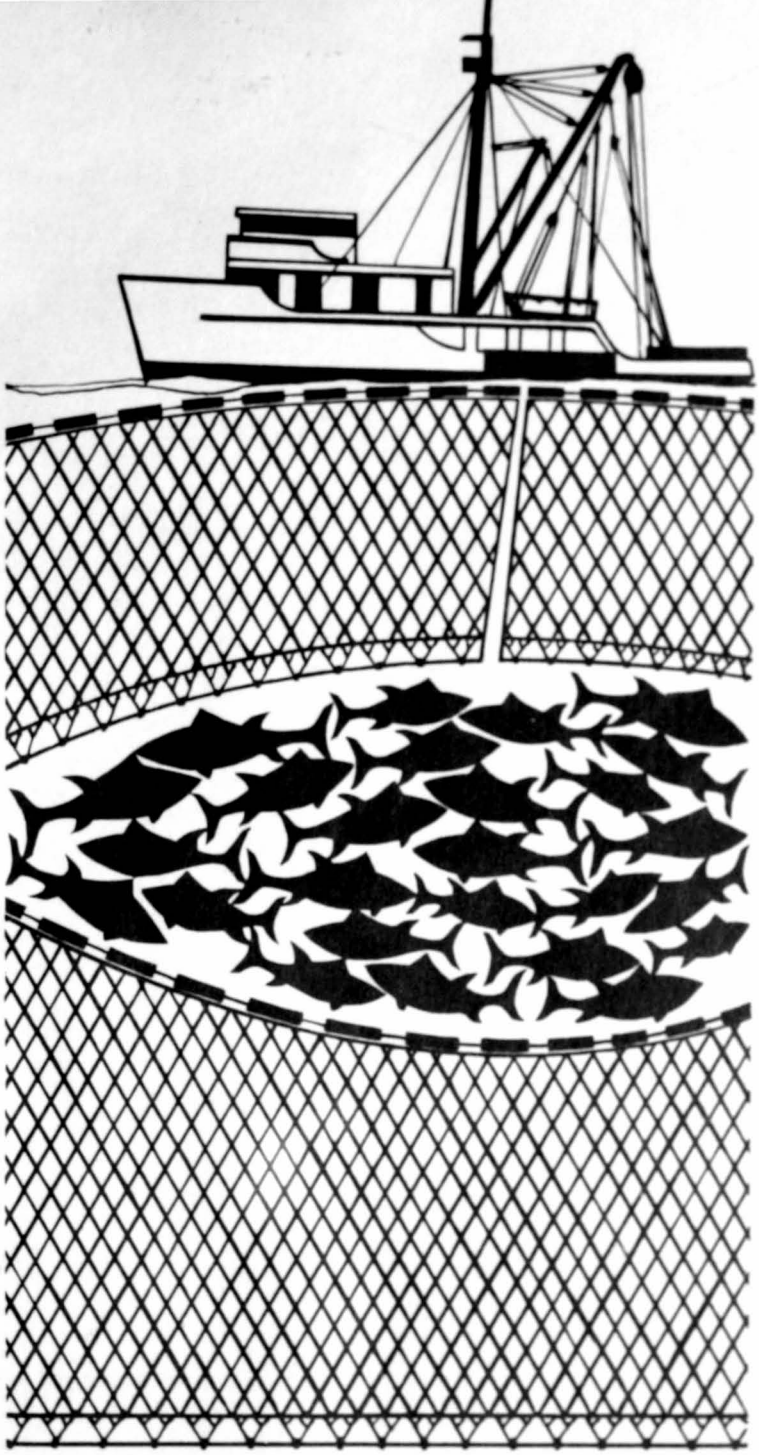

FISHERY INDUSTRIAL RESEARCH

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VALUE OF MENHADEN, *Brevoortia tyrannus*, MEAL AS A PROTEIN SUPPLEMENT TO COTTONSEED MEAL-CORN DIETS FOR PIGS

by

Robert R. Kifer and Edgar P. Young

ABSTRACT

Pig diets composed primarily of cottonseed meal and corn are deficient in the amino acid lysine. This study reports on whether supplementation by menhaden meal can supply the lysine needed. A significant improvement in rate of weight gain and in utilization of feed resulted when menhaden meal was fed as a feed supplement. No fishy flavor was detected in loins of pigs fed diets containing as much as 0.73 percent fish oil supplied by the menhaden meal.

CONTENTS

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INTRODUCTION

Although cottonseed meal has been used successfully in pig diets as a protein supplement to corn (Wallace, Albert, Ney, Combs, and Cunha, 1953; Robison, 1955), the quality of the protein in these diets apparently is not optimum for growth. For example, Miner, Clover, Noland, and Stephenson (1955) reported that supplementing a cottonseed meal-corn diet with different levels (optimum 0.1 percent) of dl-lysine produced a significant increase in the growth of pigs. Because fish meal is an excellent source of lysine (Snyder, Ousterhout, Titus, Morgareidge, and Kellenbarger, 1962; Ousterhout and Snyder, 1962), it should also improve the quality of the protein in the diet. In fact, in rat-feeding tests, the quality of the protein was increased appreciably when menhaden meal was used as a supplement to cottonseed meal-corn diets (Kifer, Young, and Leong, 1965).

Based on the known lysine content of fish meal as well as on the results obtained from the rat-feeding tests, the value of fish meal may appear to be obvious. Nevertheless, actual pig-feeding tests must be made to verify the fact that when menhaden meal is fed as a supplement in cottonseed meal-corn diets pigs grow faster. Furthermore, we must also determine if the supplementation might be undesirable for use with pigs, because fish meal, when added to diets, may impart an off-flavor to the meat (Braude, 1962).

The purpose of this study therefore was to determine the value of menhaden meal (the fish meal produced in largest quantity in the United States) as the protein supplement to cottonseed meal-corn diets for pigs. The objects of the research were (1) to verify our judgment as to the supplementary value of protein from menhaden meal for cottonseed meal-corn diets and (2) to determine if menhaden meal, at the levels of supplementation selected, give an off-flavor to the cooked meat of pigs.

I. SUPPLEMENTARY VALUE OF PROTEIN FROM MENHADEN MEAL AS A SOURCE OF LYSINE FOR COTTONSEED MEAL-CORN DIETS

In the first part of our study, we were concerned with two factors: (A) the effect of the menhaden-meal supplementation on the growth of the pigs and on their utilization of feed and (B) its effect on carcass measurements. Theoretically, a high level of protein in the diet should result in greater development of muscle and less deposition of fat. Hence, we wanted to measure not only the effect of the menhaden meal on growth and utilization but also its effect on the relative amount of muscle and fat in the carcass.

A. EFFECT OF MENHADEN-MEAL SUPPLEMENTATION ON GROWTH AND UTILIZATION OF FEED

1. Procedure

Supplementary levels of 2 and 4 percent menhaden meal were considered adequate to meet the aims of this study. Total protein levels of 16, 15, and 14 percent in the diets were used because these levels are considered to be near optimum for pigs. Described here are (a) the feed used and (b) the experimental design in the pig-feeding trials.

a. Feed used. —The three principal components of the feed used in this study were menhaden meal, cottonseed meal, and corn; these components were obtained as follows: The menhaden meal (64.73 percent protein, 11.71 percent oil) used in this study was obtained from normal commercial production. Commercially produced "degossypolized" cottonseed meal (solvent extracted) was selected from a group of about 25 samples. The selection of this meal was made on the basis of analyses for high crude-protein content (41.5 percent), low free gossypol content (0.03 percent) (Method of American Oil Chemists' Society, 1960), low total gossypol content (0.94

percent) (King, Frampton, and Altschul, 1958), and high content of lysine with free epsilon amino group (3.5 grams per 16 grams of meal nitrogen) (Conkerton and Frampton, 1959). Corn (U.S. #2 yellow) was obtained from commercial sources.

b. Pig-feeding trial. — Forty crossbred Yorkshire X Hampshire pigs of known breeding, 16.4 kilograms average weight, were purchased at a local feeder-pig sale. In addition, 16 pigs of similar breeding, weight, and age were obtained from the University of Maryland herd to give a combined total of 56 pigs. The pigs were divided into two groups and fed a 24-percent crude-protein pig-starting diet for 2 days and then an 18-percent-protein diet until selected for allotment to the experiment. From the total of 56 pigs, 36 were selected on the basis of thriftness--that is, general appearance and weight per day of age--and allotted to the experiment.

Four pigs, 2 males and 2 females, each weighing from 18.2 to 27.3 kilograms, were randomly allotted to each of nine treatments (Table 1) and fed the various experimental diets (Table 2) until they each attained about 90.9 kilograms live weight. We considered four pigs per treatment adequate for proper statistical evaluation. As is shown in Table 2, the diets fed were formulated to contain the desired levels of crude protein and were fortified (Table 2, footnotes 1-4) as needed to supply all the nutrients known to be required by growing pigs. Levels of total protein were lowered 2 percent when the pigs each attained a live weight of about 56.8 kilograms.

The dietary variables studied were levels of protein (16, 15, and 14 percent) from all sources--namely, menhaden meal, cottonseed meal, and corn. All the pigs in each lot were fed individually. Feed was offered to the pigs three times daily for a 4-week period and then two times daily for the remainder of the experiment. The pigs were held in individual crate-type pens while being fed. At each feeding, the pigs were permitted to consume feed according to appetite for a maximum time of 1 hour. Between feedings, the 4 pigs per test pen were turned out of the crates and permitted to move freely about the remainder of the pen to an automatic waterer.

