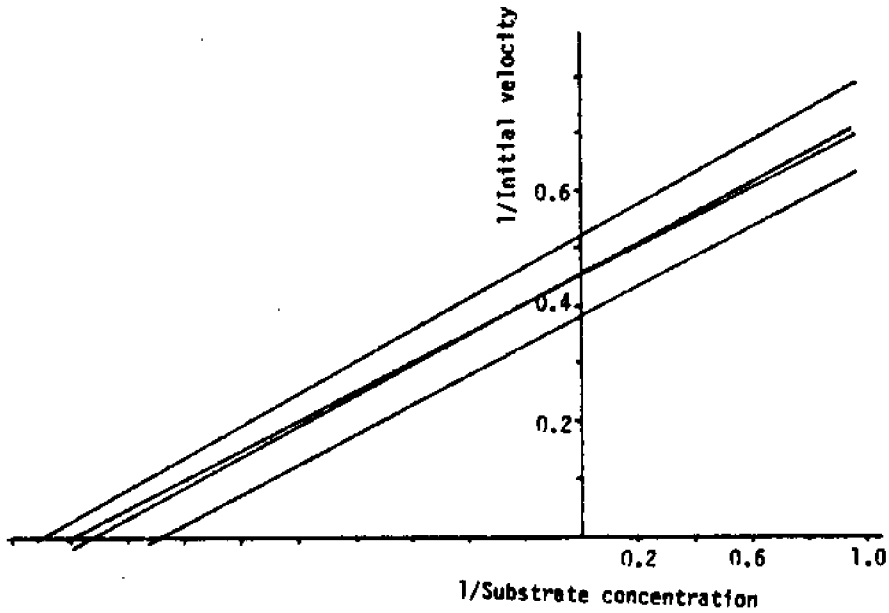

Figure 6.- Sodium Tripolyphosphate Inhibition Test

forming thioethers. This would reduce the amount of cysteine and would also reduce the initial substrate concentration, which would produce a lower initial velocity. This combination, plus the possibility of cysteine combining directly with copper, would give very complex reciprocal plots.

Figures 6, 7 and 8 show the results of the inhibition tests using phosphates. Only STP showed a definite pattern on inhibition, although it must be noted that the inhibitor concentration is very high compared to the substrate. The pattern obtained is similar to that of phenylthiourea, indicating a removal of copper by STP. The other two tests did not show any inhibition and this may be due to the pH of the reaction. At pH 6.2, the acid groups are all dissociated and its complexing ability is greatly reduced. Due to the instability of the enzyme at lower pH's, the copper-chelating effect of SHP and SAPP could not be tested in this reaction at lower pH's.

Sodium bisulfite is the most widely used inhibitor of black spot in shrimp. Several theories have been advanced to explain its activity, reducing properties, bacteriostatic ability, copper binding properties, or enzyme inactivation. Figure 9 shows that bisulfite is a competitive inhibitor for the enzyme. This fact alone could indicate the possibility of bisulfite binding to a sulfhydryl group in the active site of the enzyme. However, other sulfhydryl blocking reagents, such as N-ethylmaleimide and Ellman's reagent did not inhibit the reaction. Another possibility, although more information would be needed to confirm it, would be that bisulfite binds to the enzyme in the same way an orthodiphenol does. The configuration of the hydroxyl groups in bisulfite could allow it to "land" in the same orientation as an ortho-diphenol.

Naturally occurring substrates

Shrimp heads were extracted according to the method of Malek (1961). Thin layer plates of silica gel were run on mixtures of benzene, acetic acid and water both in one and two dimension. A maximum of three phenols were observed and one of them was identified as DOPA by comparison to a standard.

CONCLUSION

The observation of enzymatic reaction in vitro can only be taken as a primitive indication of the true nature of the reactions in vivo. The shrimp phenoloxidase examined in this study conforms with several characteristics reported for enzymes from other sources. In depends on copper for its activity and it is inhibited by sodium bisulfite. However, brown shrimp phenoloxidase is heavier than most enzymes of its kind reported. Possibly, as many other phenoloxidases, it is composed of several subunits.

The area of inhibitor research can be of great economic importance to the food industry. In the case of shrimp phenoloxidase, sodium bisulfite definitely is a better inhibitor than other food additives. Elucidating its mechanism of action on the enzyme could promote the search for better and safer black spot inhibitor.

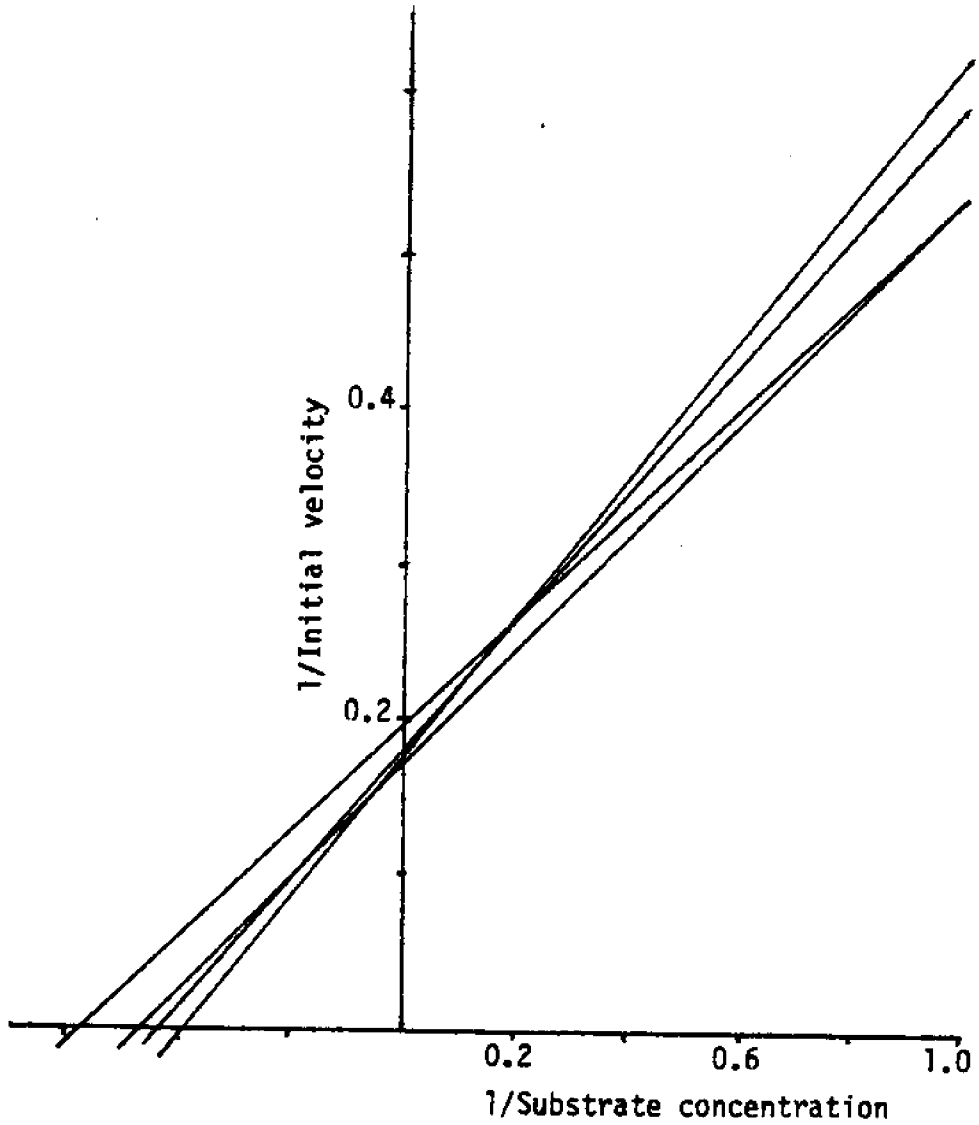

Figure 7.- Sodium Hexametaphosphate Inhibition Test

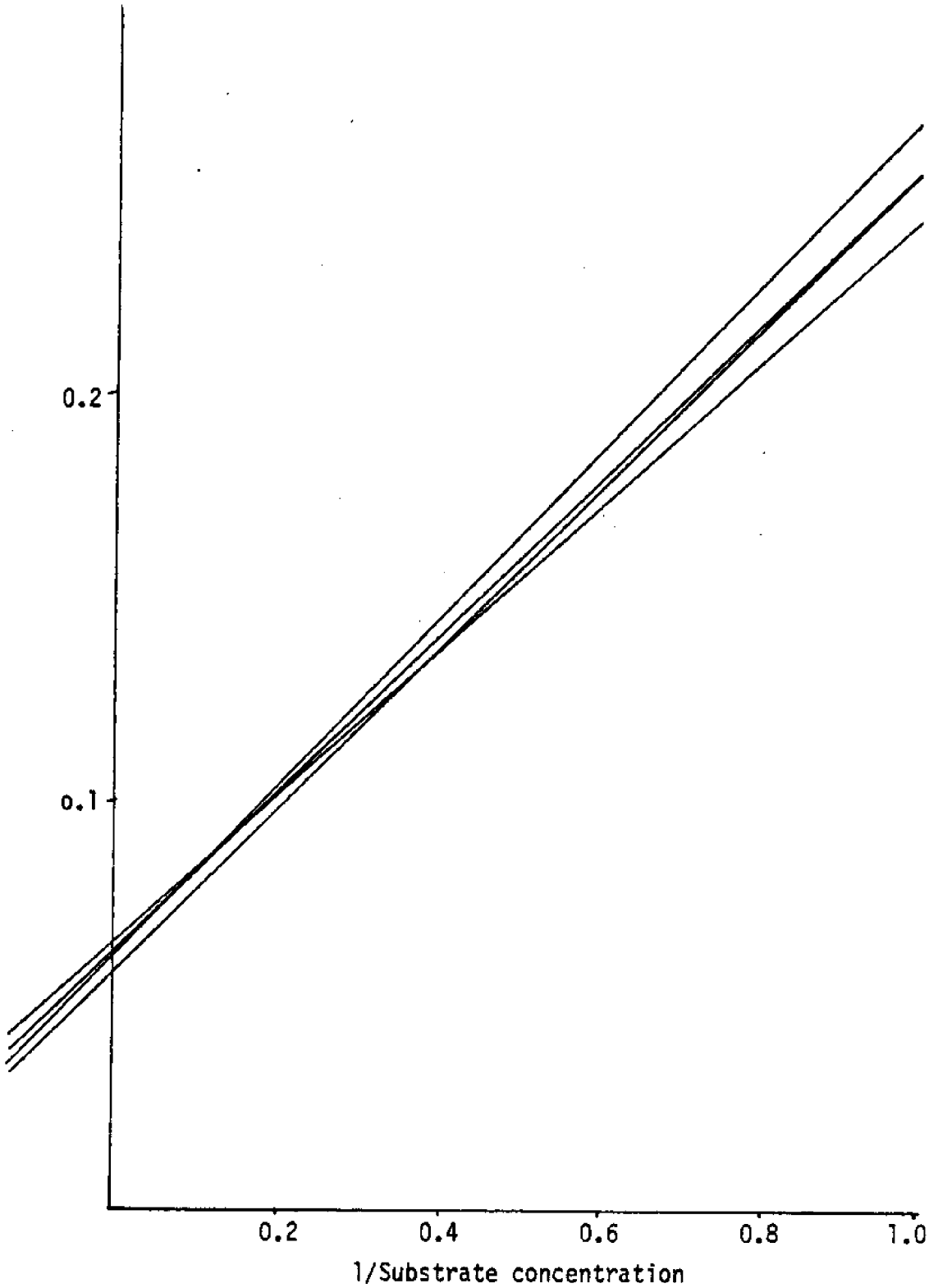

Figure 8.- Sodium Acid Pyrophosphate Inhibition Test

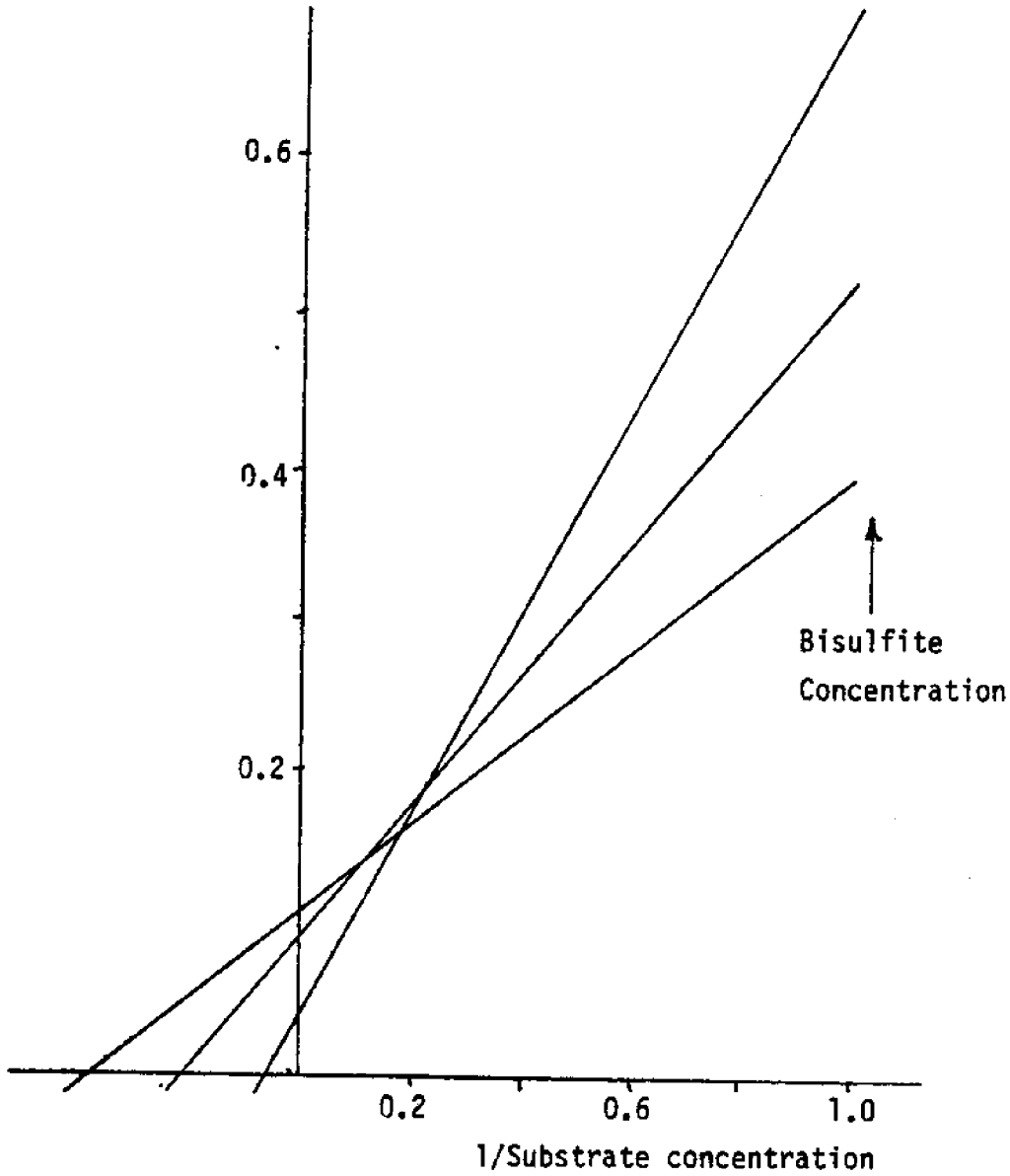

Figure 9.- Sodium Bisulfite Inhibition Test

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CONTROLLED ATMOSPHERE PACKAGING OF SWORDFISH STEAKS (FLOW THROUGH SYSTEM)

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INTRODUCTION

Modified atmosphere packaging (MAP) using carbon dioxide to replace or partially replace the air surrounding the fresh product is used to extend the shelf-life of certain foods. CO_2 is believed to delay the onset of spoilage in fresh muscle foods due to the inhibition of psychrotropic, aerobic, gram-negative spoilage bacteria (Sutherland et al., 1977; Gill and Tan, 1980).

Carbon dioxide has been used to extend the shelf-life of fish and other seafood products (Banks et al., 1980; Brown et al., 1980). However, MAP has several disadvantages: (i) the gaseous atmosphere changes as respiration occurs, (ii) the atmosphere is completely lost when the package is broken and (iii) off odors are often detected upon opening of the package. The objective of this study was to design a flow-through controlled atmosphere packaging (CAP) system offering the advantages of MAP while eliminating the disadvantages. With this system the gaseous atmosphere remains constant, individual packages can be removed without disrupting the entire system and off odors are blown away. Since fish resume their normal spoilage pattern when removed from CO_2 containing atmospheres (Banks et al., 1980), CAP offers retailers the advantage of the removal of individual packages for sale while the remainder benefit from the preservative properties of storage in CO_2.

PROCEDURE

Two fresh swordfish (Xiphias gladius), approximately 30 pounds each, were obtained, washed to remove surface bacteria and cut into one-inch steaks. The knife was rinsed in water between each cut, as were the steaks after cutting, to avoid cross-contamination and keep the initial bacterial load at a minimum. The steaks were then placed on individual styrofoam trays and overwrapped with PVC which is permeable to air and other gases. Six large barrier bags, impermeable to gas transmission, were placed in a cooler kept at 2°C. Through a tube, inserted into each of the bags, flowed one of the following gaseous atmospheres: air; 100% CO_2; 70% CO_2/30% O_2; 40% CO_2/60% O_2; 70% CO_2/30% N_2; or 40% CO_2/60% N_2. Twelve of the individually wrapped trays were placed in each of the barrier bags. Figure 1 shows a schematic representation of the CAP system.