2. Results

Table 3 gives the results of the pig-feeding trial.

a. With 16% total protein in the diet.— The pigs fed the cottonseed meal-corn diet containing 16-percent total dietary protein with 4 percent of the protein supplied by menhaden meal (CC12-MM4; CC10-MM4) gained weight at a significantly greater rate and utilized the feed more efficiently than did the pigs fed the diet without menhaden meal (CC16-MM0; CC14-MM0). Compared with this same diet (CC16-MM0; CC14-MM0), however, the

Table 1.—Experimental design of a trial to determine the value of menhaden meal (MM) as a protein supplement to cottonseed meal-corn diets (CC) fed to pigs

Diet designation	Relative amount of crude protein in the diet during:			Diet designation	Relative amount of crude protein in the diet during:		
	Relative amount of crude protein in the diet during Period 1 supplied by:				Relative amount of crude protein in the diet during Period 2 supplied by:		
	Cottonseed meal and corn	Menhaden meal	Total feed		Cottonseed meal and corn	Menhaden meal	Total feed
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
CC16-MM0	16	0	16	CC14-MM0	14	0	14
CC15-MM0	15	0	15	CC13-MM0	13	0	13
CC14-MM0	14	0	14	CC12-MM0	12	0	12
CC14-MM2	14	2	16	CC12-MM2	12	2	14
CC13-MM2	13	2	15	CC11-MM2	11	2	13
CC12-MM2	12	2	14	CC10-MM2	10	2	12
CC12-MM4	12	4	16	CC10-MM4	10	4	14
CC11-MM4	11	4	15	CC9-MM4	9	4	13
CC10-MM4	10	4	14	CC8-MM4	8	4	12

Note: Diet CC16-MMO; CC14-MMO means (1) that during Period 1, the diet contained 16 percent of protein and that all of this protein was supplied by cottonseed meal and corn and none was supplied by menhaden meal and (2) that during Period 2, the diet contained 14 percent of protein and that all of this protein was supplied by cottonseed meal and corn and that none was supplied by menhaden meal. The other diet symbols have corresponding meaning.

Table 2.—Diet formulations in a trial to determine the value of menhaden meal (MM) as a protein supplement to cottonseed meal-corn diets (CC) fed to pigs

Ingredient	Concentration of the ingredients in:								
	CC16-MM0	CC15-MM0	CC14-MM0	CC14-MM2	CC13-MM2	CC12-MM2	CC12-MM4	CC11-MM4	CC10-MM4
	Parts per hundred	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*
Corn (yellow, U.S. #2)	72.24	75.19	78.34	75.00	77.95	81.00	77.63	80.60	83.85
Cottonseed meal (41.50 percent protein)	25.00	22.00	18.85	19.50	16.40	13.45	14.25	11.25	8.00
Menhaden meal	--	--	--	3.22	3.22	3.22	6.24	6.24	6.24
Rock phosphate (defluorinated)[1]	1.58	1.50	1.57	1.02	1.20	1.37	.85	1.10	1.20
Limestone[2]	.50	.63	.56	.58	.55	.28	.35	.14	.03
Salt (trace mineralized)[3]	.50	.50	.50	.50	.50	.50	.50	.50	.50
Vitamin premix[4]	.18	.18	.18	.18	.18	.18	.18	.18	.18

Ingredient	Concentration of the ingredients in:								
	CC14-MM0	CC13-MM0	CC12-MM0	CC12-MM2	CC11-MM2	CC10-MM2	CC10-MM4	CCA-MM4	CC8-MM4
	Parts per hundred	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*	*Parts per hundred*
Corn (yellow, U.S. #2)	78.34	81.19	84.34	81.00	83.85	87.20	83.85	86.60	89.85
Cottonseed meal (41.50 percent protein)	18.85	16.00	12.85	13.45	10.50	7.25	8.00	5.25	2.00
Menhaden meal	--	--	--	3.22	3.22	3.22	6.24	6.24	6.24
Rock phosphate (defluorinated)[1]	1.57	1.50	1.57	1.37	1.20	1.37	1.20	1.10	1.20
Limestone[2]	.56	.63	.56	.28	.55	.28	.03	.14	.03
Salt (trace mineralized)[3]	.50	.50	.50	.50	.50	.50	.50	.50	.50
Vitamin premix[4]	.18	.18	.18	.18	.18	.18	.18	.18	.18

Note: Diet CC16-MM0; CC14-MM0 means (1) that during Period 1, the diet contained 16 percent of protein and that all of this protein was supplied by cottonseed meal and corn and none was supplied by menhaden meal and (2) that during Period 2, the diet contained 14 percent of protein and that all of this protein was supplied by cottonseed meal and corn and none was supplied by menhaden meal. The other diet symbols have corresponding meaning.

[1] Calcium 31-34 percent, phosphorus 18 percent (guaranteed analysis).

[2] Calcium 38 percent.

[3] The trace minerals as milligrams/kilogram of feed: Mn, 30.8 as manganese oxide; S, 12.5 as sodium sulfate; Fe, 9.99 as ferrous carbonate; Cu, 3.01 as cuprous oxide; I, 0.79 as calcium iodide; Zn, 0.51 as zinc oxide; and Co, 0.75 as cobalt carbonate.

[4] The vitamins as milligrams/kilogram of feed were: riboflavin, 3.08; pantothenic acid, 11.00; niacin, 37.40; choline chloride, 880.00; folic acid, 0.31; B_{12} (micrograms/kilogram), 11.0; Vitamin A as U.S.P. units/kilogram, 1498; and Vitamin D as U.S.P. units/kilogram, 150.

Table 3.—Weight gain and feed utilization of pigs fed cottonseed meal-corn (CC) basal diets with and without menhaden meal (MM) supplementation

Treatment designation	Average total gain in weight	Average daily gain in weight[1]	Average feed/gain
	Kilograms	*Kilograms*	
CC16-MM0; CC14-MM0	67.6	.57 b	3.97a b
CC15-MM0; CC13-MM0	69.4	.52a	4.19a b
CC14-MM0; CC12-MM0	69.4	.55a	3.85 b c
CC14-MM2; CC12-MM2	64.9	.58 b	3.83 b c
CC13-MM2; CC11-MM2	68.2	.63 b	3.64 c d
CC12-MM2; CC10-MM2	68.0	.63 b	3.47 d
CC12-MM4; CC10-MM4	64.1	.71 c	3.30 e
CC11-MM4; CC9-MM4	65.3	.68 c	3.34 e
CC10-MM4; CC8-MM4	66.8	.68 c	3.38 e

[1] Treatments with a common letter do not differ significantly from each other (t-test, $P<0.05$).

Note: Diet CC16-MM0; CC14-MM0 means (1) that during Period 1, the diet contained 16 percent of protein and that all of this protein was supplied by cottonseed meal and corn and none was supplied by menhaden meal and (2) that during Period 2, the diet contained 14 percent of protein and that all of this protein was supplied by cottonseed meal and corn and none was supplied by menhaden meal. The other diet symbols have corresponding meaning.

16-percent diet containing only 2 percent of the protein from menhaden meal (CC14-MM2; CC12-MM2) exhibited a similar rate of gain and utilization of feed.

b. With 15% total protein in the diet.— The inclusion of either 2 or 4 percent protein from menhaden meal in a 15-percent protein diet (CC13-MM2; CC11-MM2 or CC11-MM4; CC9-MM4) supported significantly greater rates of gain than did the 15-percent protein diet without menhaden meal (CC15-MM0; CC13-MM0). Similarly, more efficient utilization of feed was obtained from both diets (CC13-MM2; CC11-MM2 and CC11-MM4; CC9-MM4) compared with diet CC15-MM0; CC13-MM0.

c. With 14% total protein in the diet.— Similarly, the inclusion of the two menhaden meal protein levels (CC12-MM2; CC10-MM2 and CC10-MM4; CC8-MM4) in a 14-percent protein diet resulted in significantly improved rates of gain and utilization of feed as compared with a 14-percent protein diet lacking the menhaden meal (CC14-MM0; CC12-MM0).

3. Discussion

Supplementing the cottonseed meal-corn diets with menhaden meal did, in general, improve rates of gain and utilization of feed. This improvement probably is in response to the high lysine content in the menhaden meal. However, at the 16-percent dietary level, the inclusion of 2 percent protein from menhaden meal did not elicit an improvement in rate of gain or utilization of feed. One possible explanation for the lack of response from the 2-percent protein supplemental level would involve the marginal supplementary level of one of the amino acids not normally deficient in menhaden meal. For instance, Becker, Lassiter, Terrill, and Norton (1954) indicated that menhaden meal tends to be too deficient in tryptophan to supplement corn adequately. Therefore, increasing the dietary content of menhaden meal would overcome the deficiency quantitatively. The consumption of feed can probably be excluded as a factor affecting rates of gain, because the results of the multiple regression-covariance analysis indicate that the feed was consumed in adequate amounts (Table 4).

Table 4.—Multiple regression-covariance analysis of feed consumption of pigs fed cottonseed meal-corn (CC) diets with and without menhaden meal (MM) supplementation

Treatment designation	Initial pig weight (X_1)	Average daily feed consumption (X_2)	Estimated average daily gain in weight $\hat{Y}_1$	Actual average daily gain in weight
	Kilograms	*Kilograms*	*Kilograms*	*Kilograms*
CC16-MM0; CC14-MM0	21.95	2.27	.66	.57
CC15-MM0; CC13-MM0	19-95	2.16	.61	.52
CC14-MM0; CC12-MM0	20.50	2.14	.60	.55
CC14-MM2; CC12-MM2	24.68	2.24	.60	.58
CC13-MM2; CC11-MM2	21.95	2.32	.65	.63
CC12-MM2; CC10-MM2	21.68	2.21	.61	.63
CC12-MM4; CC10-MM4	25.50	2.36	.63	.71
CC11-MM4; CC9-MM4	24.00	2.29	.62	.68
CC10-MM4; CC8-MM4	23.59	2.31	.64	.68

[1] $\hat{Y} = 0.62 - 0.0034\ (X_1 - 22.45) + 0.1381\ (X_2 - 2.255)$.

The possibility of gossypol-toxicity interference also cannot be overlooked, although internal toxicity symptoms were not detected when the viscera were inspected routinely at the packing plant. In this study the highest gossypol content of the diets fed was 0.0075 percent, which is below the 0.01 percent free gossypol dietary level reported to cause toxicity symptoms (Hale and Lyman, 1957). However, the calculated total amount of gossypol--namely, 20 grams--consumed by the pigs in our study was only slightly lower than the 24.9-gram total gossypol consumption reported by Hale and Lyman (1957) to cause death. Perhaps the lower gain in weight and poorer utilization of feed obtained with the pigs fed the corn-cottonseed control diets is due to a pathological gossypol toxicity that is not apparent. Baliga and Lyman (1957) have postulated that gossypol will react with an amino group of the protein to form an insoluble indigestible complex. The quantity and quality of the protein in the diet were also shown to reduce gossypol-toxicity interference. So, the menhaden meal, in addition to creating the postulated better amino acid balance, could have functioned as postulated to block the deleterious effects of gossypol (Hale and Lyman, 1962).