Every other day, for a period of 22 days, one of the packages was removed from each of the gaseous atmospheres and examined for bacterial types and numbers, surface pH, total volatile nitrogen (TVN), trimethylamines (TMA) and lipid oxidation. Microbiological examination was done using surface cuts 10 cm^2 and approximately 2.5 mm deep. TVN and TMA were determined by a modified Conway procedure (Obrink, 1955). Surface pH was measured

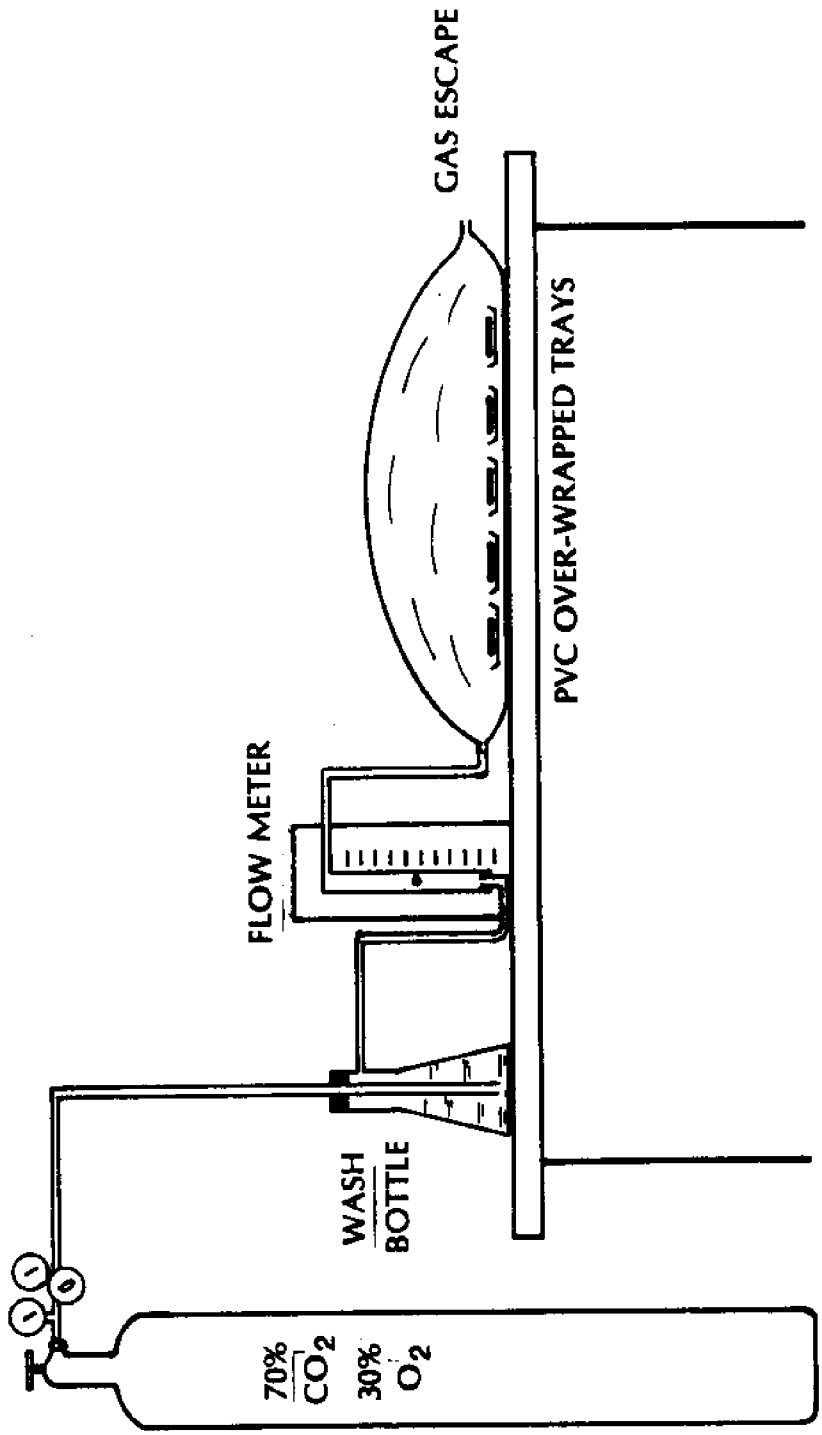

Figure 1. Schematic Representation of Flow Through Controlled Atmosphere Packaging System

with an Orion surface pH electrode. Lipid oxidation was measured as formation of malonaldehyde using the distillation method described by Tarladgis et al. (1960).

RESULTS AND DISCUSSION

Figure 2 shows the bacterial counts per cm^2. Initial aerobic plate counts (APC) were 9×10^2 per cm^2. Microflora on day 0 was dominated by Micrococcus spp., with Bacillus, coryneform bacteria, Flavobacterium, Moraxella-Acinetobacter, Citrobacter freundii and Pseudomonas spp. present in relatively low percentages. APC of samples stored in air increased rapidly exceeding 10^6 per cm^2 after 6 days. Samples stored in 40% CO_2/60% N_2, 40% CO_2/60% O_2, and 70% CO_2/30% O_2 reached an APC of 10^6 per cm^2 in 20, 14 and 22 days, respectively. APC of samples stored in 100% CO_2 and in 70% CO_2/30% N_2 were only 2.6×10^4 and 4.5×10^3 at the end of the storage period.

Pseudomonas spp., the predominant spoilage microorganism, dominated the microflora of samples stored in air for 2-14 days. Pseudomonas spp. also constituted an important part of the microflora of the samples stored in the various gaseous atmospheres. Of the 55 samples stored in these atmospheres (excluding air) for 2-22 days Pseudomonas spp. comprised 25-49% of the microflora in 11 of the samples and greater than 50% in 31 of the samples. Percentage of gram-negative microorganisms present in the various atmospheres is shown in figure 3. Initially growth of gram-negative bacteria in CO_2 containing atmospheres parallels that of the samples stored in air, however growth of gram-negatives began to drop off sharply in 12-14 days. This decline corresponds to the appearance of Lactobacillus spp.

Surface pH is shown in figure 4. Initial pH was 5.9 increasing to 6.6 in the air sample during 20 days of storage. After a slight pH increase on day 2, samples stored in the CO_2/O_2 atmospheres remained relatively constant. Samples stored in the CO_2/N_2 and 100% CO_2 atmospheres demonstrated a slight decrease in pH after 2 days with slight increases after 18-20 days of storage.

The TVN content of swordfish steaks packed in air and various gas atmospheres is shown in figure 5. TVN values for different degrees of freshness of fish have been suggested as follows: fresh fish <12mg; good quality-very slightly decomposed, 12-20; edible, 20-25; decomposed and inedible, >25 mg TVN-N/100 g tissue (Stansby, 1963). An initial value of 11.6 mg TVN-N/100 g indicates the fish was of good quality. For steaks stored in air, TVN increased rapidly reaching 25 mg TVN-N/100 g after 14 days. In contrast, TVN values increased more slowly for samples stored in CO_2-enriched atmospheres. Maximum values, reached after 22 days, did not exceed values for an edible product.

TMA produced by the reduction of TMA-O by TMA-O reductase positive microorganisms (Liston et al., 1963) is also used as a quality indicator for fish and seafood products. Farber (1965) suggested that sole was spoiled when the TMA value exceeded 4.7 mg TMA-N/100 g fish. As shown in figure 6 the TMA value increased more rapidly in air than in samples stored with CO_2. However, none of the samples exceeded the recommended values for acceptability. Since the sample stored in air had reached an APC of more than

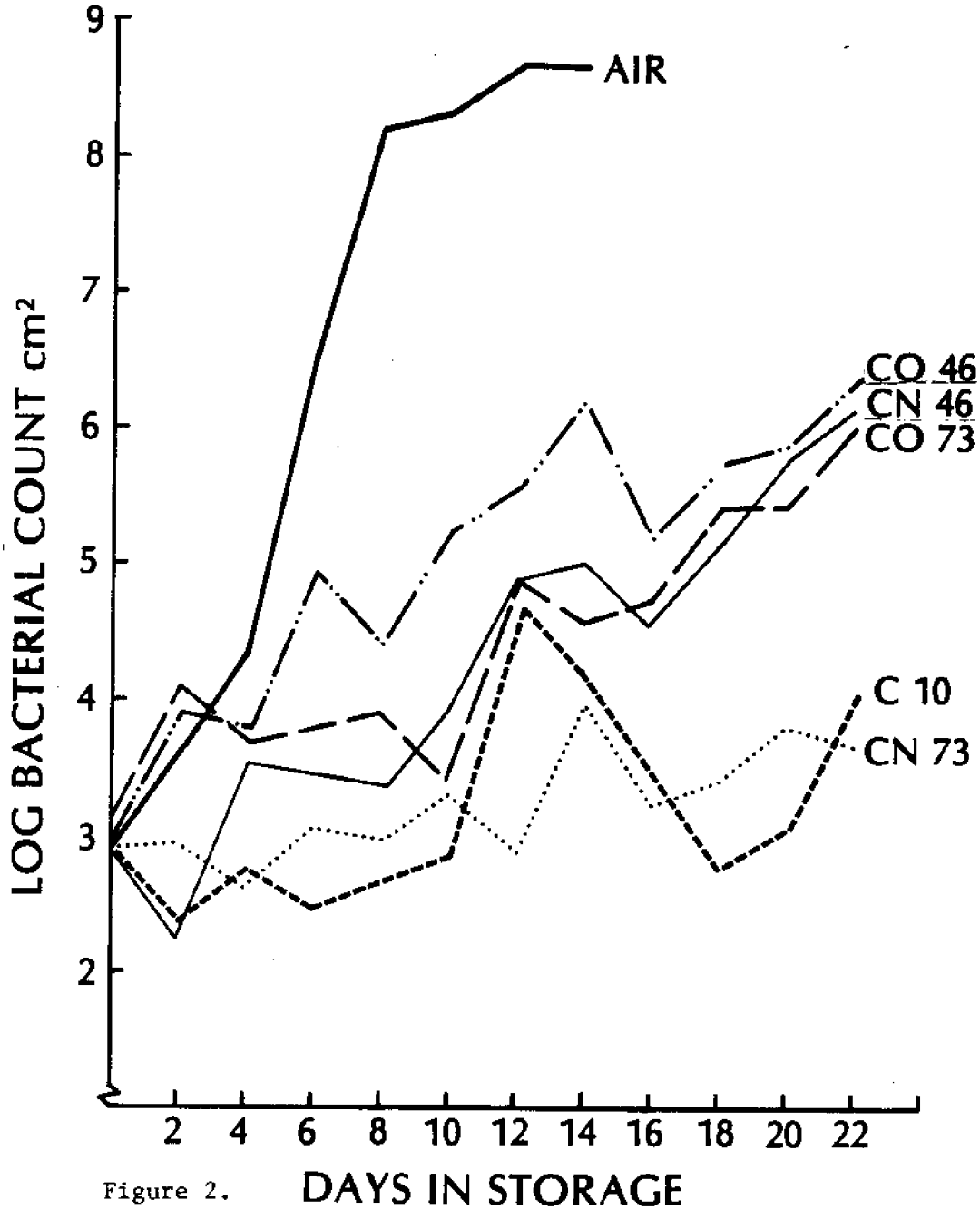
LOG BACTERIAL COUNT cm²
9
8
7
6
5
4
3
2
AIR
CO 46
CN 46
CO 73
C 10
CN 73
2
4
6
8
10
12
14
16
18
20
22
DAYS IN STORAGE

Figure 2.

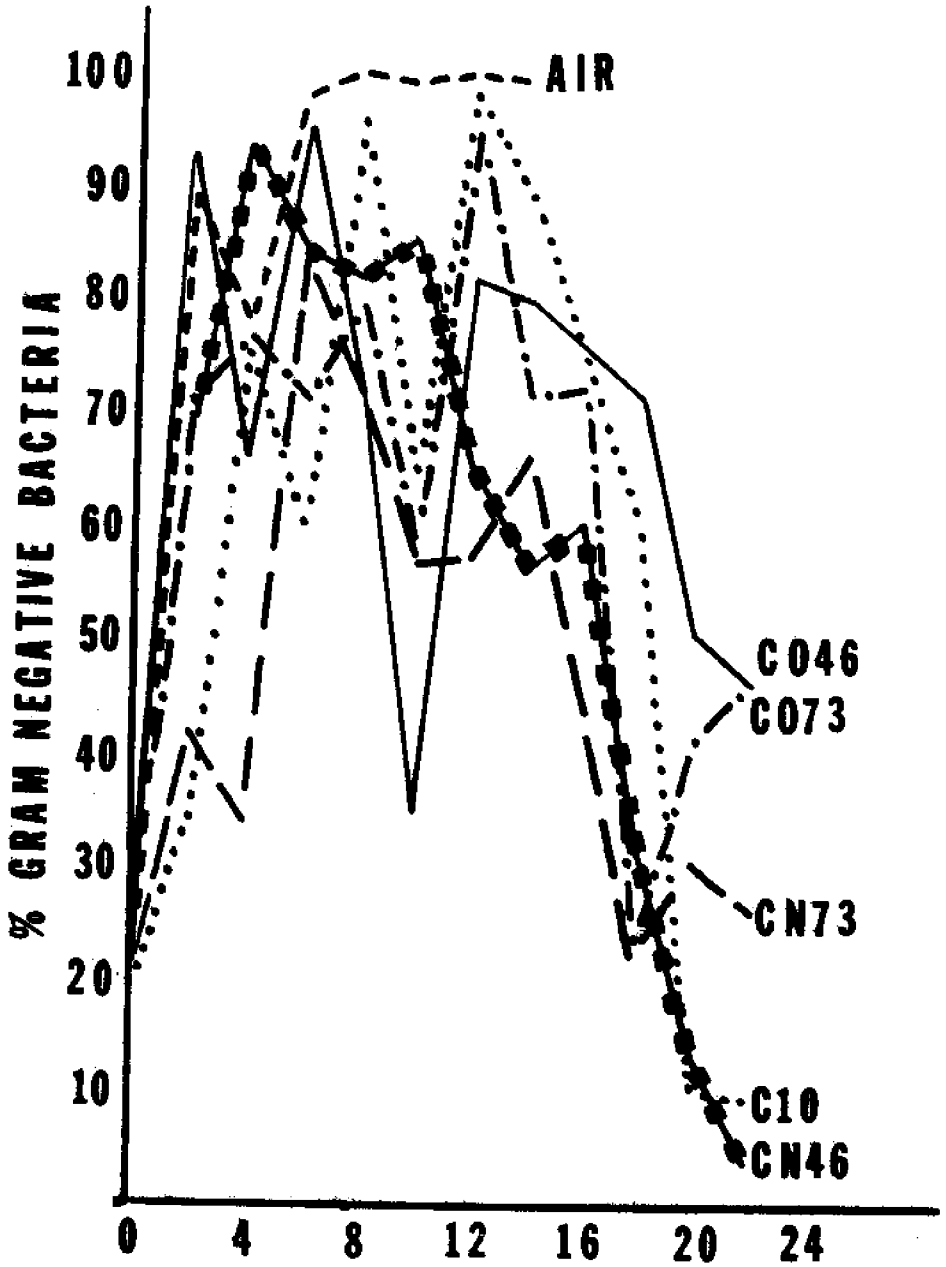
100
90
80
70
60
50
40
30
20
10
% GRAM NEGATIVE BACTERIA
AIR
CO46
CO73
CN73
C10
CN46
0
4
8
12
16
20
24
DAYS IN STORAGE

Figure 3.

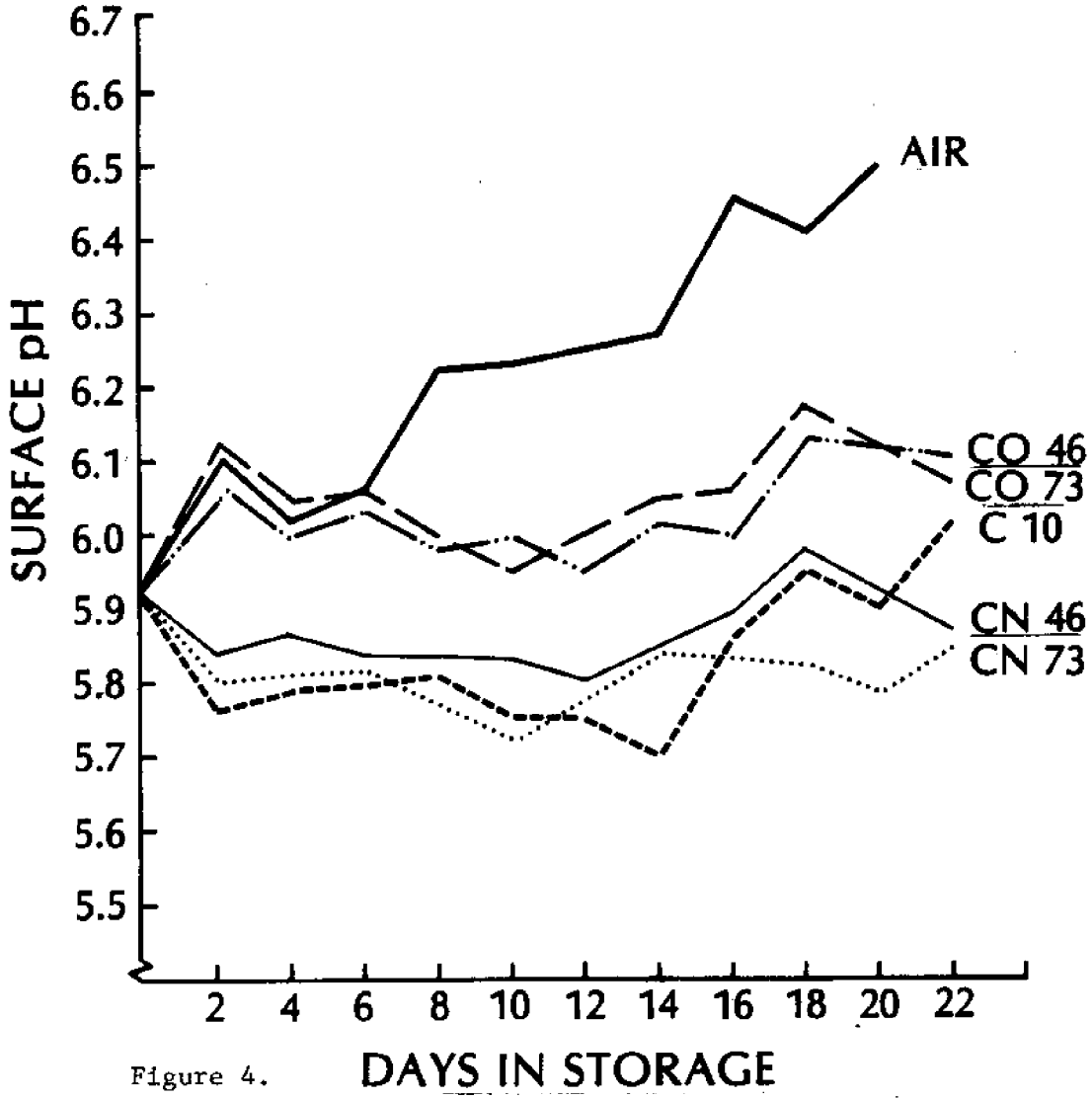
SURFACE pH
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
5.9
5.8
5.7
5.6
5.5
AIR
CO 46
CO 73
C 10
CN 46
CN 73
2
4
6
8
10
12
14
16
18
20
22
DAYS IN STORAGE

Figure 4.