Two pigs became sick during the study, one each in a group that was fed a cottonseed meal-corn diet containing either 15 or 14 percent protein. These pigs became feverish and stopped eating for 2 to 3 days. They then gradually returned to the average feed intake of their respective groups.

B. EFFECT OF MENHADEN-MEAL SUPPLEMENTATION ON CARCASS MEASUREMENTS

1. Procedure

Carcass measurements were obtained as follows: the dressing percent and lean-cut yield (see Figure 1) expressed as percent of live weight were obtained following slaughtering and butchering at a commercial slaughterhouse. Standard procedures were used to measure area of loin eye (longissimus dorsi) (Figure 2), length of body, and thickness of backfat (Figure 1).

2. Results

Although, theoretically, a high level of protein in the diet or use of a superior-quality protein should result in greater development of muscle and less deposition of fat, within the genetic limits of the animal, a statistical analysis (t-test) of the data (Table 5) did not verify this theory in this experiment.

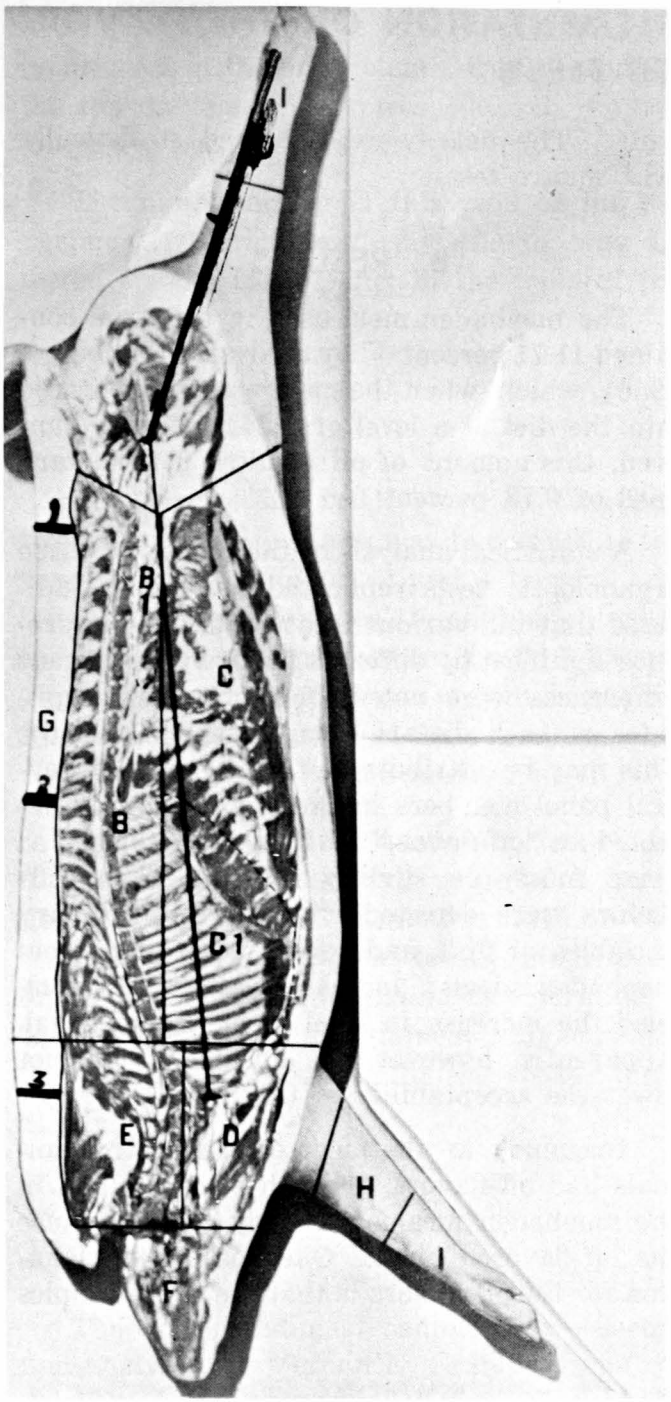
Table 5.—Carcass data obtained with pigs fed cottonseed meal-corn (CC) diets with and without menhaden meal (MM) supplementation

Diet designation	Yield		Criteria of leanness		Length of body
	Total carcass[1]	Lean cuts[2]	Area of loin eye[3]	Thickness of backfat	
	Percent	*Percent*	*Square centimeters*	*Centimeters*	*Centimeters*
1-CC16-MM0; CC14-MM0	73.9	35.67	27.60	3.25*	75.18
2-CC15-MM0; CC13-MM0	75.1	34.09	25.35	3.66	75.44
3-CC14-MM0; CC12-MM0	72.7*	33.72	22.06*	3.78	75.95
4-CC14-MM2; CC12-MM2	75.8	34.71	26.45	3.81	75.44
5-CC13-MM2; CC11-MM2	74.7	35.84	25.41	3.28*	76.71
6-CC12-MM2; CC10-MM2	75.9	34.54	25.09	3.78	73.66*
7-CC12-MM4; CC10-MM4	74.8	35.75	27.41	3.40	75.95
8-CC11-MM4; CC9-MM4	75.1	34.75	27.28	4.19	75.18
9-CC10-MM4; CC8-MM4	76.4	33.91	29.61	4.34	74.42

[1] Percentage based on live weight of aminals.
[2] Includes hams, loins, shoulders, and boston butts.
[3] Cross-section of longissimus dorsi muscle at 10th rib.
* $P<.05$.
Total carcass — Diet 3 significantly less than Diets 2, 4, 5, 6 and 7
Area of loin eye — Diet 3 significantly less than Diet 7
Thickness of backfat — Diet 1 significantly less than Diets 2 and 3
Diet 5 significantly less than Diets 3, 4 and 8
Length of body — Diet 6 significantly less than Diet 3

Nevertheless, although not statistically significant, greater loin-eye area was found if the total level of protein in the diet was increased or the level of protein derived from menhaden meal was 4 percent.

The few significant differences obtained in the various carcass measurements were not consistently related to the dietary-treatment variables. Nevertheless, these dietary variables may have elicited differences in carcass composition, but, if so, the differences were masked by the variability of the data.



AD — length of carcass, aitch bone to first rib
AI — length of leg, aitch bone to base of dew claw rear leg
A — ham
B — loin
E — shoulder (picnic)
D — boston butt
G — backfat measurements, (1) last lumbar vertabra, (2) last rib, and (3) first rib.

Figure 1.—Figure indicating positions on carcass used for various carcass measurements and of the various lean cuts.

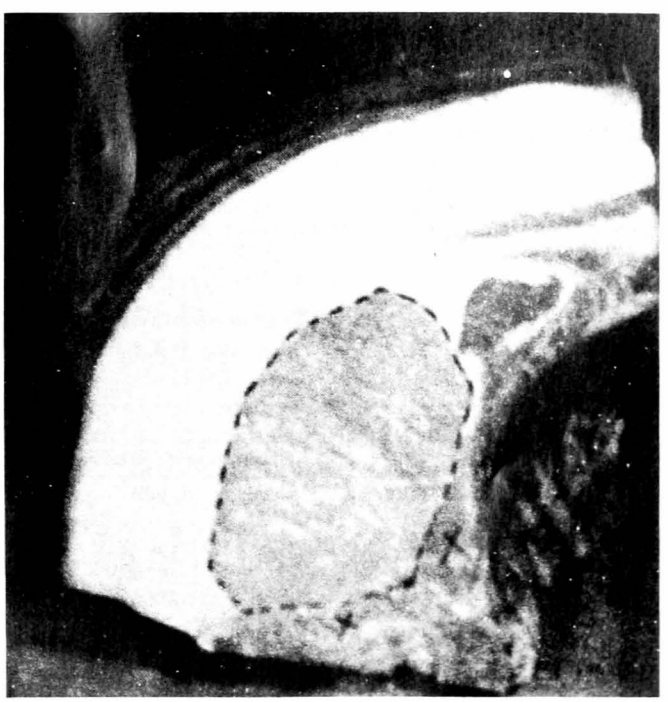


Figure 2.—Figure indicating longissimus dorsi muscle (that is, area outlined by dotted line)

II. EFFECT OF MENHADEN-MEAL SUPPLEMENTATION ON THE FLAVOR OF THE COOKED FLESH

A. PROCEDURE

After each of the pigs was slaughtered, the right loin was collected and frozen. Twelve portions, 1.3 centimeters thick, were then cut from the frozen loin, proceeding posteriorly from the 10th rib. These portions were used in a triangular organoleptic test to determine primarily if any "fish" taste was imparted to the meat of the pigs fed the diets containing the menhaden meal. Four replications of the test were made using the three experimental treatment variables of 0, 2, or 4 percent protein from menhaden meal in each replication. When 8 of the 12 panel members could pair the like samples, the difference due to the experimental variable was considered to be significant ($P<.05$).

One-half cup of water was added to each sample in an uncovered pan, and the samples were baked for 50 minutes in a gas oven at 325° F. No seasoning was added to the samples.

Upon removal of the samples from the oven, the lean meat and the fat were cut from the bone and minced together in a Hobart[1] meat slicer to a finely divided mixture. The individual samples were heated in a double boiler to 70° C., divided into about 12 equal parts, and served immediately. One test was held daily. The data were analyzed statistically (chi square test).

[1] The use of trade names is merely to facilitate the description of the experimental procedure; no endorsement is implied.

B. RESULTS

The menhaden meal used in this trial contained 11.71 percent oil by analysis (Dambergs, 1956), which, when the meal was incorporated into the diet at a level of 6.24 parts per hundred, this amount of oil resulted in a dietary level of 0.73 percent fish oil.

A statistical analysis of the results of three organoleptic tests replicated four times indicated that the various treatments did not produce significantly different flavors. Significant differences were noted for each of two replicates of each dietary comparison (Table 6). This may be attributable to the fact that several panel members in these comparisons detected an "off-flavor," which they described as being musty or slightly rancid. These off-flavors were detected with all three dietary variables of 0, 2, and 4 percent protein from menhaden meals; increased detection paralleled the increase in level of menhaden meal. Apparently, however, the off-flavors did not lower the acceptability of the meat.

Inasmuch as the meat of the control animals had off-flavors, the fish oil contained in the menhaden meal apparently did not cause the off-flavored meat. One possible explanation for the off-flavors is that the meat samples

Table 6.—Results of organoleptic evaluation of loins from pigs fed cottonseed meal-corn (CC) diets without and with supplementation by menhaden meal (MM)

Replicate	Correct identification in comparisons of:		
	Treatment 0: Treatment 2	Treatment 0: Treatment 4	Treatment 2: Treatment 4
	Number	*Number*	*Number*
1	6	7	6
2	6	6	8*
3	8*	8*	11*
4	8*	9**	6
Total	28	30	31
Average	7.00	7.50	7.75

Note 1: Treatment 0 has 0 percent menhaden meal; Treatment 2 has 2 percent fish meal; Treatment 4 has 4 percent menhaden meal.
Note 2: The taste panel consisted of 12 members.
* $P<.05$
** $P<.01$

were stored for from 2 to 3 months prior to being used in the organoleptic test. Although the storage temperature was −20° C., the fats may have become somewhat rancid.

The main finding in this part of the experiment was that no "fishy" flavor was detected by the taste panel in the loins of the experimental animals. Thus, although the samples did have off-flavors, the off-flavors did not appear to be due to the presence of menhaden meal in the diet because as was just indicated, the carcass meat from pigs fed the control had the same odors. Furthermore, the off odors were not strong enough to have masked any appreciable fishy odor.

SUMMARY

The pig-feeding trials reported here had two aims: The first aim was to determine the supplementary value of 2 and 4 percent protein from menhaden meal in cottonseed meal-corn diets at total protein levels of 16, 15 and 14 percent. The second aim was to determine if menhaden meal added at these levels imparts an off flavor to the cooked meat of pigs.

A pig-feeding trial was conducted to determine the supplementary value of 2 and 4 percent protein from menhaden meal to cottonseed meal-corn diets at total protein levels of 16, 15, and 14 percent, and to determine if these levels of menhaden-meal supplementation impart an off-flavor to the cooked meat of pigs.

The results indicated that supplementation with menhaden meal improved the rates of gain and utilization of feed when 2 percent protein from menhaden meal was added to cottonseed meal-corn basal diets. A further improvement was obtained when the menhaden meal protein level was increased from 2 to 4 percent.

No consistent trends in carcass data were observed that are related to the dietary treatment variables. In short, no relation could be found between the leanness of the meat and the quality of the protein.

No fishy flavor was detected in the loins of the pigs fed diets containing levels as high as 0.73 percent of fish oil contributed from the menhaden meal.

ACKNOWLEDGMENT

The cottonseed-meal sample was selected and analyzed by Vernon E. Frampton and coworkers, U.S. Department of Agriculture, Agricultural Research Service, Southern Utilization Research and Development Division, Seed and Meal Investigations, Oilseed Crops Laboratory, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124.

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MS #1837

UPTAKE OF OXYGEN IN REFRIGERATED RADIOPASTEURIZED FISH

by

L. J. Ronsivalli and B. L. Tinker

ABSTRACT

In cans that contained haddock fillets and that were (1) hermetically sealed at atmospheric pressure, (2) radiopasteurized, and (3) stored at about 0.5° C., the level of oxygen dropped from about 21 percent to about 2 percent within 30 days, at which time the percentage of oxygen was still falling. This decrease in the concentration of oxygen indicates that the atmospheric environment within the cans was conducive to the growth of aerobes, microaerophiles, facultative anaerobes, or anaerobes at various stages of oxygen depletion during the storage.

CONTENTS

INTRODUCTION

In the preservation of most foods, the need to exclude oxygen from packages to prevent quality-degrading reactions from occurring during storage--for example, rancidity and changes in color--is well known. Researchers who have tested the feasibility of preserving seafoods by pasteurizing them with ionizing radiation have reported undesirable consequen-

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ces of oxidative reactions in some samples packed with exposure to air. Dassow and Miyauchi (1965) have reported on such reactions.

The adverse effects of the presence of oxygen on the composition of the surviving microflora in radiopasteurized seafoods have also been of concern to researchers (Licciardello, Ronsivalli, and Slavin, 1966; Sinskey, Pablo, Silverman, and Ronsivalli, 1967; and Silverman and Sinskey, 1968).

Because of the ubiquity of spores of *Clostridium botulinum* and because of the suitability of most seafoods as a substrate for these organisms, whatever advantages are to be gained by excluding air from the package may be negated by the increased risk of botulism (should the product be mishandled and its temperature allowed to rise during storage). But the risk of botulism may not be restricted to anaerobically packed seafoods. Localized anaerobic sites can conceivably develop in containers even though air is not removed from the headspace before the container is sealed, especially when the package is not permeable to gases. Although the oxidation-reduction potential, not the oxygen tension, governs the growth of strict anaerobes, oxygen is nevertheless a factor in the establishment of the level of the oxidation-reduction potential.

Because of our growing interest in the role of oxygen in stored, radiopasteurized seafoods, the purpose of the study reported here was to determine the changes in the percentage of oxygen in the headspace of hermetically sealed cans containing radiopasteurized fillets stored at about 0.5° C.

I. EXPERIMENTAL METHODS

Described here are the methods used in the preparation of the samples contained in the hermetically sealed cans and the methods used in the measurement of the concentration of oxygen in the headspaces of these cans.

A. PREPARATION OF SAMPLES

This section reports on the source of the fillets used as samples and the method of handling these fillets and the cans in which they were packed.

1. Source of Fillets Used as Samples

Because haddock (*Melanogrammus aeglefinus*) have been investigated extensively in this laboratory, they were used as the source of the fillets studied in this work.

2. Method of Handling Fillet Samples and Cans

This subsection describes the method of packing the fillets in cans, the modifications of the cans to permit the headspaces to be sampled, and the method of irradiating and storing the cans.

a. Method of packing the samples in cans. —Commercially cut fillets of haddock were packed in modified No. 2½ C-enameled cans in such a manner that the headspace of each can was about 75 milliliters.

b. Modification of cans to permit sampling of headspace. — Before we sealed a can, we modified its lid by punching out a small hole in the center and by sealing an injection-port septum over the hole. The purpose of this hole was to permit us to sample the headspace gas of the can, by means of a syringe, without destroying the hermetic seal.

c. Irradiation and storage of the cans.— The cans were irradiated at a level of 250 kilorads and stored for up to 30 days at about 0.5° C. Connors and Steinberg (1964) have described the details of irradiation and storage.

B. MEASUREMENT OF THE CONCENTRATION OF OXYGEN IN THE HEADSPACE

To check on our measurements of the change in the percentage of oxygen in the headspace

of each can during storage, we used two independent methods for determining the percentage: namely, the polarographic method and the gas chromatographic method.

The polarographic method, with an accuracy of ±1 percent, was the simpler of the two to use. Furthermore, its use required no calculations. On the other hand, the gas chromatographic method, with an accuracy better than ±1 percent (see Figure 3, Peaks A and B), made possible nondestructive multiple analyses, which could not be made with the equipment that we used in the polarographic method. In addition, the gas chromatograph was better suited for analyzing for carbon dioxide simultaneously, if we had decided to do so.

1. Polarographic Method

The equipment used was a Beckman Model 777 Oxygen Analyzer[1] featuring a patented polarographic oxygen sensor, which measures the partial pressure of oxygen directly. The sensor was connected to a Beckman headspace sampler, which in turn was attached to the can as is indicated in Figure 1. Details of the equipment and of the method of use are available from the manufacturer.

2. Gas Chromatographic Method

Described in this subsection are (a) the equipment used, (b) the method of sampling

[1] Trade names are used merely to simplify descriptions; no endorsement is implied.

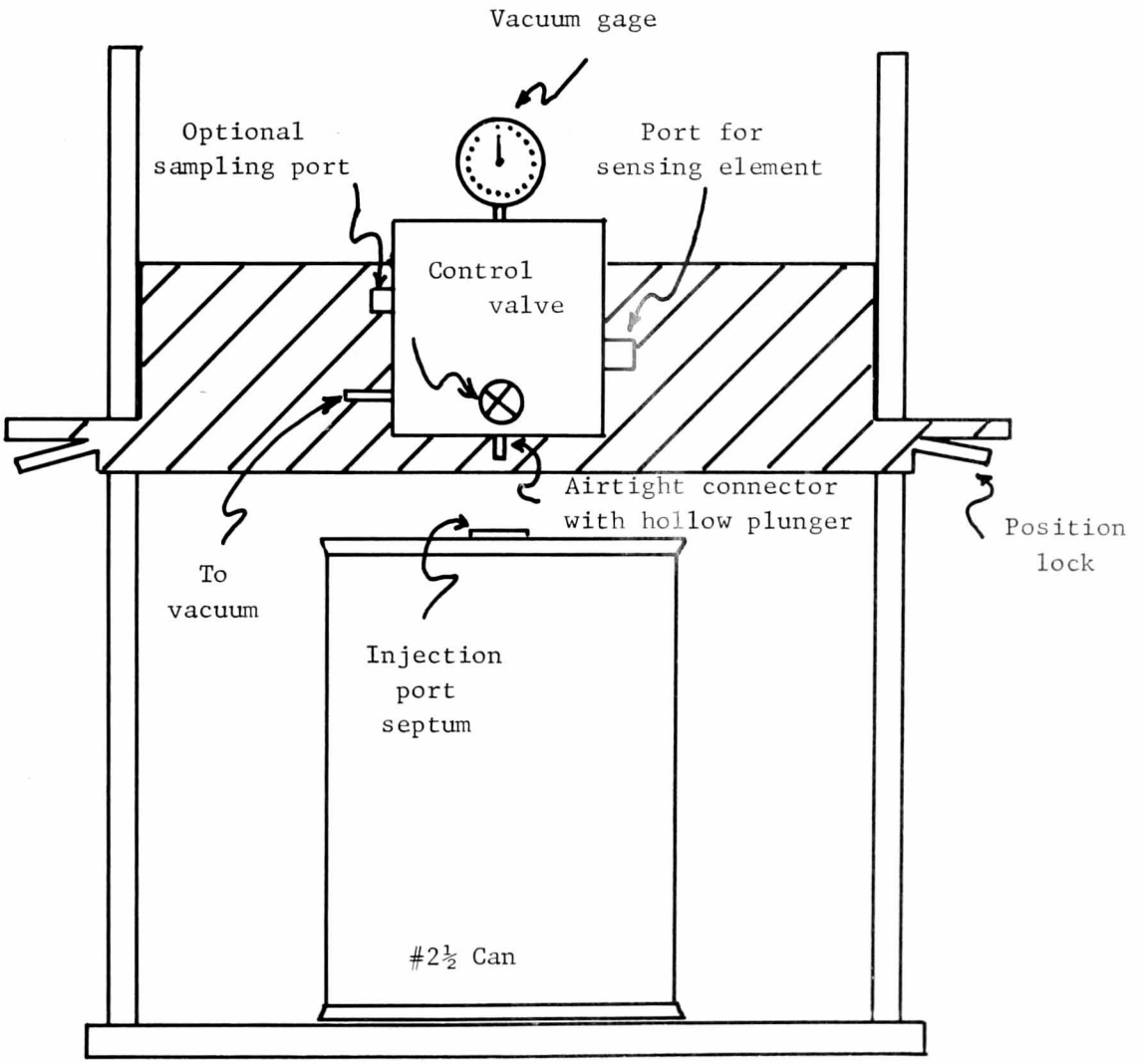


Figure 1.—Beckman headspace sampler used in the polarographic method.