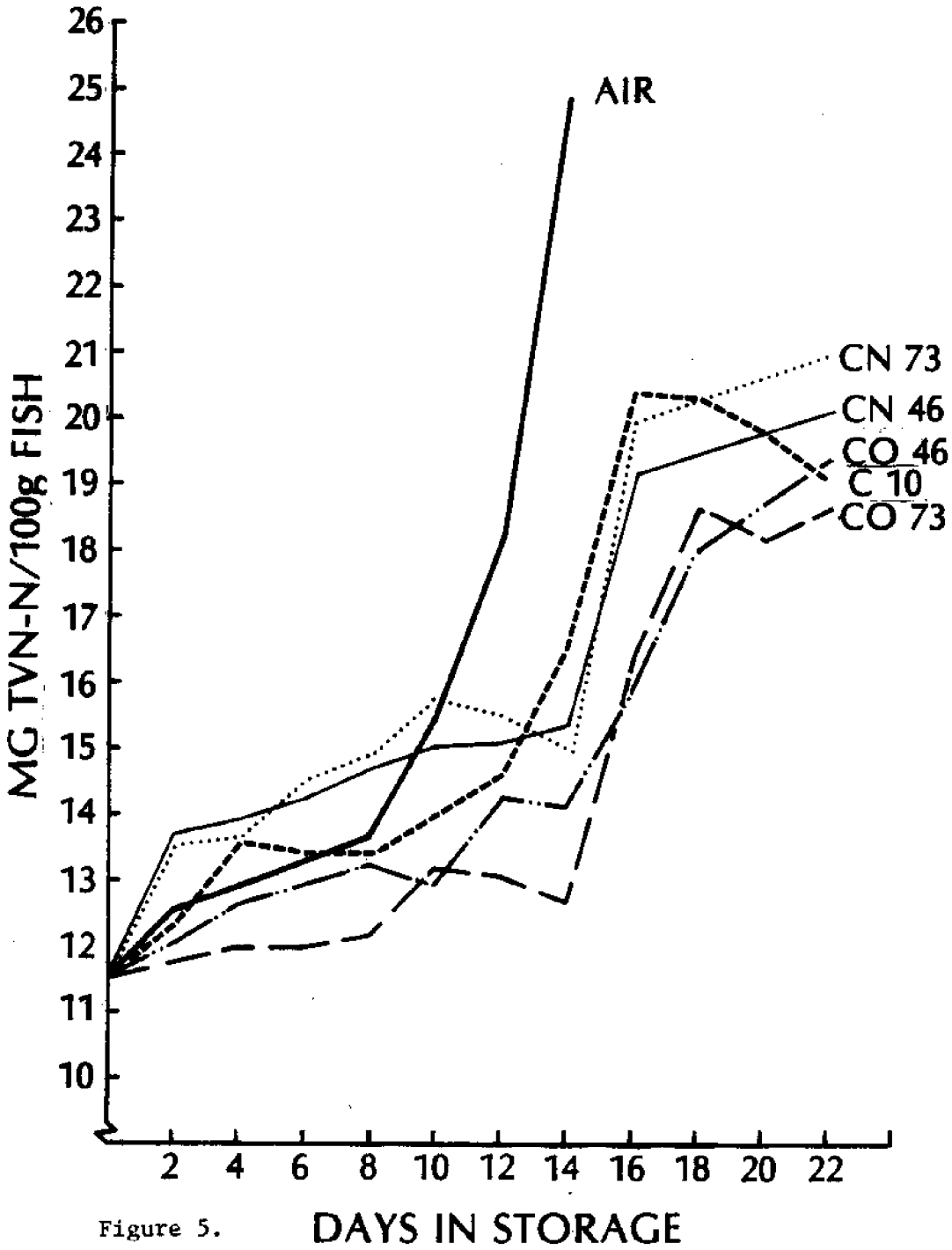
MG TVN-N/100g FISH
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
AIR
CN 73
CN 46
CO 46
C 10
CO 73
2
4
6
8
10
12
14
16
18
20
22
DAYS IN STORAGE

Figure 5.

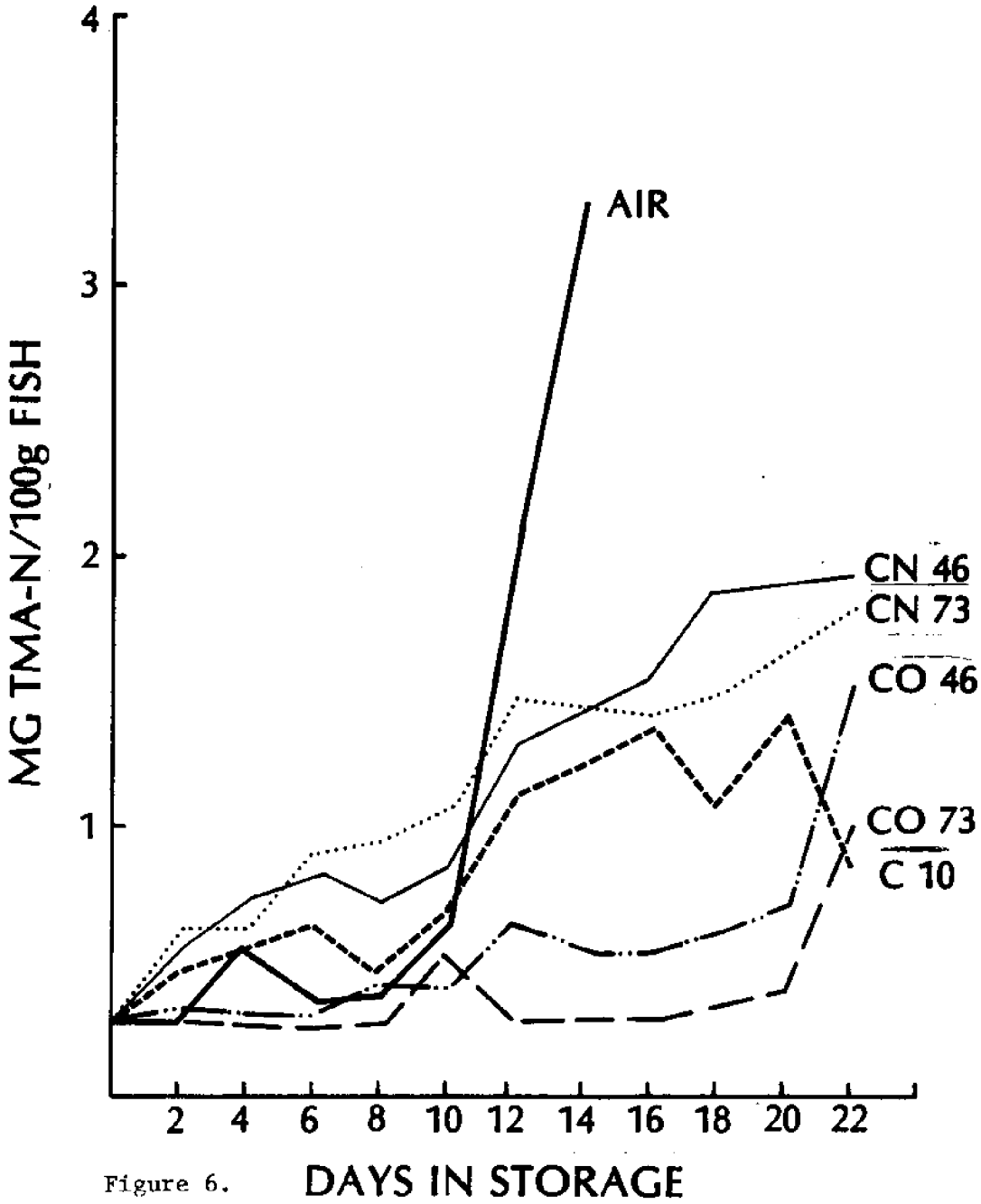
MG TMA-N/100g FISH
4
3
2
1
AIR
CN 46
CN 73
CO 46
CO 73
C 10
2
4
6
8
10
12
14
16
18
20
22
DAYS IN STORAGE

Figure 6.

10^8 per cm^2 after 8 days of storage and was obviously spoiled, this test may not be a good indicator of quality.

Lipids in fresh fish seldom exhibit a tendency toward oxidative rancidity (Liston et al., 1963). However, with modified or controlled atmospheres containing high levels of O_2 rancidity could be a potential problem. Development of oxidative rancidity expressed as mg malonaldehyde/kg fish is shown in figure 7. Although the samples stored in air and O_2 containing atmospheres exceeded 4 mg malonaldehyde/kg fish, a maximum value suggested by Sinnhuber and Yu (1958), no rancid odors were detected organoleptically at any time during the 22 days of storage.

After two days of storage the samples stored in the absence of O_2 exhibited a slight browning of the red muscle while the swordfish steaks stored in air or O_2 containing atmospheres retained the bright red color. However, after 6 days of storage the samples stored anaerobically had a better overall appearance, a trend which continued for the remainder of the storage period. Within 6 days the sample stored in air had a definite off odor. By day 14 the samples stored in the presence of O_2 had a very slight "fishy" odor and within 18 days had developed a slight surface slime. Other than a mild stale odor after 22 days, the samples stored anaerobically did not exhibit any undesirable organoleptic characteristics.

CONCLUSIONS

Carbon dioxide was effective in inhibiting bacterial growth in all concentrations tested. Even though Pseudomonas spp. comprised a high percentage of the microflora present in all samples, reduction of total numbers of microorganisms was effective in extension of shelf-life.

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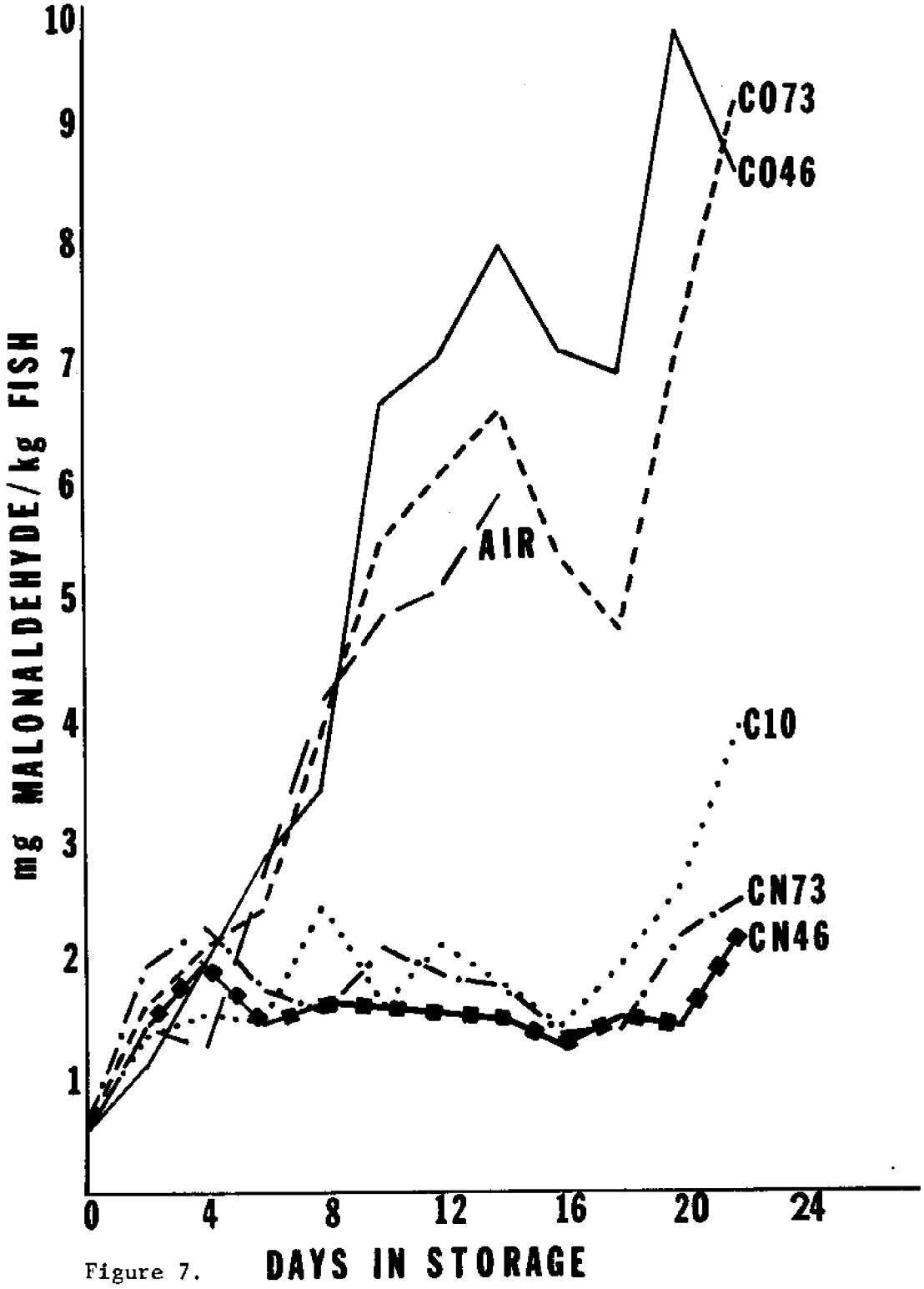
mg MALONALDEHYDE/kg FISH
10
9
8
7
6
5
4
3
2
1
0
4
8
12
16
20
24
DAYS IN STORAGE
CO73
CO46
AIR
C10
CN73
CN46

Figure 7.

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SPOILAGE CHARACTERISTICS OF FRESH SHRIMP STORED IN VACUUM AND ATMOSPHERES CONTAINING CO_2

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INTRODUCTION

Texas shrimp processors have requested product quality information relative to marketing shrimp at the retail level. Except for fresh or thawed shrimp available in seafood stores and a few supermarkets in the U. S., most shrimp is marketed in the traditional five pound carton. Such a package is expensive to the consumer as a unit item, does not satisfy the demand for a family size portion, and does not display the product. This last point is especially important when displaying shrimp along with red meats, pork and poultry in the retail store as a competitive main course item.

Additionally, one processor is interested in converting to a new packaging system for institutional sales as well as the frozen bait market, based on his evaluation of energy savings in processing and transportation as well as purchaser acceptance.

Considering the need to develop a packaging system which will deliver a quality product at predictable prices and in sufficient quantity to satisfy the retail market demand, seafood technologists at Texas A&M University have been evaluating various packaging techniques including controlled and modified gas atmospheres as well as vacuum systems.

Previous work by Lannelongue et. al. (1) in which Gulf of Mexico shrimp were packaged under modified gas atmospheres containing carbon dioxide, nitrogen, and oxygen showed that the refrigerated shelf life of shrimp could be extended several days when compared with shrimp held in air. These samples were packaged in molded trays with an approximate 3:1 gas volume to product ratio. Korhonen and Lanier (2), however, showed consumer preference of fish which were vacuum or heat shrink packaged as compared with an over wrapped product.

The purpose of this research therefore was to compare the storage characteristics of vacuum packaged shrimp with shrimp in the same bags but held under modified gas atmospheres. Additionally, loose shrimp on ice were evaluated.

METHODS

One day old white shrimp (Penaeus setiferus) purchased from Texas bay shrimp fishermen were packaged in Curlon® barrier bags. Using a Multivac AG machine the shrimp were packaged under vacuum; 100% CO_2; 70% CO_2: 30% O_2; and air (approximately 2:1 gas to product ratio) and subsequently placed in a refrigerator at 4°C.

Loose shrimp held on ice served as a control. At regular intervals each treatment was analyzed for bacterial numbers (APC), total volatile nitrogen (TVN), trimethylamine (TMA), and "black spot" formation.