the headspace gases, (c) the method of transcribing the detector responses, (d) the conditions and procedure used, and (e) the method of calculating the data.

a. Equipment used. — An Aerograph A-90P gas chromatograph with a thermal-conductivity detector and two stainless-steel columns, 0.25 inch (6.35 millimeters) in diameter, placed in series, was used. The first column, which was packed with silica gel, isolated carbon dioxide from oxygen, nitrogen, and other gases. The second column, which was packed with molecular sieve, absorbed carbon dioxide and separated the oxygen from nitrogen and other components.

b. Method of sampling headspace gases. —The headgases were sampled with a 1-milliliter gastight syringe through the sampling port of a Zahm tester (Figure 2), which is similar to the Beckman headspace sampler and which was used to facilitate the transfer of the sample.

c. Method of transcribing detector responses.—The detector responses were transcribed on a Leeds Northrop strip-chart recorder.

d. Conditions and procedure used. — Details of the gas chromatograph conditions and the procedure were the same as those described by Karel, Issenberg, Ronsivalli, and Jurin (1963).

e. Method of calculating data. — The values for the percent oxygen in each sample was calculated according to the formula:

$$O_S = \left[\frac{s}{a}\ \frac{X_S}{X_a}\ (0_a + A)\right] - A$$

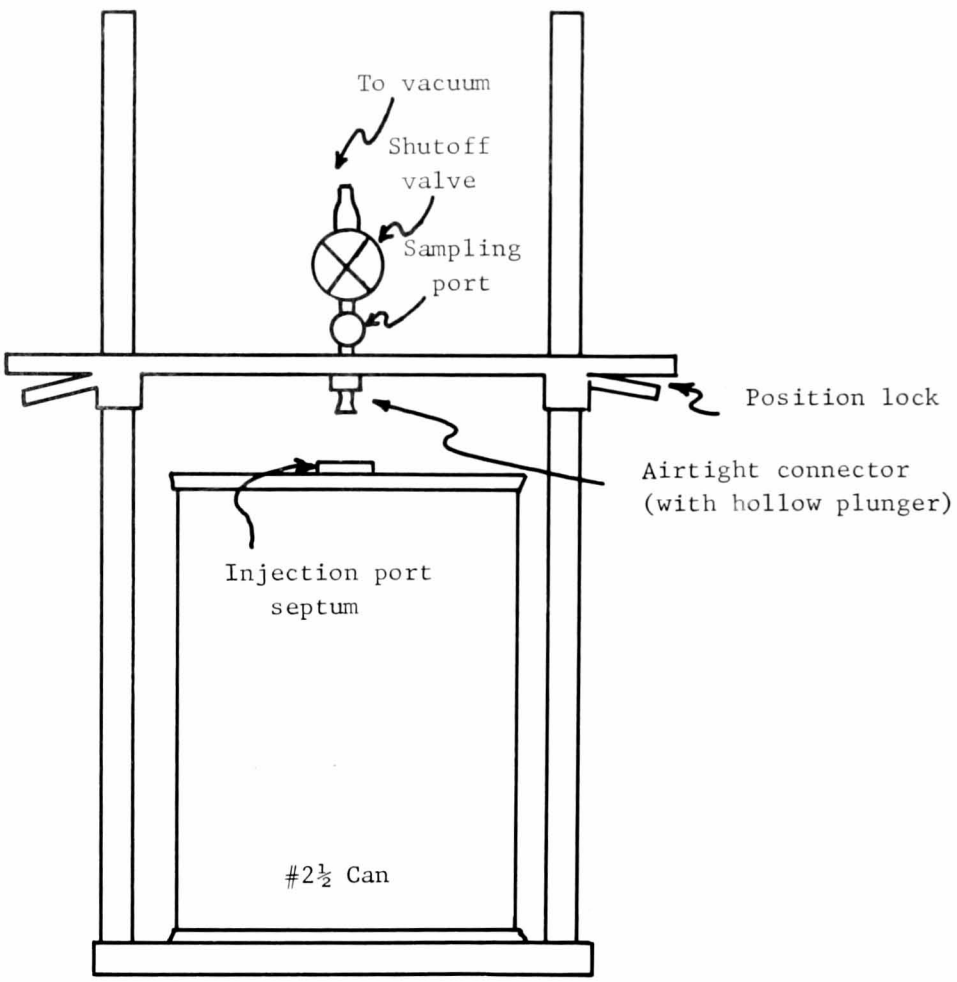


Figure 2.—The Zahm tester.

where: O_S = percent oxygen in the test sample.

s = measured height of the oxygen peak of 1 milliliter of headspace gas.

a = measured height of the oxygen peak of 1 milliliter of air.

X_a = sensitivity setting used in the air analysis.

X_S = sensitivity setting used in the analysis of the head gas.

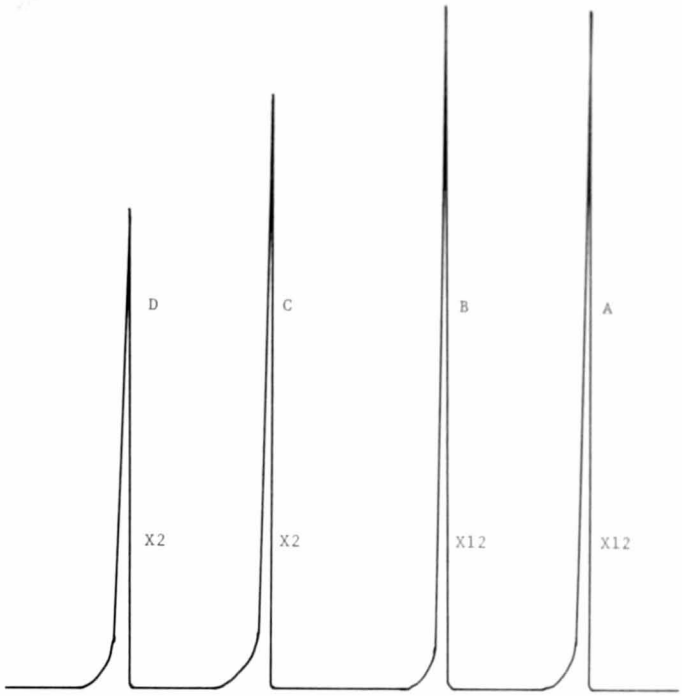
O_a = percent oxygen in air.

A = percent argon in air.

The presence of argon had to be taken into account, because, under the conditions of the test, its retention time was the same as that of oxygen.

In our calculations, we used the peak height in the chromatogram as a quantitative measure of the volume of oxygen represented by the peak, even though the standard procedure is to use the peak area as a measure of the volume. Our justification for using the peak height is the correlation that we established earlier for the volume of oxygen, the peak area, and the peak height. This relation was true provided the operating conditions (for example, temperature of the column and rate of flow of the carrier gas) were not changed so as to alter the width of the peak.

Figure 3 illustrates typical chromatograms (1) for oxygen obtained from the headspaces of cans that contained radiopasteurized haddock fillets and that were stored for 30 days at 0.5° C. and (2) for oxygen as it occurs in air.



A,B = oxygen-argon in 1 ml. of air
C = oxygen-argon in 1 ml. of headspace gas, Sample 1.
D = oxygen-argon in 1 ml. of headspace gas, Sample 2.
X2 = because of required attenuation of the response, the actual volume of the sample is twice the size represented by the peak areas.
X12 = actual volume of the samples is 12 times the size represented by the peak areas.

Figure 3.—Traces from gas chromatograms of air and of headspace gases in two cans of irradiated fillets stored for 30 days at about 0.5° C.

The calculation for determining the percent oxygen in Sample D (Figure 3) is shown below:

$$O_S = \left[\frac{s}{a}\ \frac{X_S}{X_a}\ (0_a + A)\right] - A$$

$$= \left[\frac{4.4}{6.3}\ \frac{2}{12}\ (20.9 + 0.9)\right] - 0.9$$

$$= 1.8 \text{ percent}$$

II. RESULTS

Figure 4 shows the results of 16 analyses from a typical experiment, using the polarographic method.

Results by the two methods were in excellent agreement. Compare, for example, the value of the last point on the graph (polarographic) and the value obtained in the calculation for Peak D in Figure 3, (chromatographic).