RESULTS AND DISCUSSION

The initial bacterial load of 1.2×10^5 per gram (Figure 1) indicated that perhaps the shrimp had not been properly iced on the boat. However, the numbers would not be unusual for Gulf shrimp held on ice several days prior to offloading. As expected, the air sample showed the most rapid increase in bacterial outgrowth, reaching 10^8 per gram between 5 and 7 days. The product had a definite "off" odor after three days, and smelled quite spoiled after seven days. The shrimp held loosely on ice showed a similar but slower bacterial outgrowth due to the lower temperature and washing action of the melting ice. The characteristic spoilage odor of the iced shrimp did not develop in the open container (ice chest) until sampling was discontinued at day 10.

Shrimp stored under 100% CO_2 showed the least bacterial outgrowth, increasing only one log unit over a 14 day period (Figure 1). Also, these shrimp had only a slight "musky" ordor at the end of the experiment. Bacterial growth on shrimp in the CO_2:O_2 atmosphere jumped dramatically at first only to somewhat level off between days 7 and 14 (Figure 1). Although these bacteria were not typed, representative colonies were picked, restreaked on slants, and gram stained. Results showed a definite shift from gram negative to gram positive organisms between days 7 and 14. Banks et. al. (2) found a similar shift in bacterial flora on fish stored under modified atmospheres. They identified the organisms as shifting from predominately gram negative spoilage bacteria (primarily Pseudomonas and Moraxella) to gram positive Lactobacillus and Coryneforms. Interestingly, even with the relatively high bacterial numbers after three days, the shrimp in this mixed gas atmosphere did not begin to smell spoiled until the 12th day of storage.

Vacuum packaged shrimp showed an increased lag time in bacterial outgrowth relative to all treatments except 100% CO_2, with only a one log increase in 7 days (Figure 1). These shrimp smelled fine on day 5, but would be definitely unacceptable to a consumer at 7 days.

In the study referred to earlier where shrimp were stored under various gas atmospheres (1) the authors showed a close relationship between the bacterial outgrowth of grom negative spoilage organisms and TVN production. Results from the present experiment are similar for atmospheres containing CO_2 and for air (Figure 2), but quite different for shrimp held on ice (i.e. high bacterial counts but relatively low TVN values) and for vacuum packaged shrimp where after 7 days bacterial outgrowth was only 10^6/gram but TVN was 51 mg N/100 grams shrimp. Using TVN values suggested by Stansby (3) of 20-25 as edible, but greater than 25 as decomposed these shrimp would not be acceptable after 3 days (Figure 2) much less 7.

TMA values showed similar results (Figure 3). Using the suggestion of Cobb et. al. (4) of 5 mg N/100 grams shrimp as the limit of acceptability, the vacuum packaged shrimp would be good at 5 days but unacceptable at 7 days.

Minimal black spot development occurred in any of the samples prior to concluding the experiments. This was in contrast to a prior similar experiment

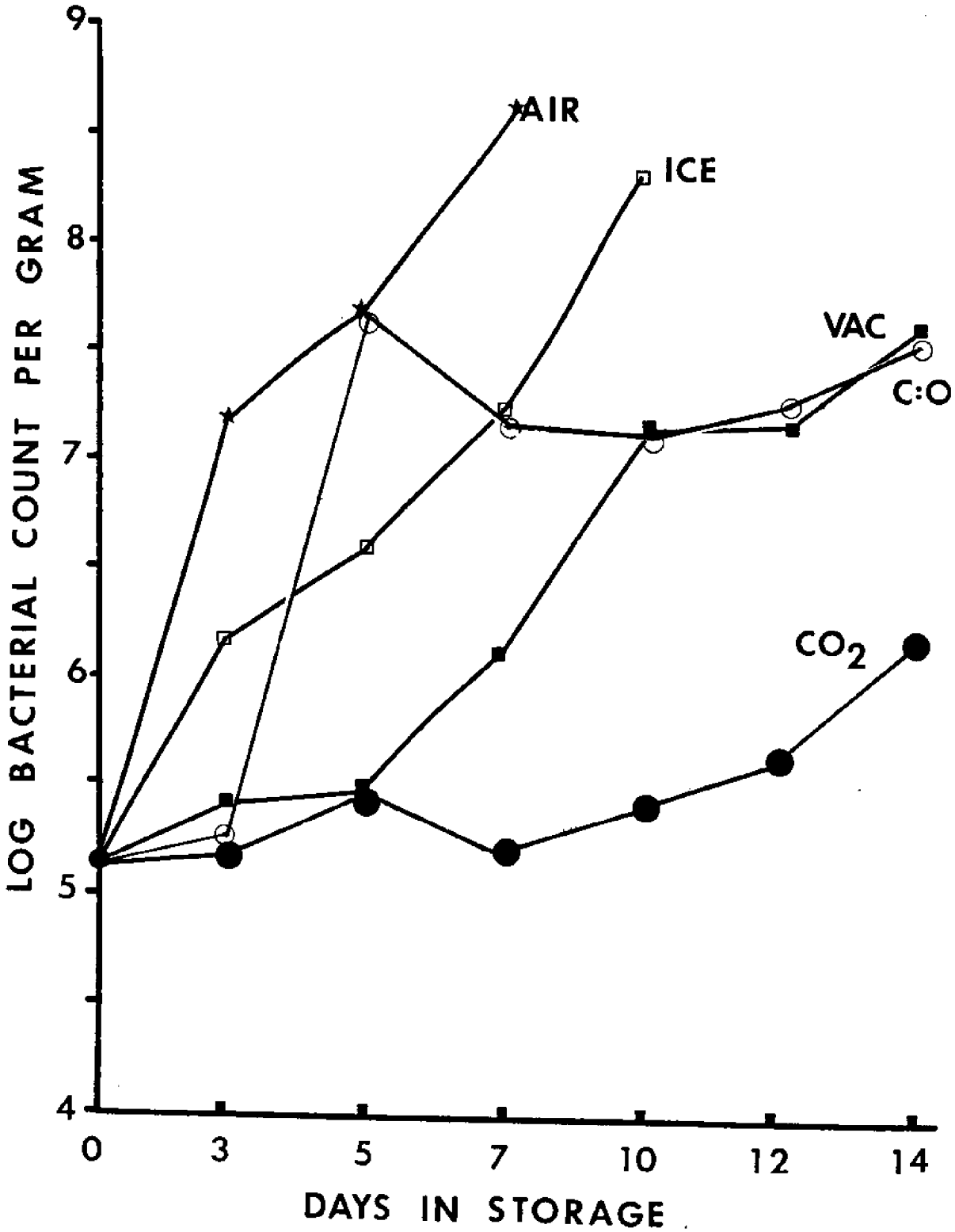
LOG BACTERIAL COUNT PER GRAM
9
8
7
6
5
4
AIR
ICE
VAC
C:O
CO_2
0
3
5
7
10
12
14
DAYS IN STORAGE

Figure 1

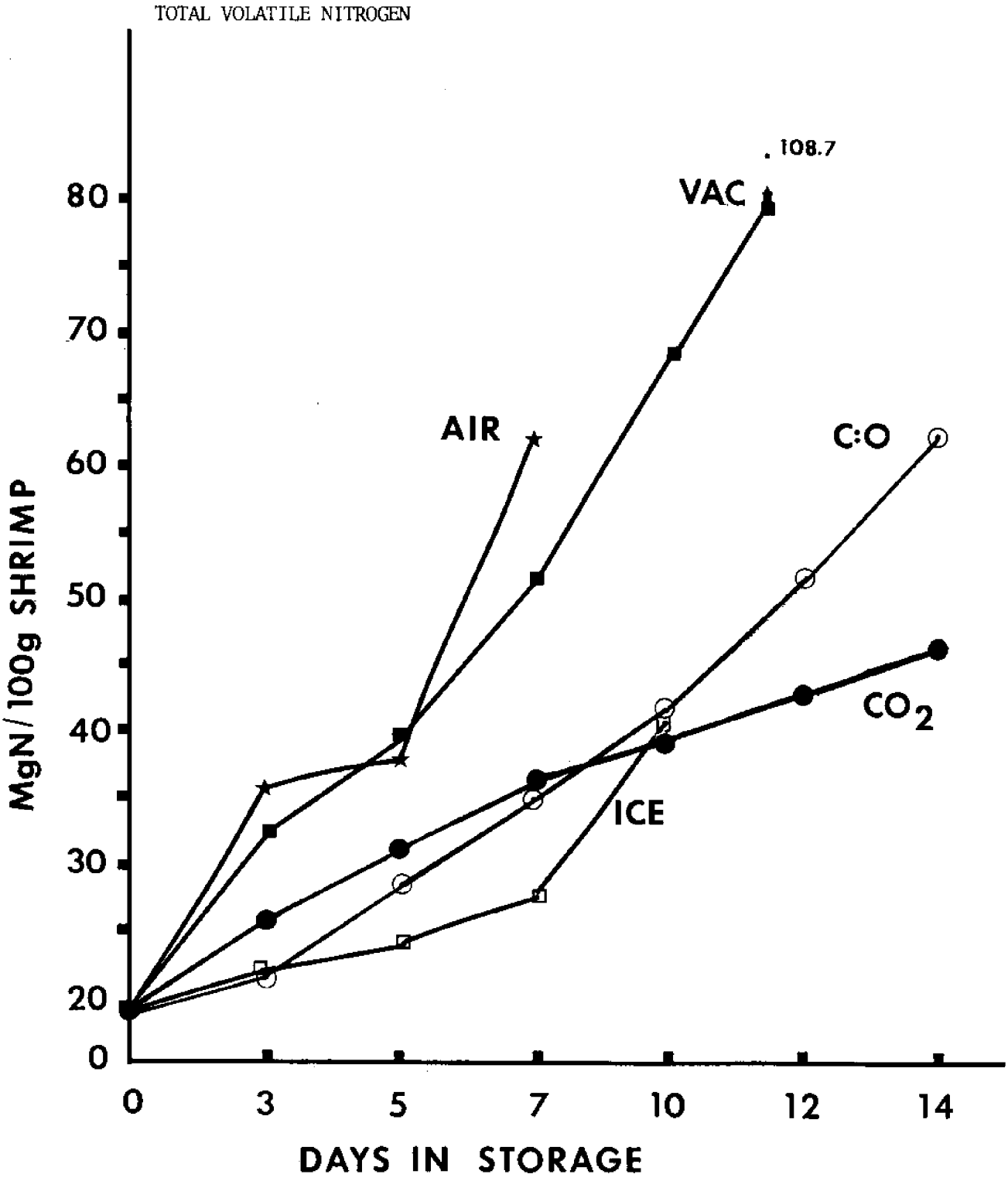
TOTAL VOLATILE NITROGEN
108.7
VAC
AIR
C:O
CO2
ICE
MgN/100g SHRIMP
80
70
60
50
40
30
20
0
0
3
5
7
10
12
14
DAYS IN STORAGE

Figure 2

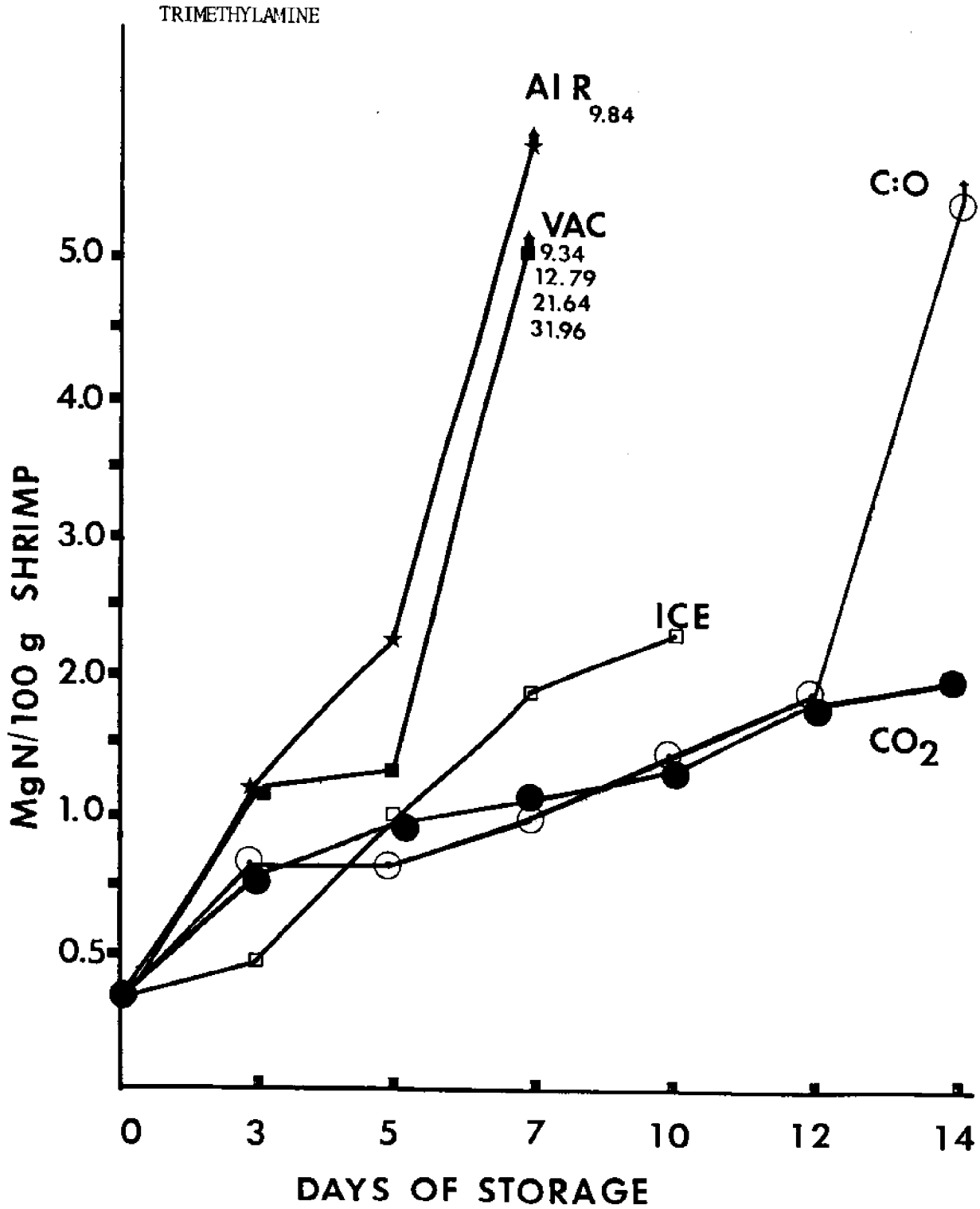
TRIMETHYLAMINE
AI R
9.84
VAC
9.34
12.79
21.64
31.96
C:O
ICE
CO_2
5.0
4.0
3.0
2.0
1.0
0.5
MgN/100 g SHRIMP
0
3
5
7
10
12
14
DAYS OF STORAGE

Figure 3

using pond raised shrimp, indicating the necessity for further research into the mechanism of black spot formation-especially as it relates to the initial handling of shrimp.

SUMMARY

Seafood technologists at Texas A&M will continue to evaluate various packaging systems for the seafood industry concentrating particularly on the following methods for the retail market.

1. Gas permeable vacuum or shrink bags held under modified or controlled gas atmospheres in master cartons.

2. Gas impermeable vacuum bags for fresh or frozen products. Such bags might be flushed with a CO_2:O_2 gas mixture prior to sealing to increase shelf life.

Results from this experiment indicate that vacuum packaged shrimp have a 3-5 day refrigerated shelf life. Therefore, assuming consumer acceptance of such a packaging system, we would envision marketing a frozen product which could be rapidly thawed at home.

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ICE CONSUMPTION DURING FISH STORAGE

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1. INTRODUCTION

The amount of ice needed to keep fish fresh is economically important in sub tropical and tropical areas, because ice melts more quickly in warm climates. Proper insulation of fish boxes and containers is also important as well as the handling and transporting of iced fish as they affect the ice consumption.

The introduction of new fish handling methods has not always been successful, very often due to the high cost of ice. Moreover, the practical rules (e.g. 50% fish + 50% ice) usually found in technical publications (5),(6),(11), cannot be applied to tropical and semi-tropical conditions.

In 1950, Jaeger (8),(1), developed a computational method to evaluate the amount of ice melted up to time t. In Australia, this method was applied to refrigerated rail cars cooled by ice. More recently, other computational methods have been developed (2),(3),(4).

And even though, computers are more accessible nowadays, developing countries still need an easier and safer way to estimate ice consumption.

This paper describes a simple method to estimate ice consumption during fish storage. This method was tried with plastic boxes and hake (Merluccius hubbsi) when ice losses were caused by heat transfer due only to natural convection. Nevertheless, it can be used to test any kind of box or container, and can give valuable indications of other heat transfer conditions.

2. MATERIALS AND METHODS

Three different types of plastic (polyestirene) boxes were used. The accepted net contents of each box and the external surfaces are given in Table 1.

Flake ice normally used by the fish industry at Mar del Plata was used. Some trials used crushed block ice. Ice "chips", not used by the fish industry, produced by a small ice machine was also used in some experiments.

TABLE 1. Characteristics of plastic boxes used

Box type	Net content (Kg)	Side area (cm^2)	Bottom area (cm^2)
1	35	3001,6	2731,3
2	40	4506,6	2730,7
3	45	4513,2	2793,5

An industrial balance (±20g) was used to weigh the boxes and the ice. Temperature around the boxes was measured by Fe - Constantan thermocuples and registered in a strip-chart recorder. An experimental cold storage room with automatic temperature control (±0.5°C) was used. Some experiments were carried out in industrial cold storage rooms and ship holds in order to test the viability of the estimations; in this case a max-min thermometer (±0.5°C) was used to assess a mean temperature.

The fish used was fresh hake (Merluccius hubbsi). Trials without fish were also conducted in order to take into account the weight losses of the fish during the experiments.

3. PROPOSED MODEL

Tentative experiments have shown that there could be a lineal relationship between melted ice and time when external temperature remained constant (9).