A. DECREASE IN THE CONCENTRATION OF OXYGEN

The sudden, small decrease in the concentration of oxygen at the start of the experiment

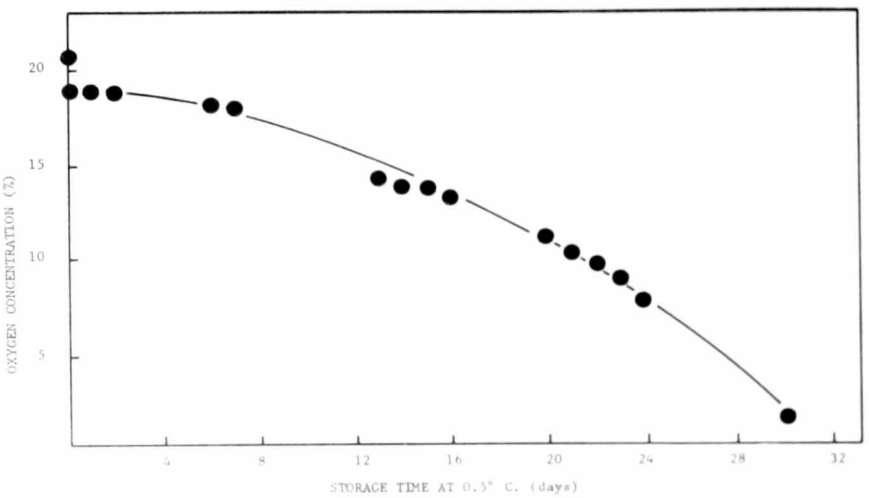


Figure 4.—The percent oxygen in the headspace of Sample D containing haddock fillets irradiated at 250 kilorads (polarographic method).

is due presumably to oxidative reactions catalyzed as a result of irradiation.

The further decrease in the concentration shown in Figure 4 implies that hermetic sealing of nonsterile fillets can be expected to have a major effect on the composition of the microflora whether the fillets are pasteurized or not. The reasons that the composition of the microflora is likely to be affected are twofold. First, the environment goes from aerobic, through diminishing values of free oxygen, to anaerobic. Second, the oxygen requirements (Ingram, 1952) of microorganisms lie in different ranges according to types of microorganisms (that is, according to whether they are aerobes, microaerophiles, facultative anaerobes, or anaerobes). According to the data of Ingram, the atmospheric conditions within the cans after 1 month of storage at 0.5° C. were conducive to the growth of anaerobic microorganisms.

B. SHAPE OF THE CURVE

A second implication from the curve is related to speculation regarding the method by which oxygen is consumed. In a simplified hypothetical reaction: $0_2 + FR \rightarrow EP$,

0_2 = free oxygen
FR = flesh reactants
EP = end product

Because this reaction results in a lowering of the concentration of free oxygen, one would expect that the rate of consumption of oxygen would be constant or that it would decrease during storage, provided that the concentration of the flesh reactants was not limiting initially. But if that were the case, the slope of the curve might be constant, or the slope might have its greatest value at the beginning, and it would follow a concave path. (In mathematical terms, the second derivative would be positive.) The slope of the curve in Figure 4, however, followed a convex path, suggesting that either a reactant other than oxygen or a group of reactants was limiting initially and new reactants were produced that caused a greater consumption of oxygen. We suggest that the role of the microbes surviving after irradiation, or of the microbial enzymes produced prior to irradiation, or of both the surviving microbes and the enzymess should not be overlooked in any hypothesis explaining the decrease in the concentration of the headspace oxygen.

ACKNOWLEDGMENTS

David Wallace of Massachusetts Institute of Technology assisted in the gas chromatographic analyses.

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MS. #1904

TEST-TANK STUDIES OF SHRIMP-POT EFFICIENCY

by

Doyne W. Kessler

ABSTRACT

How spot shrimp and dock shrimp escape from shrimp pots and how they react to each of five designs of shrimp-pot entrances were studied. Observations of pot efficiency--that is, of the number of shrimp entering and escaping each type of pot in a given time--indicate that a long conical tunnel was the most effective of the entrances tested.

CONTENTS

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INTRODUCTION

Fishermen in Southeastern Alaska have used shrimp pots for many years; however, little information is available on the factors that affect the efficiency of shrimp pots. Each fisherman has his own idea of the most effective design. As a result, opinions conflict on which design is best.

Butler (1963) and Dahlstrom (1963)[1] were among the first to study factors that affect the efficiency of shrimp pots. Butler demonstrated that traps made with a solid covering, such as metal, may be more effective than web-covered traps. Dahlstrom reported that size and shape may also influence their effectiveness.

During Cruise 65-3 of the research vessel *John R. Manning*, the staff of the Bureau of Commercial Fisheries Exploratory Fishing and Gear Research Base in Juneau, Alaska, made additional studies of shrimp pots. This preliminary work indicated that the design of the entrance to the shrimp pot may also be an important factor affecting the efficiency of the pots.

In the past, the efficiency of shrimp pots was evaluated by catch analyses rather than by observations. Use of catch analyses is unquestionably indispensable for the final evaluation of fishing equipment. An observational approach, however, does answer some of the questions of design that arise during developmental stages of gear research.

These studies of shrimp pots seem particularly adaptable to an observation technique. Observations on how known numbers of shrimp react to various types of pots can be made under controlled conditions in a large test tank. Pots can be modified and tested without the need to make and fish the large numbers of pots that would be required for testing in the field. The final design can then be field tested. Thus, much of the time that would be lost in field testing intermediate modifications can be eliminated.

The studies on shrimp-pot efficiency presented in this paper represent the first use of the observational technique by the staff of the Base at Juneau, Alaska. During February, March, and April 1966, shrimp pots were studied under controlled conditions in a large test tank at Little Port Walter, Alaska. Primary goals were (I) to determine how shrimp escape from the pots and (II) to measure the relative efficiency of the pots in terms of the number of shrimp entering and escaping pots that had different types of entrances.

I. DETERMINING ESCAPE BEHAVIOR OF SHRIMP

A. PROCEDURE

Described in this section are (1) the shrimp, (2) the test tank, (3) the lighting, (4) the shrimp pot, and (5) the observational technique.

1. Shrimp

The shrimp were caught by small shrimp pots set on the bottom in Little Port Walter Bay in 10 fathoms or less. The shrimp were too small to be of commercial importance, averaging 80 whole shrimp per pound. Commercial sized shrimp averaging 14 or less whole shrimp per pound were not available during these experiments.

2. Test Tank

The experiments were made in a 4- by 4- by 8-foot plywood test tank lined with fiberglass and having a 2-foot square window on one side. This tank was filled to a depth of 34

[1] Dahlstrom, Walt. 1963. Cruise report 63-A-1 prawn–Shrimp. California Department of Fish and Game, Fisheries Laboratory, Terminal Island, California, 3 pages, 2 figures. Unpublished manuscript.

inches with water that was pumped from the bottom of the bay through plastic pipes. A standpipe at one end of the tank controlled the level of the water.

3. Lighting

Lighting in the test tank was held to a minimum during all tests. A black "Visqueen"[2] tent over the test tank produced nearly total darkness between observations. Illumination during observations was by two 40-watt incandescent lights, one above each end of the test tank.

4. Shrimp Pot

A circular, collapsible shrimp pot baited with herring, which had been thawed, was used. The Base previously had used this type of pot, covered with 1¼-inch stretch mesh, in exploratory fishing in Alaska.

5. Observational Technique

Ten spot shrimp, *Pandalus platyceros,* averaging 0.7 inch in carapace length were put in the test tank about 1 hour before the baited collapsible pot was added. Shrimp were observed continuously for the first hour and then intermittently at ½-hour or 1-hour intervals throughout the 8-hour test. Lights were on continually for the first half of the test but were turned off between observations during the second half.

B. RESULTS

The observations indicated that the 1¼-inch stretch mesh of the collapsible pot was too large to retain the spot shrimp tested. Shrimp entered and left the pot at will through the web instead of through the tunnel entrances. Shrimp backed tailfirst through the web, using their walking legs to force themselves slowly through and stretching the mesh to allow the carapace to pass through the web. The escape behavior resembled the settling phase of the burrowing movements of pink shrimp, *Penaeus duorarum,* as described by Fuss (1964). The entire process took several minutes.

The activity of the shrimp was inversely related to the amount of illumination. During a 4-hour period of constant illumination, the shrimp remained motionless on the web of the pot, under the pot, or in the shadow of the standpipe. After 1 hour of darkness, the shrimp were actively swimming or crawling about the test tank. The general decrease in the activity of the shrimp during periods of illumination agrees with the observations by Fuss and Ogren (1966), who reported a decrease in activity of pink shrimp with increasing levels of light.

The shrimp had little interest in the bait. Once inside the pot, they tended to remain motionless on the pot webbing. No shrimp was seen on or near the bait during this phase of the experiment.

II. DETERMINING EFFICIENCY OF SHRIMP-POTS WITH VARIOUS ENTRANCES

The relative efficiency of five designs of pot entrances was tested in two experiments concerning (A) ease of shrimp entry and (B) ease of shrimp exit.

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

A. POT EFFICIENCY, MEASURED BY EASE OF SHRIMP ENTRY

The experiment on ease of entry was designed to determine the number of shrimp that would enter pots through each of five different types of openings in 4 hours.

Figure 1.—Shrimp pots with five types of entrances used in efficiency tests: A. Short tunnel; B. Long tunnel; C. Top loader; D. Ramp; E. Plastic pipe.

1. Experimental Procedure

Each of the five shrimp pot entrances was tested four times. The order of these tests was randomized so that each entrance type was tested on two different mornings and two different afternoons. The five types of shrimp-pot entrances are shown in Figure 1.

Tunnels of four different designs were incorporated in the ends of 12- by 12- by 24-inch wooden lath pots with 1- by 2-inch wooden frames. These tunnels were made of ⅜-inch nylon bobbinet. The fifth entrance was made from a 1-pound tin can set in the top of a 10½- by 18- by 24-inch wooden lath pot. Figure 2 shows the details of construction for the various tunnels.

The shrimp were taken in small shrimp pots set on bottom in Little Port Walter Bay in 10 fathoms or less. These shrimp were from 0.5 to 1.1 inches in carapace length and averaged 0.7 inch, or about 80 shrimp per pound. Spot shrimp were used whenever possible; however, when catches of spot shrimp were insufficient to supply the need, dock shrimp, *Pandalus danae*, were used.