A lineal model can be easily developed if we suppose that ice is melting inside the boxes due to steady heat transfer from the exterior. In this situation, the heat transferred will be given as :

$$q_t = A.U. (T_i - T_e) \quad \text{(Kcal/day)} \qquad (1)$$

where :

A = area of transference (cm^2)
U = overall heat transfer coefficient (Kcal/day.cm^2.°C)
T_i = temperature inside the box (usually taken as 0°C)
T_e = temperature outside the box (°C).

This heat will be counterbalanced by the heat resulting from the fusion of ice. It follows that :

$$q_f = \lambda_f \cdot \frac{dM_i}{dt} \quad \text{(Kcal/day)} \qquad (2)$$

where : λ_f = latent heat of fusion (usually taken as 80 Kcal/Kg).

M_i = mass of ice (kg)
t = time (in this case, it was found useful to take days as unity).

In this steady state approach, quantities (1) and (2), should be equal. It follows that :

$$\frac{dM_i}{dt} = \frac{A.U}{\lambda_f} (T_i - T_e) \qquad (3)$$

Equation (3) can be easily integrated :

$$\int_{M^o_i}^{M_i} dM_i = - \frac{A.U}{\lambda_f} (T_e - T_i) \int_o^t dt \qquad (4)$$

where :

M^o_i = initial mass of ice (kg)

Finally :

$$M_i = M^o_i - \left[\frac{A.U}{\lambda_f} . (T_e - T_i) \right] . t \qquad (5)$$

or

$$M_i = M^o_i - K.t \qquad (6)$$

where K is the expression between brackets in equation (5).

The equation (5) or (6) is the simplest one that can be used to study ice melting inside a fish box. The amount of ice needed to chill to °C the fish right after capture should be calculated separately.

4. EXPERIMENTAL RESULTS

4.1 Ice melting rate

In Figure 1, typical results of the weight changes observed in a 5 box stack are shown. The tested stacks were surrounded by other stacks during the trials. These results suggest that the location of the box in a stack is important. As a matter of fact, the top and bottom boxes in a stack are the most affected by the heat transfer, due to the greater surface area; also the air speed at the top is higher than that existing around the intermediate boxes. For experimental purposes, intermediate boxes can be considered as having the same melting rate.

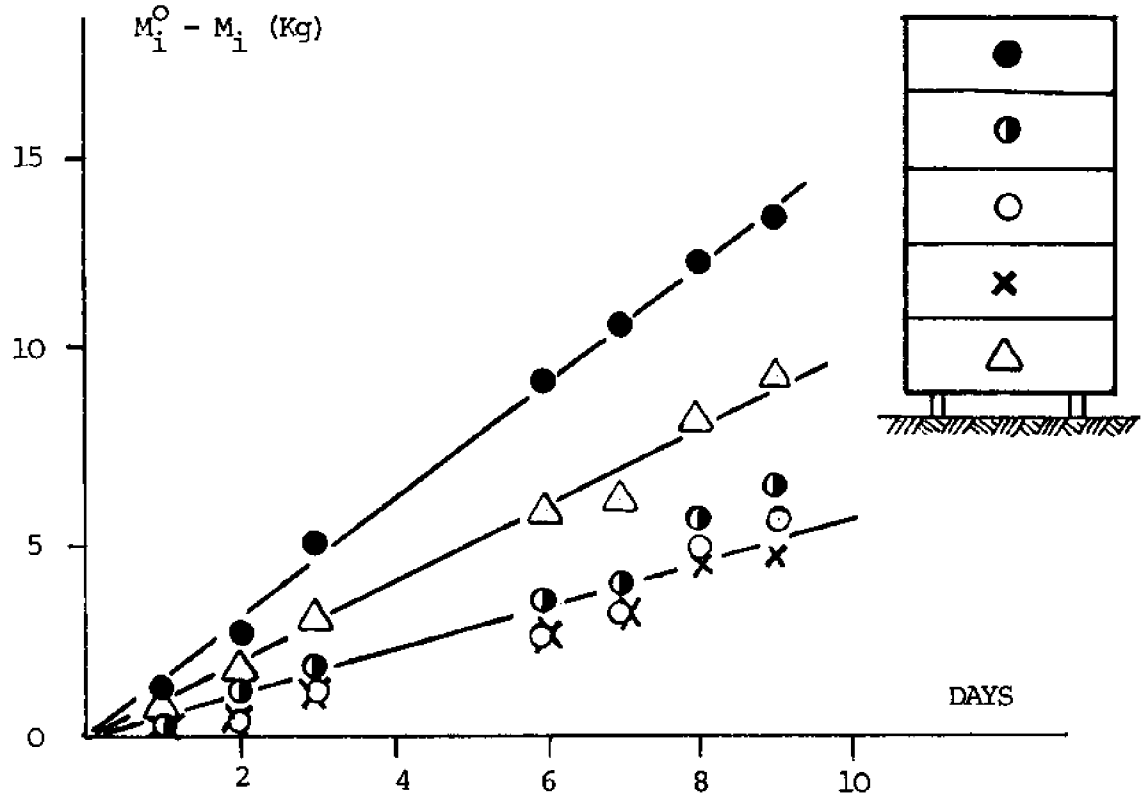

FIGURE 1. Ice melting rate during storage in a stack of plastic boxes (35 Kg net). Flake ice. $T_e = 5^oC$. Natural convection on the sides of the boxes. Industrial conditions.

Experiments were carried out at different temperatures, between 1 and 9°C, for the three types of boxes. Figure 2 shows the typical results obtained from trials using 40 Kg (net) boxes in intermediate positions.

The ice melting rate can be taken as a constant for a given external temperature. The correlation coefficients of the straight lines were always equal or higher than 0.98. Table 2 shows the K values - Eq.(6) -, obtained for the three types of boxes placed in the three positions - top, intermediate and bottom - for an external temperature of 5°C.

The ice melting rate in the top boxes was always higher than in the middle or bottom boxes. The difference with intermediate boxes is not only due to bigger area exposed, but also to the air temperature gradient existing between top and bottom and different heat transfer conditions (different heat transfer coefficient) for horizontal and vertical surfaces. In our experiments air was also allowed to circulate under the bottom boxes (racks on pallets).

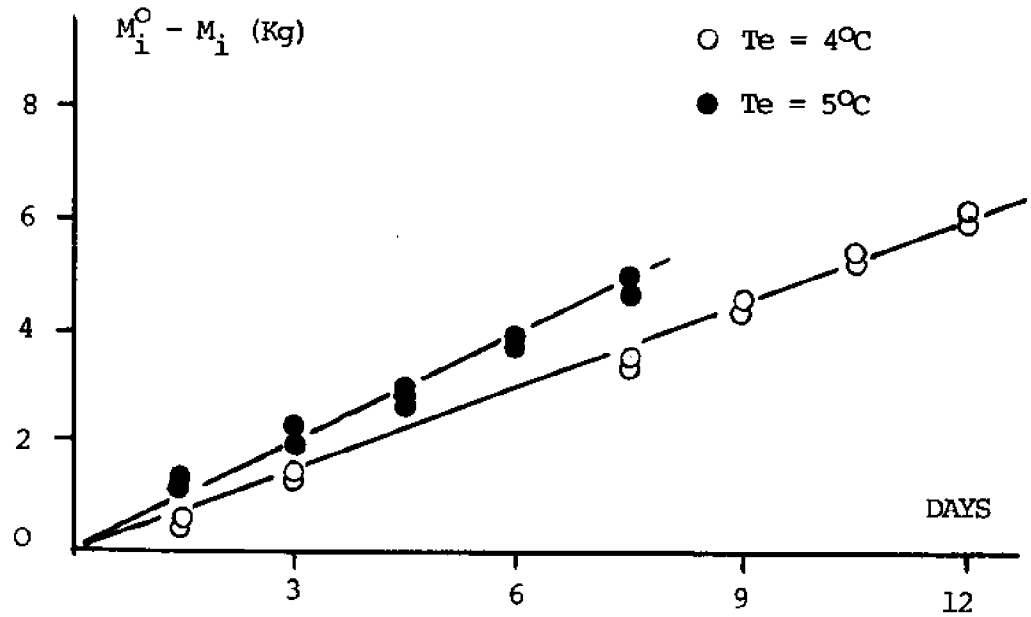

FIGURE 2. Ice melting during storage at different temperatures. Flake ice. Plastic boxes (40 Kg net). Natural convection (intermediate boxes). Laboratory conditions.

TABLE 2. Experimental K values (Eq.6). Flake ice. $T_e = 5^oC$

Box type \ Pile position	Top	Intermediate	Bottom
1. (35 Kg net)	1.65	0.85	1.25
2. (40 Kg net)	2.15	1.38	1.64
3. (45 Kg net)	2.57	1.30	1.63

4.1 Ice melting rate and temperature

It is interesting to verify the relationship given by Eqs. (5) and (6).

$$K = \frac{A.U}{\lambda_f} \cdot T_e \qquad (7)$$

assuming that $T_i = 0^oC$

Figure 3 shows the experimental relationship between K and T_e, in one of the boxes studied.

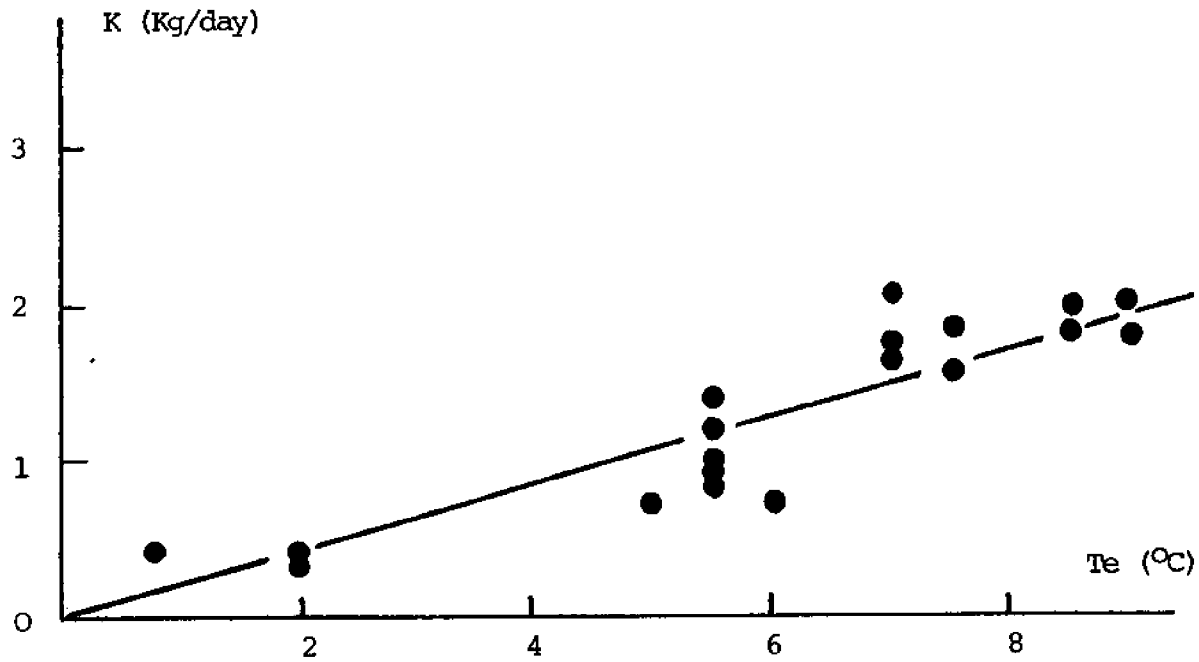

FIGURE 3. Experimental relationship between K and T_e. Plastic boxes 40 Kg net placed in intermediate positions. Flake ice.

The results of Figure 3 can be adjusted by the following expression :

$$K(Kg/day) = 0.22 \cdot T_e \ (^oC) \qquad (8)$$

with a correlation coefficient r = 0.98

The most scattered points in Figure 3,and in general in all the experiments were those obtained under industrial conditions. This can be attributed to the difficulty in keeping constant temperature under industrial conditions, and also to the difficulty in appropriately defining an average temperature in these conditions. Nevertheless, the linear relationship between K and T_e can be accepted for practical purposes as in the case of ice melting in boxes, when heat is transferred by convection.

4.3 Ice melting rate and the experimental overall heat transfer coefficient

The ratio between two K values corresponding to two different boxes stored under the same conditions can be given as :

$$\frac{K_2}{K_1} = \frac{A_2 \cdot U_2}{A_1 \cdot U_1} \qquad (9)$$

but if the overall heat transfer coefficients are the same, it may be :

$$\frac{K_2}{K_1} = \frac{A_2}{A_1} \qquad (10)$$

As the experiments with the different boxes were carried out under the same conditions, Table 3 compares the ratio between the areas exposed and the ratio between the corresponding experimental K values.

TABLE 3. Ratios between heat transfer areas in boxes and ratios between experimental K values

Box Position	A_2/A_1	K_2/K_1	A_3/A_1	K_3/K_1	A_3/A_2	K_3/K_2
Top	1.26	1.36±0.20	1.27	1.57±0.23	1.01	1.17±0.17
Bottom	1.26	1.21±0.39	1.27	1.16±0.14	1.01	0.97±0.21
Middle	1.50	1.48±0.16	1.50	1.51±0.10	1.00	1.04±0.11

1 = box 35 Kg net ; 2 = box 40 Kg net ; 3 = 45 Kg net
Flake ice - Natural convection - Temperature range : 1-9°C

Table 3 suggests that for intermediate boxes in the middle of a stack, the equation (10) holds, and only one transfer coefficent can be taken in this case. For bottom boxes, the relationship given by Eq. (10), can be accepted even though the deviations are higher than for boxes in the middle (percentage differences reach 9.5%). For top boxes, the differences reach 20% and a composed overall heat transfer coefficient should be used to analyse the results.

The experimental average value of the overall heat transfer coefficient for the intermediate boxes was :

$$U_{exp} = 46.28 \cdot \frac{Kcal}{m^2 \cdot {}^oC \cdot day} \qquad (11)$$

on a total of 162 experiments with a correlation coefficient of 0.90.

4.4 Estimated overall heat transfer coefficient

The overall heat transfer coefficient can be calculated as the summing-up of all the particular heat transfer coefficient involved.

This calculation was used to estimate the overall heat transfer coefficient for the box's side.

The general expression of the overall heat transfer coefficient is :

$$U = \frac{1}{\frac{1}{h_{ea}} + \frac{L}{K} + \frac{1}{h_{ia}} + \frac{1}{h} \cdot \frac{A_L}{A_i}} \qquad (12)$$

where :

h_{ea} = film heat transfer coefficient, air external side of the box (Kcal/h.m^2.^{o}C)

h_{ia} = film heat transfer coefficient, air internal side of the box (Kcal/h.m^2.^{o}C)

L = box wall thickness (m)

K = thermal conductivity box wall (Kcal/h.m.^{o}C)

h = film heat transfer coefficient of the air on ice (Kcal/h.m^2.^{o}C)

A_L = side area of the box (m^2)

A_i = side area of the mass of ice (m^2)

The value of h_{ea} can be calculated from the following expression (10) :

$$h_{ea} = 1.27 \left(\frac{\Delta T}{l}\right)^{0.25} \qquad (13)$$

where :

ΔT = difference of temperature between interior and exterior of the box (^{o}C), in our case, we took ΔT = 4.86^{o}C as the average temperature in our experiments.

l = box wall height (m) ; in our case l = 0.20 m

from that :

$$\frac{1}{h_{ea}} = 0.3546 \ \frac{m^2 . h . {}^oC}{Kcal} \qquad (14)$$

The box's thickness is 0.003 m, and the thermal conductivity of polietylene or poliesterene can be taken as 0.0280 Kcal/m.h.^{o}C ; then:

$$\frac{L}{K} = 0.1071 \ \frac{m^2 . h . {}^oC}{Kcal} \qquad (15)$$

The estimation of the internal film transfer coefficients is the weakest point in this procedure. Inside the box, natural convection is in a closed space, thereby it is more difficult to obtain data and correlations to estimate the film heat transfer coefficients. One possibility is to use the method of the equivalent thermal conductivity (7). In this case :

$$\frac{1}{h_{ia}} + \frac{1}{h} \cdot \frac{A_L}{A_i} = \frac{\delta_o}{k_o} \qquad (16)$$

where :

δ_o = distance between the ice and the box wall (m)

k_o = equivalent thermal conductivity (Kcal/h.m.^oC)

Taken δ_o = 0.001 m (value at the beginning of the storage)

and

k_o = 0.0208 (Kcal/h.m.^oC) - from graphics in reference (7) and according to our conditions -

$$\frac{\delta_o}{k_o} = 0.0480 \ \frac{m^2 \cdot h \cdot {}^oC}{Kcal} \qquad (17)$$

Finally, from (12),(14),(15) and (17), we have :

$$U = \frac{1}{0.3546 + 0.1071 + 0.048} = 1.962 \ \frac{Kcal}{m^2.h.{}^oC} = 47.08 \ \frac{Kcal}{m^2.dia.{}^oC} \qquad (18)$$

The U value obtained from the calculation compares quite well with the average experimental value given by (11). Nevertheless, this result was influenced by the fact that we knew beforehand the experimental value of U.

From (18), it is easy to see that in this case the overall transfer coefficient is mainly determined by the air film outside the box and the box wall. In insulated containers, the preponderant factors will be the same, but wall heat transfer resistance will be the most important.

Probably, in insulated containers, the internal heat transfer resistance (δ_o/k_o) could be left out in calculations without introducing large errors.

5. DISCUSSION AND CONCLUSIONS

When we started to carry out the experiments, we expected large deviations from the simplest model described above, mainly due to the complexity of the models reported earlier on and to the well known difficulty arising from heat transfer problems involving phase change. Nevertheless, the results obtained here are quite acceptable from a practical point of view. The more elemental approach has proved useful in studying the problem of ice melting inside fish boxes or containers.

Although it is feasible to estimate the overall heat transfer coefficients, experimental trials are recommended mainly when materials and conditions are not well known. Although calculations were not included here, it is possible to obtain experimental values of the overall heat transfer coefficients for tops and bottoms of boxes, using the geometry of the boxes and the well determined overall heat transfer coefficient for the box walls.

During the experiments, deviations were observed when ice stored in the open air ("old ice") was used. In these cases, ice in boxes did not last as long as was predicted. Calorimetry determinations have shown that this ice contained liquid water when experiments started. This means that a percentage of the initial ice weight was not ice, but water around and inside the ice flakes or pieces. Table 4 shows the experimental values of water in "equilibrium" with ice.

TABLE 4. "Equilibrium" water content of ice stored at 27°C

Ice type	"Equilibrium" water % (w/w)
Flake ice	12 - 16
Crushed block ice	10 - 14
Ice 'chips'	16 - 20

Water in "equilibrium" with ice is a function of ice type, flake or piece sizes, and storage temperature. There is no water in "equilibrium" at or below 0°C, and of course, water in "equilibrium" is negligible in ice bars independent of temperature (12), but water in "equilibrium" becomes important when area per unit of weight and temperature increase. This point can really be a problem for ice utilization under tropical and semi-tropical conditions and deserves a more in-depth analysis.

In the derivation of Eq. (6), only convection (outside and inside the boxes) and condution through the walls were taken into account radiation was not considered.

Of course, radiation is negligible when boxes or containers are in the shade, but it can be important when they are handled and stored on decks or jetties directly exposed to the sun, mainly under tropical and sub-tropical conditions. In any case, it will mean an increased melting rate. In these cases, it is convenient to cover boxes or containers with a clean wet canvas (and keep it wet). This technique already used in some developing countries will reduce losses not only due to radiation, but also due to conduction/convection since the temperature outside the box or container will be the wet bulb temperature.

The only possible way to reduce ice melting due to conduction/convection is by insulation and/or cold storage. In this case, ice melting could be estimated using the method outlined in this paper.

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BACTERIA DETECTION IN SEAFOOD THROUGH NUCLEIC ACID HYBRIDIZATION

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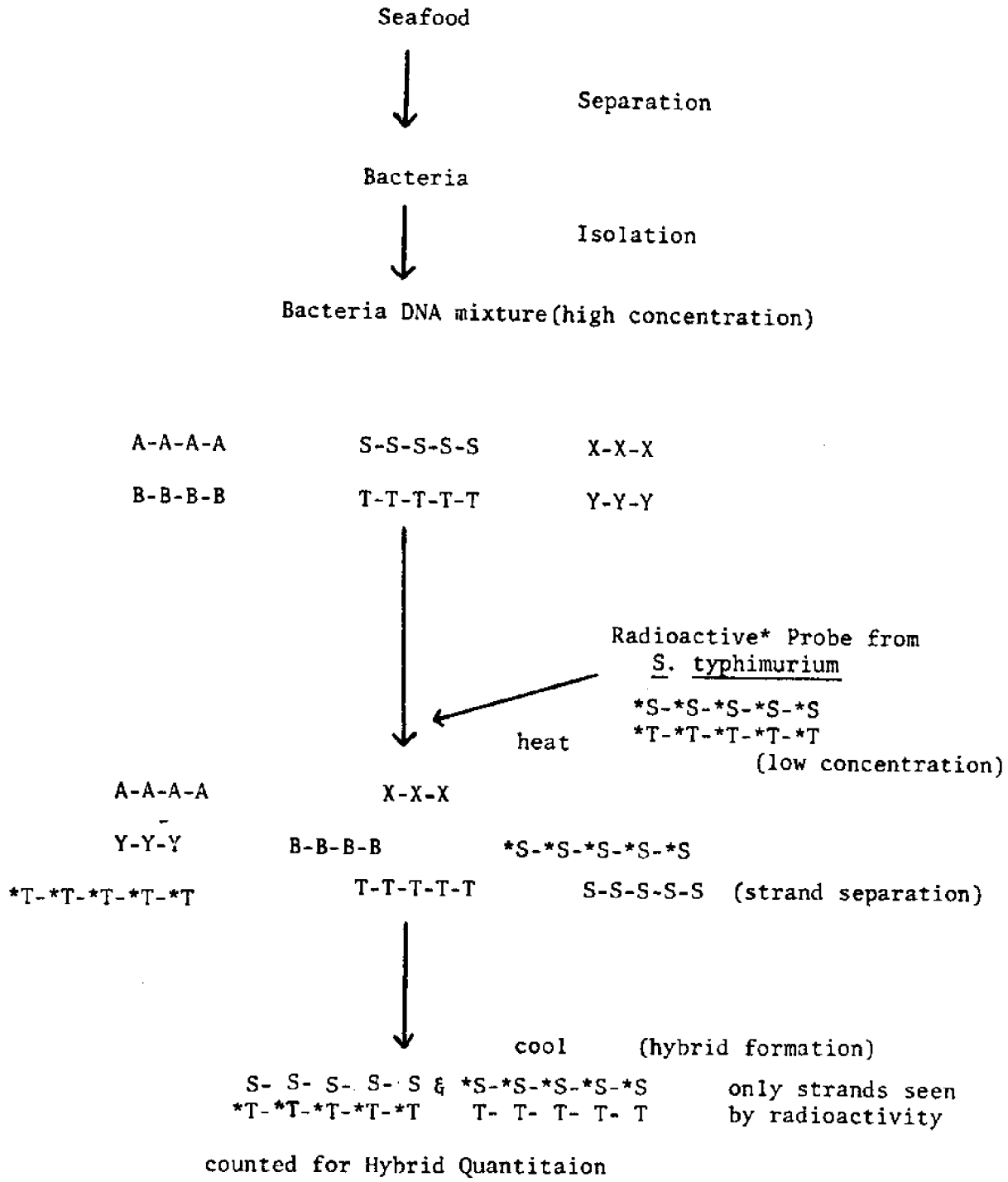
Increased screening of seafood products for microbial contamination may lower the incidence of foodborne illness attributed to seafood consumption. Current screening methods involving cultural analyses may require from two to five days for completion (1). With the restraint that long test times have on seafood production it would be more desirable to have a rapid method of bacteria detection.

While rapid methods of bacteria detection are being developed, they may not necessarily be suited for seafood screening. New rapid tests(2) include immunoassay systems such as fluorescent antibody or ELISA tests, rapid kits for biochemical analysis, gas chromatography, impedance analysis and radiometric methods. These procedures, while having some advantages over the strictly cultural methods, can still be lengthy and may require sophisticated instrumentation. Since these tests are usually based on detecting a biochemical or antigenic character of the bacteria they are susceptible to missing atypical strains.

An approach to rapid identification of bacteria that can overcome some of the problems of standard techniques and also work with atypical bacterial strains is the detection of specific bacterial DNA. In order to detect specific bacterial DNA a nucleic acid hybridization technique can be used. This technique is based on the ability of separated complementary single strands of DNA to reanneal to form a stable double stranded complex (hybrid). Since the formation of a complex (hybridization) is accomplished through the matching of base sequences, a nucleic acid strand can be used as a sensitive probe to detect its complementary base sequence. Thus a radioactive labelled DNA probe can be used to locate and bind the same bacterial nucleic acid sequence in an unknown sample.

In this study, radiolabelled DNA, isolated and purified from *Salmonella typhimurium*, was used as a screen for the same bacterial DNA in seafood and laboratory samples. If *Salmonella*, with its DNA, were present in the samples, a radiolabelled hybrid formed which was easily collected and quantitated (see Figure 1).

FIGURE 1. *Salmonella* DNA Hybridization Test



METHODS AND MATERIALS

Preparation of DNA Probe Specific for Salmonella

Radiolabelling of DNA was accomplished by growth of *Salmonella typhimurium* LT2 (ATC #15277) in media containing [^{3}H-methyl] - thymidine (New England Nuclear) in a manner similar to Mahler (3). Growth of *Salmonella* in 500 ml of minimal media (M9) containing 250μ Ci of [^{3}H-methyl]-thymidine, specific activity 20 Ci/mmol provided adequate labelling of DNA.

Labelled DNA was isolated and purified from the *Salmonella* after the manner of Miura(4). This involves: (a) lysis of labelled *Salmonella* with lysozyme and detergent treatment, (b) phenol extraction to remove and denature proteins, (c) RNase treatment to remove RNA, and (d) isopropanol fractionation to remove low molecular weight contaminants. Yields of DNA probes were about 1.5 mg from 3 grams of bacteria cell paste at a purity of at least 96%. Specific radioactivities were as high as 1×10^7 cpm/mg.

Shearing of the labelled probe to a smaller uniform size was performed by blending. Use of an Eberbach micro-blender permitted the shearing of small volumes (3-15 ml) of DNA. The DNA samples were for 5 minutes at 4°C, and frozen for storage. Probe prepared in this manner was stable for at least six months.

Isolation of Pure Salmonella DNA

Pure DNA from *Salmonella* was isolated after the manner of Miura(4). After shearing as described for the radiolabelled probe, the pure DNA was stored frozen in 0.14 M sodium phosphate buffer, pH 6.7.

Rapid DNA Isolation

Bacteria from 50 ml of broth medium were harvested by centrifugation and suspended in 0.5 ml of a 10 mg/ml lysozyme, 0.15 M NaCl - pH 8.0 solution. After a 10 minutes at 37°C the suspension was placed in a 75°C bath. Three ml of a hot (75°C) Tris-SDS solution (0.1 M Tris, 1% sodium dodecyl sulfate (w/v), pH 9.0) was added and vortexed. After an additional 15 minutes at 75°C the lysate was chilled on ice. Three ml of phenol was added and the tube was shaken for 5 minutes. After centrifugation, the upper 2 ml of the aqueous layer was removed into a separate tube. The DNA was precipitated from the aqueous 2 ml by addition of 4 ml of cold (-20°C) ethanol. (Addition of 0.1 volume of 3 M sodium acetate was sometimes used to aid in the precipitation before ethanol addition). The DNA precipitate was collected by centrifugation and dissolved in 1.0 to 1.5 ml of 0.14 M sodium phosphate buffer, pH 6.7.

DNA Hybridization

Hybridizations were performed typically in 1 ml volumes in 1 dram screw-top glass vials. Both probe (about 0.5 ug) and sample DNA (typically 0.1-0.2 mg but varied with experiment) in the vial were heated at 100°C in a boiling water bath for 10 minutes to melt the DNAs. The vials were removed and incubated at 75°C until quantitation of hybrid formation.

Quantitation of Hybrid

Hydroxyapatite(HAP) was used to separate double stranded hybrid from unreacted DNA probe (single stranded). When the hybridization mixture was mixed with hydroxyapatite in 0.14 M sodium phosphate, pH 6.7, only the hybrid was adsorbed to HAP while the single stranded probe (unreacted) was washed free. The hybrid was elutable with 0.48 M sodium phosphate, pH 6.7. All buffers were kept at 75°C. The separation was accomplished either through thermal column chromatography or a simpler and more rapid batch filtration method (5). The radioactivity in the hybrid or unreacted probe was quantitated by scintillation counting to determine the % of hybrid formation.

RESULTS AND DISCUSSION

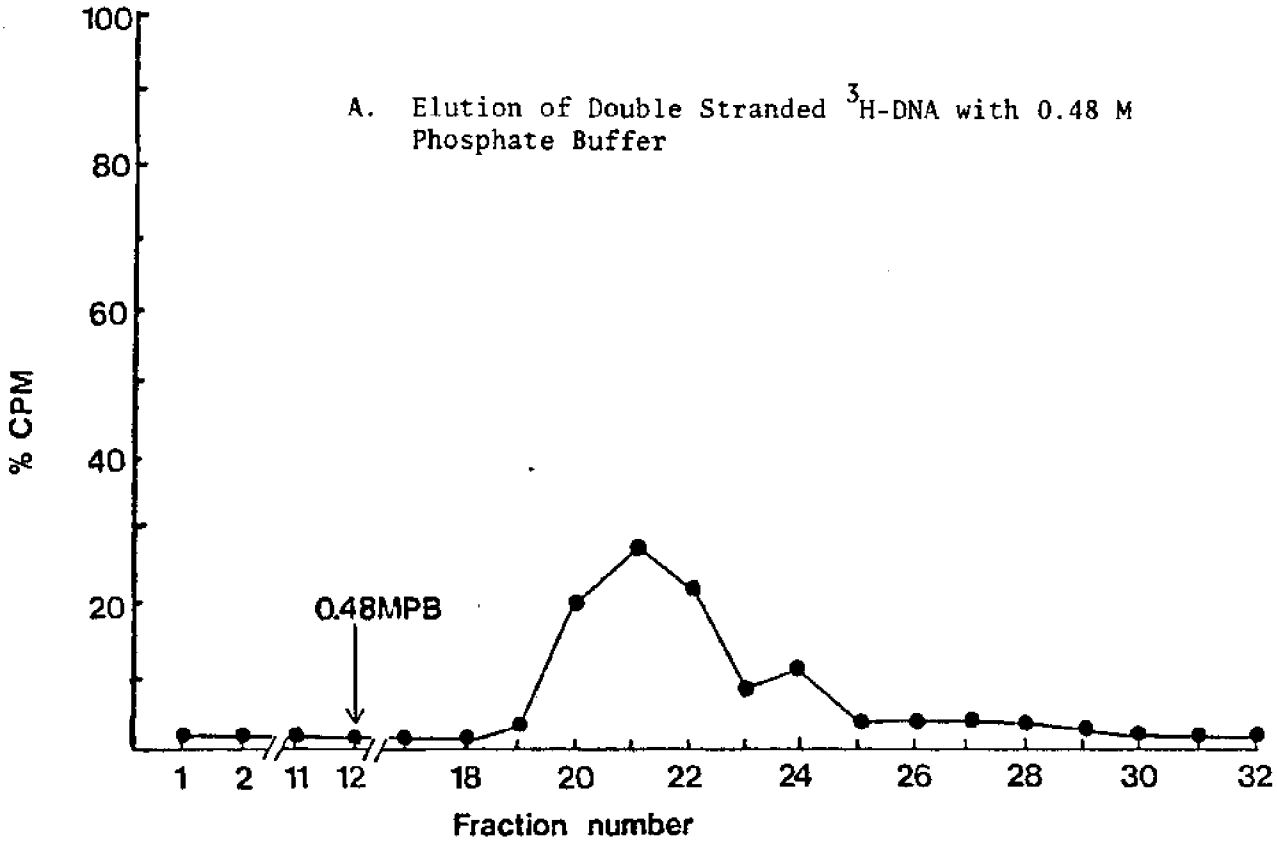
Initial Testing of Probe on Hydroxyapatite

After preparation of the [^{3}H]-labelled DNA probe from *Salmonella* we tested its behavior on hydroxyapatite. When the labelled probe was applied to a HAP column all the radioactivity (cpm) remained bound to HAP until 0.48 M sodium phosphate buffer (PB) was applied (Figure 2A). This indicated all the ^{3}H label was in double stranded DNA probe, as desired. If the labelled probe was first melted to single strands by boiling and then applied to the HAP column all the probe (cpm) eluted with the starting buffer, 0.14 M PB (Figure 2B). This finding meant we could use hydroxyapatite as a means of distinguishing whether labelled probe was single stranded or double stranded.

Hybridization Curve with Purified Salmonella DNA

The ability of the ^{3}H-labelled DNA probe to form hybrid DNA was tested with purified *Salmonella* DNA. After melting the probe, it was allowed to hybridize with melted pure *Salmonella* DNA of increasing Cot value. Cot was defined as the concentration of DNA times the length of hybridization incubation. As can be seen from Figure 3, the % reassociation (hybrid formed) increases with increasing Cot values to almost 100% of original probe bound in hybrid formation. Hybrids were quantitated by HAP thermal chromatography. Therefore, the labelled probe does recognize and form hybrid with *Salmonella* DNA and the amount of hybrid can be controlled by either the concentrations of DNA or length of incubation in the hybridization test.