To minimize differences in the physical condition of the animal that might affect the results, we used uniform procedures in handling all shrimp. Until needed for a test, shrimp were held for several days without food in live cages on the bottom of the bay. Although considerable effort was made to keep uniform the length of time that the shrimp were held be-

fore a test, this uniformity could not always be achieved, owing to the variability of daily catches. The 20 shrimp used in each test were transported from the live cage to the test tank in a plastic pail previously filled with water from the test tank. These procedures were

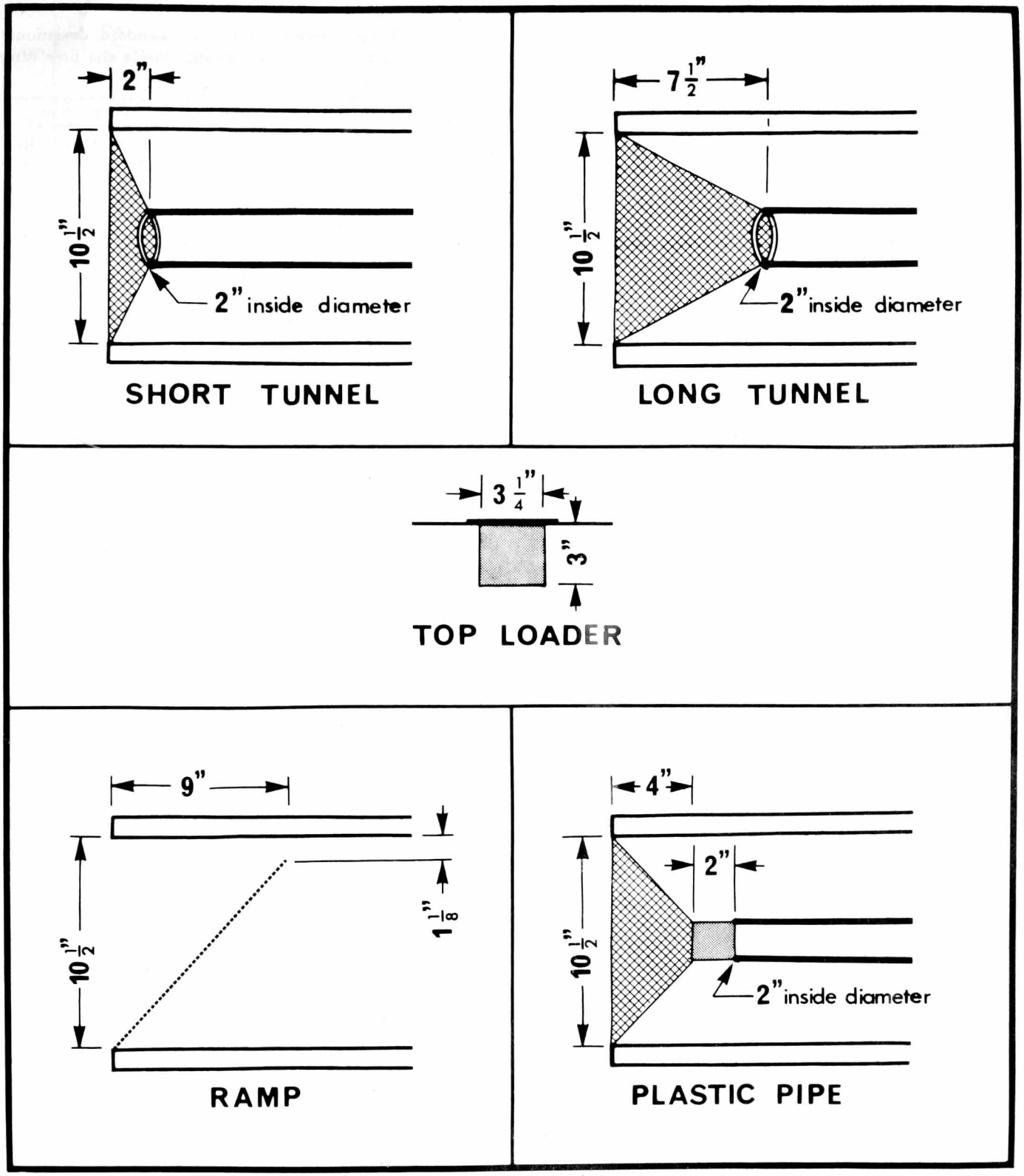

Figure 2.—Schematic diagrams of the five types of opening used in the pot-efficiency tests.

necessary to minimize prolonged exposure of the shrimp to the fresh surface water from Sashin Creek, which usually killed them.

For each test, 20 shrimp were placed in the test tank and allowed to become accustomed to their new environment for one-half hour. When a pot baited with thawed herring was put into the tank, the observations began. How the shrimp reacted to the entrance of the shrimp pot was observed during the first half-hour of the test. The circulation of water then was stopped for the rest of the test. The lights were turned off except for the short time needed to count the number of shrimp inside and outside the pot after ½, 1, 2, 3, and 4 hours. The test ended after the fifth count.

Results of the experiment were analyzed statistically by analysis of variance and Duncan's new multiple-range test (Steel and Torrie, 1960). Because the variances were not homogeneous, it was necessary to perform a log transformation on the raw data before performing these tests.

2. Results

Table 1 summarizes the results of the ease-of-entry experiment, and Figures 3 and 4 display them graphically.

Table 1.—Ranges, means, and SD (standard deviations) of the number of shrimp inside the pots after 4 hours

Kind of pot entrance	Tests made	Total shrimp tested		Shrimp in pot		
		Spot shrimp	Dock shrimp	Range	Mean	SD
	No.	*No.*	*No.*	*No.*	*No.*	*No.*
Long tunnel ...	4	70	10	3-7	5.25	2.06
Plastic pipe	4	70	10	2-7	3.75	2.22
Short tunnel ...	4	80	0	2-3	2.75	0.50
Top loader	4	78	2	0-4	1.25	1.89
Ramp	4	70	10	0-1	0.25	0.50

a. Long-tunnel pot. — More shrimp entered the long funnel-shaped web tunnel then entered the other entrances. Twenty-three shrimp entered and two left this pot. The shrimp entered by crawling slowly forward or

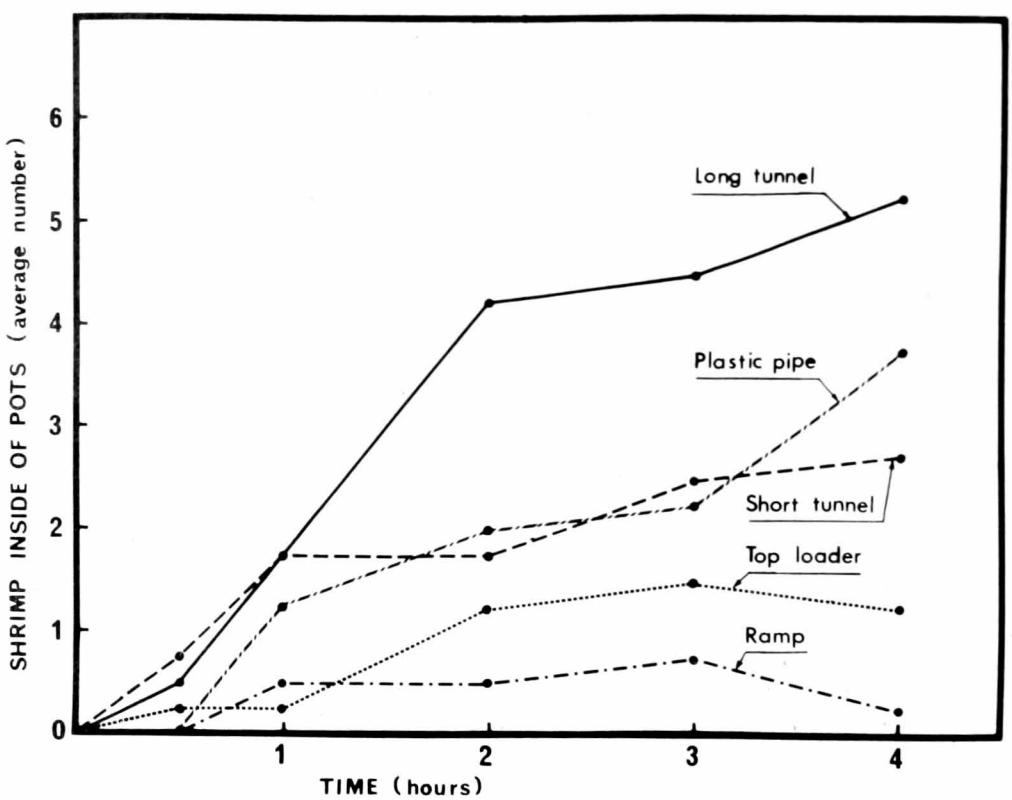

Figure 3.—Number of shrimp captured by each type of shrimp-pot entrance at the end of ½, 1, 2, 3, and 4 hours.

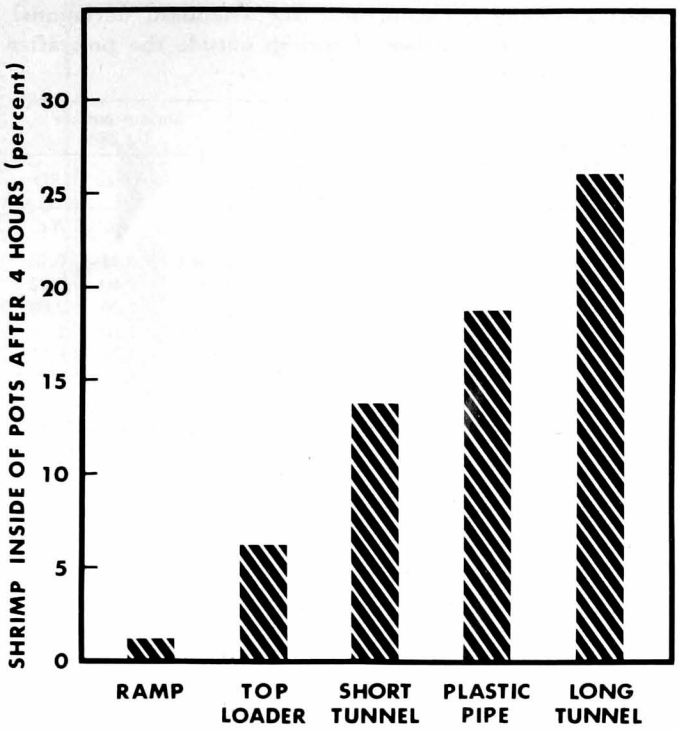

Figure 4.—Shrimp-pot efficiency based on ease of entry.