Figure 2 - Hydroxyapatite Chromatography of ^{3}H-DNA Probe



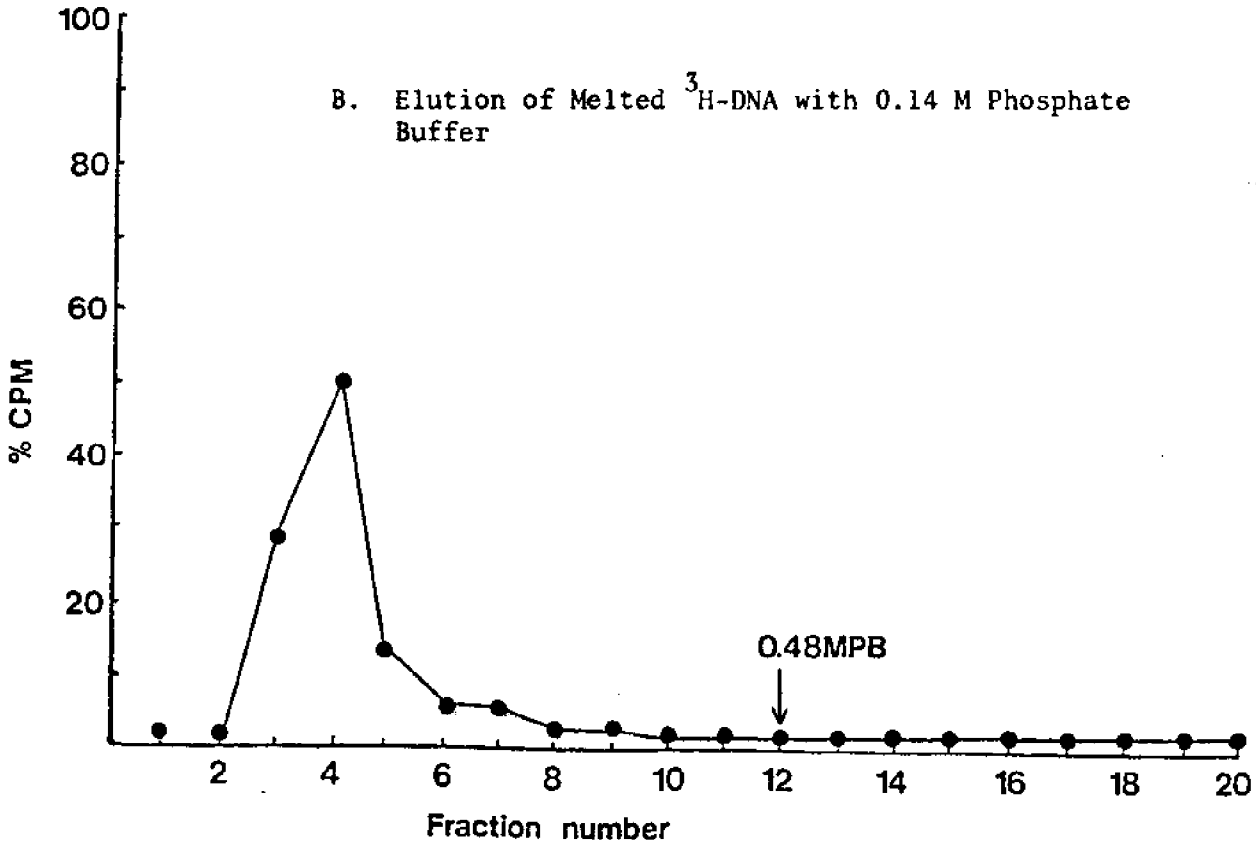
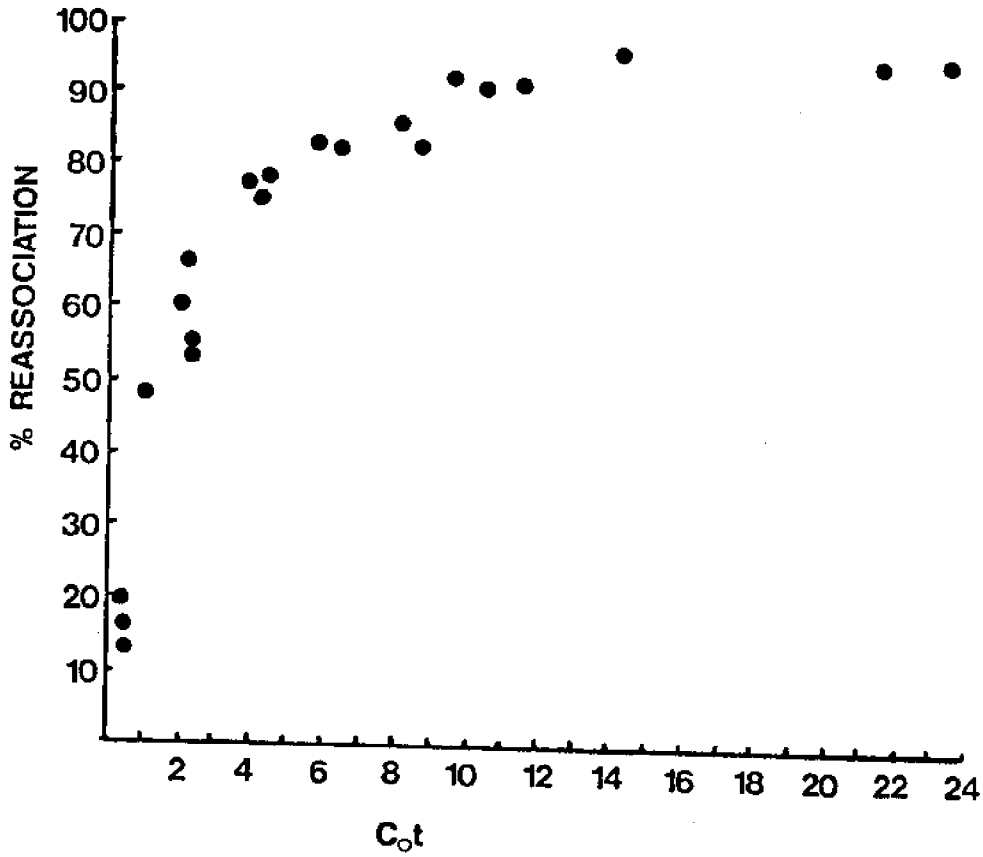


Figure 3 - Reassociation of ^{3}H-DNA Probe with Purified *Salmonella* DNA.

Cot = 10 x mg/ml of DNA x hours of incubation



Specificity of Probe with Non-Salmonella Bacteria

The ability of eight non-Salmonella enteric bacteria to hybridize to the ^{3}H-labelled DNA probe from Salmonella typhimurium. A typical experiment is shown for Citrobacter diversus (Figure 4). In this test labelled DNA probe was melted with DNA isolated by the rapid method from Citrobacter diversus and allowed to hybridize at increasing Cots. Hybrid was measured by HAP batch filtration method. As seen by the closed circles (Figure 4) there was, at maximum, only 30% cross-reactivity even at extremely high Cot values. The positive control for the experiment (open circles) demonstrated that DNA rapidly isolated from Salmonella still formed adequate hybrid. The seven other enterics tested - Pseudomonas, Edwardsiella, Klebsiella, Enterobacter, E. coli, Shigella and Proteus species yielded similar low cross reactivities with the Salmonella probe (5).

Therefore our test has the capacity to detect Salmonella DNA rapidly isolated from a sample and descriminate between it and DNA from other common enteric bacteria.

Recognition of Salmonella Related Bacteria

The hybridization of ^{3}H-DNA probe from Salmonella typhimurium with DNA rapidly isolated from an Arizona species revealed a similarity in hybrid formation (Figure 5). This was in agreement with the work of Brenner (6) suggesting a close genetic relationship with Salmonella and Arizona.

The close relationship of the Salmonella typhimurium probe to Salmonella typhi was found in a further experiment (Table I). Hybridization of the ^{3}H-probe with DNA's of Cot's over 60 demonstrated the almost equal genetic character of the two Salmonella species and, additionally, the close similarity to Arizona.

TABLE I. S. TYPHIMURIUM PROBE HYBRIDIZATION WITH BACTERIA DNA[a]

Bacteria	% Hybrid
S. typhimurium	89
S. typhi	85
Arizona	70
Shrimp flora	8

a. DNA was isolated from the above sources of bacteria by the rapid isolation technique. Hybridization analysis of the DNA's was carried out at Cot's over 60 with ^{3}H-labelled Salmonella DNA probe. Hybrid formation was monitored by the batch filtration method.

Detection of Salmonella Seeded on Shrimp

As a test of our technique's ability to detect Salmonella contaminated seafood, we analyzed shrimp either seeded or non-seeded with Salmonella typhi-

Figure 4 - Cross-reactivity of ^{3}H-DNA Probe from Salmonella with Citrobacter diversus DNA.

● - Citrobacter diversus DNA

o - Salmonella typhimurium DNA

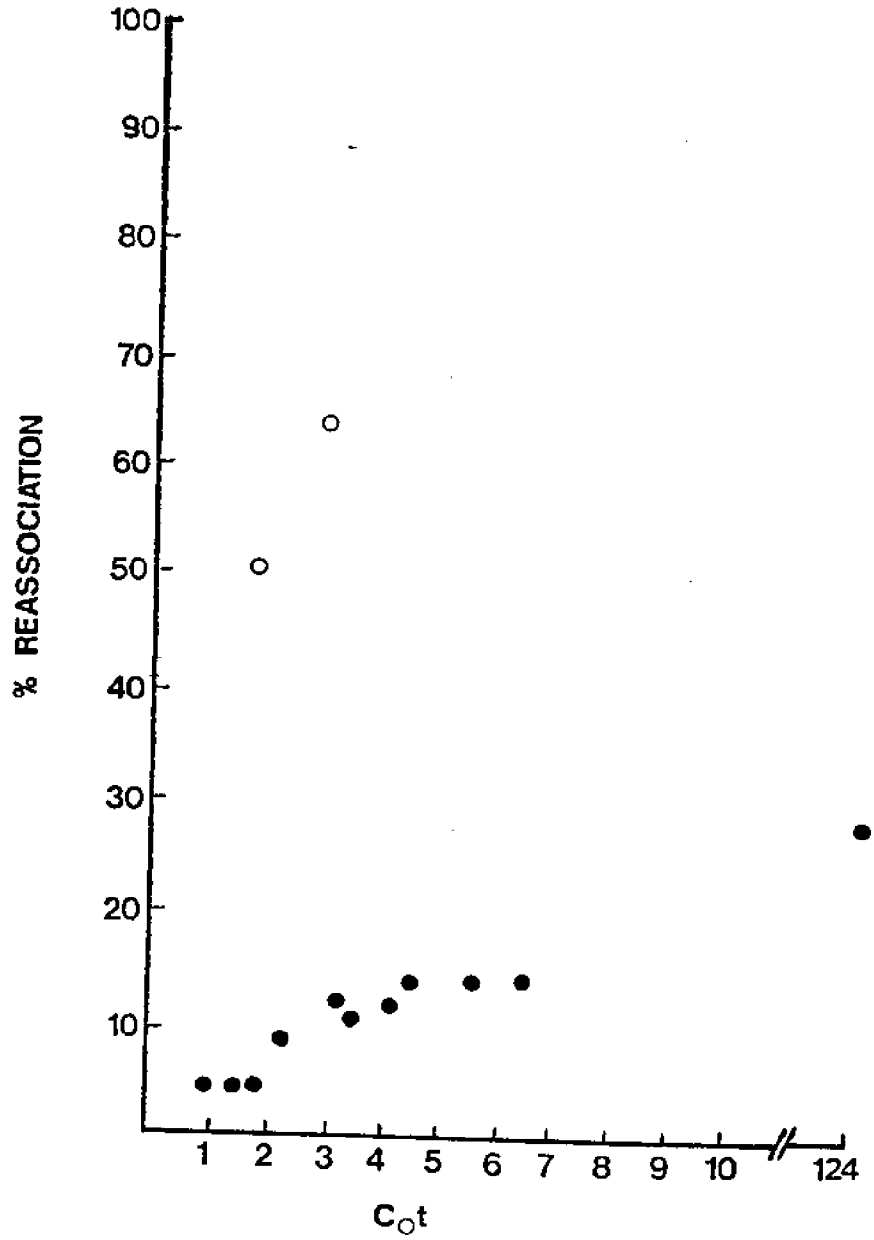
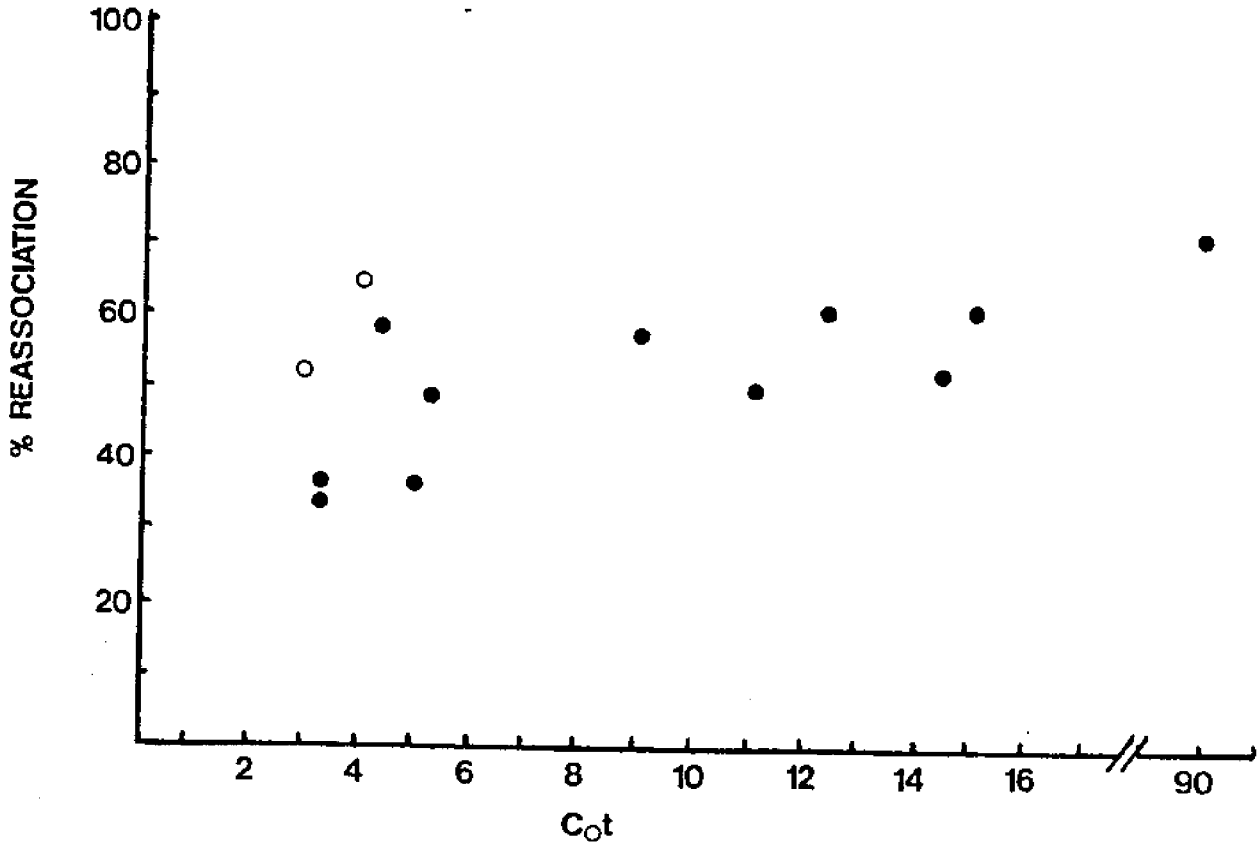


Figure 5 - Cross-reactivity of ^{3}H-DNA Probe from Salmonella with Arizona DNA

● - Arizona DNA

o - Salmonella typhimurium DNA



murium. As shown in Table II, Salmonella containing shrimp could always be distinguished from non-seeded shrimp. The test was also rapid, results were obtained only fifteen hours after the original seeding of the shrimp. These preliminary results indicated the good potential the hybridization technique has for the rapid screening of seafood.

TABLE II. Rapid Test of Seeded and Non-Seeded Shrimp for Salmonella[a]

Experiment	# Shrimp Analyzed	Seeded	Non-Seeded
		% Hybrid Formed	
1	6	70 ± 1	18 ± 1
2	6	48 ± 1	8 ± 5
3	10	35 ± 7	9 ± 4

a. Separate shrimp samples of 8 to 10 g were either seeded or not seeded with 20-50 Salmonella/g. After incubation in 50 ml of MacConkeys for 11 hours the bacteria were harvested. DNA was isolated from the bacteria by the rapid isolation technique. The hybridization analysis was carried out with ^{3}H-labelled Salmonella DNA probe.

CONCLUSION

Nucleic acid hybridization has potential as a rapid screening tool for Salmonella in seafood products. It is specific for Salmonella related bacteria, having low cross-reactivities with other enterics. The test works with mixed cultures and requires less than 24 hours for completion.

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THE POSTLARVAL OCEAN MANAGEMENT STUDY: HABITAT BASED FISHERY MANAGEMENT - AN ALTERNATIVE

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INTRODUCTION

It is a well known fact that man cannot continually take from any type of environment without replacing in one form or another what he has taken. In the case of our present marine fisheries, this is especially true.

To date, our fisheries have been managed by a wide range of laws and regulations, which according to the legislators that passed them, are designed to prevent overfishing, stabilize, and increase productivity. Unfortunately, these laws and regulations have not been entirely successful in Florida, as is evidenced in a steady decline of fishery landings over the years (7).

Excluding oysters and other marine species that are starting to be "farmed", our fisheries are still based on the age old hunter/gatherer approach of securing a food source. It is difficult, to say the least, to attemp to manage wild animals that are simply hunted and then left to their own accord to maintain their populations. Nevertheless, we attemp to secure dwindling fish stocks by implementing more laws and more regulations.

Why hasn't it worked? Why won't it work? Maybe it is because we have been concentrating on managing man the fisherman instead of fish the food source, or maybe it is because we have interfered with nature's management program by destroying countless miles of nearshore nursery habitat that produces next year's "crop". Whatever the real cause, one thing is for certain: if our fishing industry is going to keep up with the demand our ever growing population presents, then radical new approaches to fishery management must be implemented.

Making an analogy here, we know that there are three major factors that dictate how productive an agricultural endeavor will be: (i) the number of seeds that are planted, (ii) the composition of the soil they are planted in, and (iii) the climate in which the seeds are planted. Likewise, there are three major factors that dictate what future fishery productivity will be: (i) the size of the spawning population, (ii) the condition of the currents and water column that feed and transport the larvae from spawning ground to nursery ground, and (iii) the survival and growth of the postlarvae once they

have entered their respective nursery areas.

The first factor, size of the spawning population, is one that we have attempted to make the best of by closing seasons on (a very few) species which are spawning, or restricting fishing in spawning grounds. Although functional, and most certainly necessary, it just so happens that it is generally during the spawning season (when most species congregate) that the fisherman has his best catches, thereby making it difficult, if not impossible, to close season for those species. Florida's west coast mullet roe fishery, which has shown an incredible drop in productivity (7), is a prime example. If standing stocks continue to decline however, we will be forced to close such seasons for the preservation of the species, no matter how many fishermen, wholesalers, packers, and the like are put out of work.

There is absolutely nothing we can do about the second factor controlling fishery productivity: current and tidal dispersment of eggs and larvae from spawning ground to nursery ground. It would be very nice indeed if we could somehow funnel larvae and metamorphosing postlarvae into calm productive nursery areas, because it is this stage of development that represents the number of "seeds" that are "planted" for the future fishery.

For the nearshore species that require it*, nursery habitat, the third and probably most important factor controlling what future fishery productivity will be, is one that we are capable of taking full control of and yet have done very little with.

Year after year, coastal development in the form of seawalls, dredge and fill operations that kick up habitat (seagrasses, etc.) choking silt, and pollution has been destroying shallow water nursery by the thousands of acres, and year after year we have to hunt harder and harder to catch the same fish we did the years before. I cannot present any hard data correlating habitat loss with subsequent declines in adult populations (to the best of my knowledge no such study has been done), and I'm not claiming that loss of grass beds, intertidal zones and drops in water quality are the <u>only</u> factors responsible for declining (pertinent) stocks. The point I'm getting at is that many of our fisheries are starting to get in trouble and that there's a good chance nursery habitat has something to do with it.

The Postlarval Ocean Management Study (POMS) is based upon a simple fact coupled with a simple theory and is designed to

*The rest of this paper deals with species whose offspring require nearshore nursery habitat.

answer a basic question concerning the role that nursery habitat plays, and can play, in fishery productivity. Rates of highest mortality in the marine environment occur during the early life stages of the animal. Therefore, it stands to reason that if these early life stages were protected and ensured a high survival, that the adult populations would benefit.

In other words, can a man-made structure significantly increase a fisheries seed stock survival to a point where the adult population is directly increased? Can man-made nurseries replace those destroyed by coastal development? Can a fishery be managed with critical life stage habitat? We know that artificial reefs increase productivity on barren sand bottoms (10), so why can't artificial "reefs" be used to increase nursery productivity? Not that I'm all for an artificial ocean or anything like that, but it's the only logical approach we have to make up for lost ground, and to increase production to meet the demand our growing populace presents.

The Postlarval Ocean Management Study is directly analogous to agricultural research conducted many years ago - research that was aimed at finding that magic combination of nutrients which sprouted the most seeds and grew the largest plants. Instead of requiring special nutrient composition for good growth and survival as plants do, young (nearshore) fish/shellfish require special habitat composition (water quality will be discussed later). Thus I've been experimenting with various habitat combinations to see which design "sprouts" and grows the most postlarvae (PL's) for different species, and have been looking at roughly how many "seeds" might be planted per acre (in an open embayment) and what the fisherman's harvest might be.

In essence, the goal of POMS is to develop a "soil" in which wild seeds and cultivated seeds alike can be planted in the ocean for harvesting, not hunting ... A blue revolution that is not based on more boats, more hooks, and more regulation.

METHODOLOGY

In order for such a nursery system to work, several criteria must be met.

First, the habitat must be an attractive "settling" substrate for the metamorphosing postlarvae. For oysters and most other bivalves, this is the stage immediately prior to spat settlement; with crabs, the megalopa stage; spiny lobsters, the puerulus stage; fish, shrimp and clawed lobsters, the postlarval stage. All in all, it is the postlarval animal that is

dependent on such habitat for survival and growth. On land, wind blown seeds will sprout when they settle in suitable soil. In the water, current drifted "seeds" will "sprout" when they settle in suitable habitat (or else get eaten).

Second, the habitat must be ecologically viable from a bio-conversion viewpoint. For every kilogram of fish produced, anywhere from 2.5 to 5 kilograms of food must be available (depending on whether or not the species is carnivorous, herbivorous, etc.). The food supply must be easily accessible, fluctuate very little, and last throughout the nursery grow-out period year after year.

Third, the habitat must provide adequate protection from predation until the animals are old enough to leave and better fend for themselves in the wild - to a size in which a calculable number could be expected to reach the adult ranks.

Last but not least, the nursery and its anchoring system must be strong enough to withstand the worst weather conditions of the area it is located in, have a lifespan of no less than ten years, and provide sufficient fishery recruits to make the entire system cost effective (including maintainance) within five years.

Looking at the first three criteria - attractiveness, biomass conversion and predation protection - and using the spiny lobster fishery (palinuridae) as an example, we find that the work of Witham et. al. (15), Serfling and Ford (9) and Phillips and Olsen (6) has met about 50 percent of required criteria. All of these researchers have developed habitat "collectors" to monitor recruitment of puerulus (postlarval) lobsters into nearshore nursery areas. Although the collectors were developed for three different species (same genus) in three different parts of the world - Florida, California and Australia, respectively - and were all of different designs (showing species specificity), they all were attractive substrates to the PL's and floated on the surface away from bottom dwelling predators.

Concentrating on a single fishery now, the Florida spiny lobster (Panulirus argus) fishery, Little and Milano (4) found that their Witham collectors developed extensive macrofloral and faunal fouling assemblages after a few months in shallow water. Little (personal communications) had to strip foulers off the habitats when they became too dense.

Going on the assumption that P. argus PL's would settle on other habitats that were well fouled and floated on the surface, I started testing designs that would (i) maximize fouling substrate, (ii) maximize niches within the habitat, and (iii) provide maximum protection from predation.

Roughly speaking, the habitat had about ten times the surface area of a same size Witham habitat (¼ m^2), and looked much like a condominium without the walls.

Since the invention of the Witham habitat collector in 1968 (15), it has been a well known fact that P. argus PL's will settle on it (3,4,14,15), and that habitat location is important (4,15): it must be fairly close to shore.

Based upon this data, I conducted a simple 2½ month experiment (zoology class, Fla. Keys Comm. College) to see if P. argus PL's would settle on a different type of floating structure, and if so, determine roughly what the survival might be to a stage in which the animals could leave the habitat and better fend for themselves in the wild.

Essentially, what I found was that the habitat (placed in shallow near-shore water) developed a very lush fouling assemblage, attracted just under the number of PL's one could expect to settle on a Witham habitat (4), and provided what appeared to be sufficient food for the young lobsters (the third criterion).

To follow growth and survival of the young lobsters, a notch was cut out of the telson. This notch was recognizable for at least two molts. To follow the growth of other species* (on similar habitats) such as the stone crab, Menippe mercenaria, and blue crabs, Callinectes sapidus, the 2 and 3 mm animals were soaked in a neutral red solution for three days. For blue crabs, the stain was recognizable for up to seven molts, for the stone crabs, at least two molts (after which time they are retagged).

RESULTS AND DISCUSSION

Although my spiny lobster habitat experiment was somewhat weak (time wise), it did show that P. argus PL's would settle on the habitats in numbers close to those recorded for Witham collectors (4), and that at least 20 percent of the PL's (7 mm carapace length, CL) survived (stayed) on the habitats for about 2½ months, at which time they averaged about 20 mm CL - just under one third mature size. Throughout the study period, PL's were constantly settling on the habitats during new moon phases (conforming with lunar regulated settlement reported by all others in the field (3,6,9,15), and by the end of the study, the ¼ square meter habitats were supporting anywhere from 15 to 37 animals each, ranging in size from 7 to 21 mm CL. One sampling trip yielded a 100 percent tag return (20 days, 14 out - 14 in) from one of the habitats (of 4 tested).

In essence, what I found was a habitat that met the first three aforementioned criteria by (i) being attractive to the metamorphosing PL's, (ii) providing an apparently abundent food supply in the form of an extremely lush floral and faunal fouling

* B.A. Thesis, New College

assemblage, and by (iii) apparently eliminating predation by floating on the surface away from bottom dwelling predators. The fourth criterion, cost effectiveness, could possible be worked out through plastic research, or maybe even bamboo. My habitats were made out of fiberglass and rope, materials alone ran well over $50.00 per square meter of habitat.

Biological considerations concerning the carrying capacity of the habitats and biomass conversion (to lobsters) is a detailed and complex matter to say the least. Addressing this matter briefly, I have found that the habitats' carrying capacity is governed by (i) nutrient availability for algae growth, (ii) phytoplankton, zooplankton, and suspended organic matter availability for faunal fouling assemblages and associated (target species food) organisims, and (iii) the physical (niche) characteristics of the habitat itself.

What all this boils down to is selecting the proper location for habitat deployment and designing the physical characteristics of the habitat to meet the needs of the target species. Locations within three to four hundred meters of shore near areas which are conducive to detrital-based food chains (mangrove or seaweed) should be ideal. For species that cannot be reared from the egg to postlarva in the lab, such as P. argus, the habitats must be placed where natural PL recruitment occurs (this can be determined with the Witham habitat). For species which can be spawned and reared to postlarvae in the lab (virtually all other species of commercial importance) for "seeding", natural PL recruitment is not mandatory, but would be desireable. Physcial characteristics of the nursery habitat should be species specific so that (hopefully) the target species is at the top of the systems food pyramid. For example, one would not want to deploy a nursery system which is meant for grouper (serranidae) that also happens to be attractive for snapper (lutjanidae) but doesn't provide the right cover for the snapper. What would happen is that the grouper would probably eat the young snapper and all you end up with is grouper while the snapper are wasted (or something like that). So, a happy medium must be found to meet the specific needs of both fish.

Of major importance here is the possibility of using such nursery habitat as a "soil" in which PL's can be planted. Mariculture technology today is at the point where spawning and rearing of larvae is becoming somewhat of a minor concern compared to the actual growout of the animal, and seeding programs are not by any means new ... They just haven't met with alot of success yet. I'm sure the release sites for seeding programs are the best the biologists can find, but are they really sufficient? Can they be improved?

When we think about it a little, we would hardly plant corn in an area conducive to rice culture or potato culture. Or would we? Not too many hundreds of years ago farmers had to make this discovery for themselves. Likewise, the ocean farmers of the future will have to make the same discoveries if we're going to have any ocean harvesters left ... The fishermen.

Concentrating on a single fishery again, the Florida spiny lobster fishery, Little and Milano (4) show that 4, 125 collector sampling days covering three different areas (sand, rock, and grass) produced 16,465 PL's. This represents about 4 PL's settling per ¼ square meter habitat per day, during their 6 day lunar sampling quarter. Close to the same number of PL's settled on my habitat designs. Because of the apparently significant number of collector sampling days present in Little and Milano's work, I will use this data for theoretical considerations involving an artificial nursery habitat designed specifically for this species.

Prior to getting into theoretical considerations, I must caution the reader to keep in mind that it has not been determined if a full square meter habitat is four times as productive as four ¼ square mater habitats, or if a meter wide strip of habitat one hundred meters long is a hundred times as productive as a single square meter habitat.

Assuming that 24 PL's will settle on a ¼ square meter habitat per (lunar) month, and that 288 would settle in a year, then we could expect about 1,152 PL's to settle on a full square meter habitat in a year. Looking at a square kilometer of habitat, just over a billion PL's should settle, with 2 square kilometers, about 2.3 billion (don't scream yet). If one percent of the spawn of 2 million female lobsters (about ½ of landings) returned to the Florida fishery, this would represent about 20 billion PL's (not including possible recruitment from the Caribbean (2,5)).

Naturally, this theoretical habitat would have to be spread out in "plots", each plot covering perhaps a hectare. Thirty percent of each hectare would be habitat. The habitat itself would have to be arranged "strips" a meter wide (to allow for good circulation within the habitat) and perhaps two hundred meters long. Distance between each strip should be about 3 meters to allow for good water circulation and PL accessibility. The habitats would be placed over sand bottoms, and ideally have sea bird perches every couple meters to aid in algae growth. Materials and anchoring need to be worked out.

A question to consider here is whether or not the artificial nursery would take PL's away from natural nursery habitat. If it did, this would be fine because it would take some of the work load off of the natural nursery habitat, possibly allowing for better growth and survival of the PL's that did settle there.

What about hurricanes? The entire system must be designed to "flow" with the swells and yield as little resistance as possible. One possibility would be to have the floatation system air filled so that a plug can be pulled and everything sinks to the bottom.

There are many "what if's" and "what about's" that need to be looked at, for sure. So without getting off the track trying to figure out if such a system is possible or not, let us look at what the theoretical possibilities are.

If only 5 percent of the PL's survived for the first three months after settlement (not an unreasonable assumption) and reached about 20 mm CL, and if 5 percent of the 20 mm animals reached maturity (.25% survival from PL), then just under five million adult (pounds) lobsters worth somewhere around 12 million dollars ('82 dockside) would enter the fishery.

Todays Florida lobster fishery lands about 5 million pounds annually (Fla. Dept. Nat. Res.), so theoretically, two square kilometers of properly constructed and deployed nursery habitat could double the landings - assuming that virtually all harvestable animals are caught at the end of the season. Japan has used highly structured species specific artificial reefs to increase fishery landings anywhere from two to ten fold ( ). Why can't we? Actually it's ironic to say that such a system could double our lobster landings ... it would just bring it back to what it was about ten years ago (7).

Dealing with the same type of floating nursery habitat, but applied to a different species - the stone crab, Menippe mercenaria - in-depth data on megalopa recruitment into the habitat, and subsequent juvenile emmigration out of the nursery, indicates that such a system could indeed impact the adult fishery (B.A. thesis). What's really exciting about this habitat though, are its diverse applications.

Unlike the spiny lobster nursery which is dependent on wild PL recruitment, the stone crab nurseries could easily be seeded with megalopa or first crab animals. With stone crabs this is most important because carrying capacity of the habitat appears to be limited by cannibalism, i.e., it looks as though no matter how many PL's go in, only a set number of juveniles will come out. Laboratory experiments show it is cannibalism. If a fishery was to be based on such seeding of habitat with very limited carrying capacity, then the seeding effort would be a minimal task.

Based on the field evaluation of four protype nurseries and wild PL recruitment, I would estimate that 80 PL's seeded per square meter of habitat (each square meter of habitat has about 10 square meters of surface area) per month (20 PL's per

week) would produce 16 juveniles of 25 mm carapace width (CW) per month after a three month nursery growout, or a 20 percent PL to juvenile survival (water temp. 19° C or higher). Juveniles are habitat dependent (1), so if a post-nursery habitat yielded a 10 percent survival to maturity, then a single square kilometer of nursery habitat seeded at 80 PL's per month for six months of the year (April through September, mid-west coast of Florida or south) could theoretically produce about 9.5 million crabs worth about 6 million dollars a year (using 5 claws/pound @$3.00, both claws taken). Two square kilometers of such seeded habitat could theoretically double the pounds of stone crab landed in 1977 (averaging the landing figure given by Bert et al (1) and Sullivan (13)).

Looking at nursery habitat which might augment the "wild" stone crab fishery is difficult because there is a total lack of PL recruitment data for the fishery other than what I've collected for a single site. Fortunately, a quantitative collecting device, which acts exactly like a Witham PL collector for P. argus but is for stone crab PL's, was developed and can be used in the future to indicate areas and rates of recruitment for a wide geographical area (B.A. thesis). If the collectors indicate that stone crab PL's settle in the required geographical area for a large scale nursery, and that they settle in sufficient strength, then the same theoretcial results for seeded habitat should apply.

An interesting application of the stone crab habitats, which is presently getting under way, is to install them into one of Sarasota Bay's latest coastal development projects - 41 piers, 40 to 120 feet in length. The corporation, which is a major coastline developer in Florida, is willing to make this and future developments work for the marine environment, thereby attempting to offset nearshore nursery habitat destruction resulting from past (and future) construction. Based on natural PL recruitment and prototype nursery testing at the proposed pier site, it appears as though the nursery habitat installed into the piers could produce about 70,000 25 mm CW juvenile stone crabs per year. With juvenile/sub-adult habitat (in development) on about 7 percent of the bottom adjacent to the piers, this coastal development project could theoretically produce about 17,000 adult stone crabs annually, worth about 11 thousand dollars. However, this depends on whether or not the post-nursery habitat yields a final growout of 25 percent, which it is ideally designed to do. Cost for the entire project initially was estimated at about 60 thousand dollars, but if design and material modifications keep on the right track, this estimate could possibly be cut in half.

A third application of this stone crab nursery would be to use it to replace natural nursery habitat which has been destroyed by not only coastal development, but man's encroach-

ment upon the coastlines as a whole.

In Sarasota Bay alone, our activities have destroyed as much as 40 percent (12) of the seagrass beds used by a wide range of commercially important juvenile and adult species. Subsequently, sport fishermen blame poor catches on the commercial fishermen; there are more sport fishermen than commercial fishermen, so the commercial fishermen blame it on the sport fishermen. All fishermen blame poor fishing on pollution, on coastal development, on dredge and fill ... whatever they can. One fact is for sure though: we have a problem. It must be dealt with before it's too late, and to do so we must gain a better understanding of our last and least understood earthly frontier, and what she requires to stay alive ... and keep us alive.

Although I've only mentioned two species which would be applicable to this type of a system, two other species, the blue crab, *Callinecties sapidus*, and the bay scallop, *Pectin irradians*, both settled on and utilized the floating stone crab PL collector habitats for at least a month indicating applicability to the concept; shrimp, *Penaeus* spp., appear as promising as the clawed lobster, *Homarus americanus*, because the late larva and metamorphosing postlarva swim in near-surface waters (10). For species whose postlarvae are strictly benthic and never enter the shallows, benthic nurseries can be developed once the requirements of the particular species are identified.

In conclusion, I would like to state that it is my firm belief that virtually all fisheries of importance to man can be "managed" with the proper habitat system, extremely polluted areas excluded (but challenging). The pelagics present the greatest challenge, and perhaps with wave powered upwelling pumps to provide intensive nutrients for phytoplankton which is at the base of an intensive fishery, the pelagics too can be managed. As we have had our green revolution, so must we have a blue revolution!

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