backward on the web, toward the plastic ring forming the inner tunnel opening. Most of the shrimp remained for awhile part way through the ring before continuing, either head or tail first, into the center of the pot. An average of 5.25 shrimp per pot, or 26.2 percent of the shrimp, were inside the pot at the end of 4 hours.

b. Plastic-pipe pot. — Fifteen shrimp entered the funnel shaped web lead-in modified with a 2-inch piece of plastic pipe; none escaped during the four tests. The shrimp entered this type of pot in the same manner as they did in the long-tunnel pots. An average of 3.75 shrimp per pot, or 18.8 percent of the shrimp, were captured by the pot of this type.

c. Short-tunnel pot. — Thirteen shrimp entered and two escaped through the short flat, web entrance with a shallow lead-in. Although none were actually seen entering the pot, one shrimp was seen on the plastic ring forming the inner entrance opening. One tried to enter the pot by repeatedly charging head first against the lower web of the entrance. An average of 2.75 shrimp per pot, or 13.8 percent of the shrimp, were captured by the pot of this type.

d. Top-loader pot.—Seven shrimp entered and two escaped through the tin-can opening of the top-loader entrance. None of these shrimp were observed entering or leaving the pot. Although shrimp were seen crawling and swimming around the sides of the pot, none were observed on or near the top of the pot, where the entrance was located. An average of 1.25 shrimp per pot, or 6.2 percent of the shrimp, were captured by the pot of this type.

e. Ramp pot. — Fewer shrimp entered through the flat, sloping ramp than entered the other entrances. Of the six shrimp that entered, five left, leaving only one shrimp captured during four tests. Shrimp entered this type of tunnel by crawling slowly up the ramp head first. Once at the top, they either dropped into the center of the pot or continued crawling slowly down the underside of the ramp. An average of 0.25 shrimp per pot, or 1.2 percent of the shrimp, were captured by the pot of this type.

B. POT EFFICIENCY, MEASURED BY EASE OF SHRIMP EXIT

The experiment on ease of exit was to determine the number of shrimp that would escape pots through the entrance openings used in the ease-of-entry experiment.

1. Experimental Procedure

For each of the tests in this experiment, 20 shrimp were placed inside the pot at the start of the test, and bait (thawed herring) was placed outside the pot. All other procedures were the same as those used in the ease of entry experiment.

2. Results

Shrimp were seen trying to escape the pots by means other than through the entrance tunnels. They tried to escape through the small cracks between the wooden slats and through small openings between the web and the side of the pot. Sometimes they attempted to escape by wedging the carapace through head first,

but they usually inserted the tail through the crack first. The walking legs were then used to force the carapace through the narrow opening. Usually, this procedure was slow. The movement of the tail and walking legs resembled a burrowing type of behavior. Occasionally, the shrimp became violent, flipping their tail rapidly and straining their walking legs in an effort to force themselves through. In one test, several shrimp were seen escaping in this manner; therefore, the test was repeated after the pot had been repaired.

Escape of shrimp through the tunnel entrance openings of pots in good repair are summarized in Table 2 and Figures 5 and 6.

a. Long-tunnel pot. — Fewer shrimp escaped from the long funnel-shaped web tunnel than from the other entrances. Only two shrimp left this type of pot, but one of them went back in, which left only one shrimp outside the pot after four tests. This entrance limited escape to an average of 0.25 shrimp per pot, or 1.2 percent of the shrimp that were inside the pot at the start of the tests.

Table 2.—Ranges, means, and SD (standard deviations) of the number of shrimp outside the pots after 4 hours

Kind of pot entrance	Tests made	Total shrimp tested		Shrimp outside the pot		
		Spot shrimp	Dock shrimp	Range	Mean	SD
	No.	*No.*	*No.*	*No.*	*No.*	*No.*
Long tunnel ...	4	80	0	0-1	0.25	0.50
Plastic pipe	4	78	2	1-3	2.00	0.82
Top loader	4	80	0	1-5	2.00	2.00
Short tunnel ...	4	80	0	2-5	3.50	1.73
Ramp	4	80	0	7-20	13.25	6.70

b. Plastic-pipe pot. — Eight shrimp escaped from the plastic pipe tunnel entrance during the four tests. Although no shrimp were actually seen leaving, several did go into the plastic pipe, where they remained for long periods of time--more than an hour in one instance. The entrance limited the escape of shrimp to an average of 2.0 shrimp per pot, or 10.0 percent of the shrimp that were inside the pot at the start of the tests.

c. Top-loader pot. — Eight shrimp also escaped from the top loader entrance during the four tests. Thus, this entrance also limited

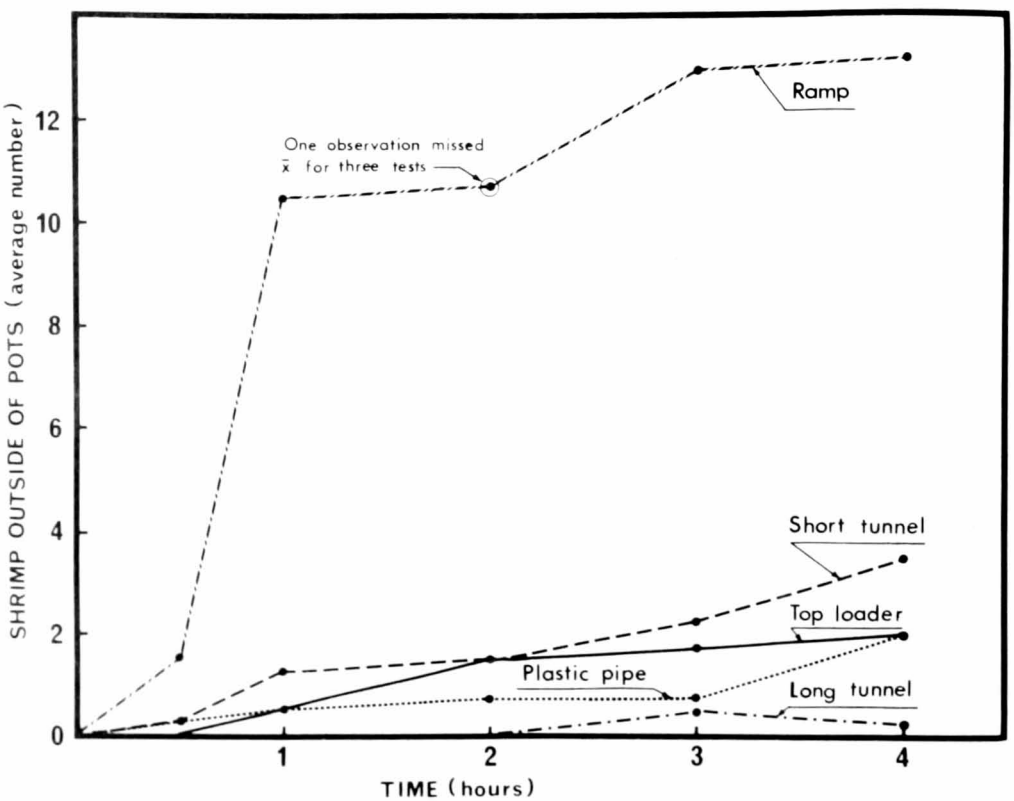

Figure 5.—Number of shrimp escaping from each type of shrimp-pot entrance at the end of ½, 1, 2, 3, and 4 hours.

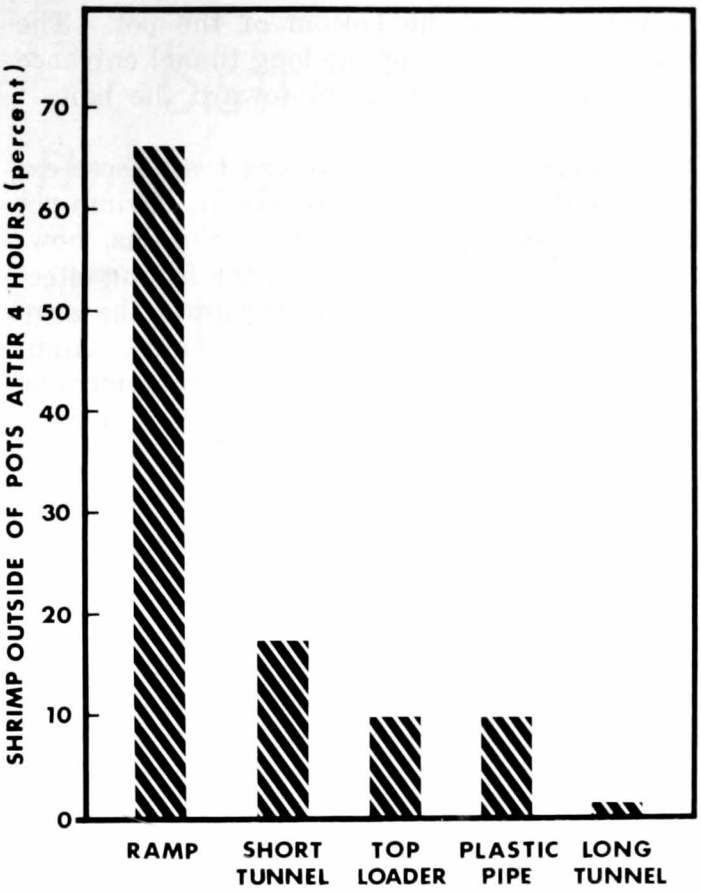

Figure 6.—Shrimp-pot efficiency based on ease of exit.

escape to an average of 2.0 shrimp per pot, or 10 percent of the shrimp that were inside the pot at the start of tests.

d. Short-tunnel pot. — Fourteen shrimp escaped from the short-tunnel entrance during the four tests. Although, no shrimp were actually seen leaving the entrance, an average of 3.5 shrimp per pot, or 17.5 percent of the shrimp did escape and was counted outside this pot at the end of the experiment.

e. Ramp pot. — More shrimp escaped from the ramp entrance than from the other entrances. Fifty-five shrimp left and two entered this type pot during the four tests, although only one shrimp was actually seen leaving. This one shrimp escaped by swimming out of the entrance along the top of the pot. An average of 13.2 shrimp per pot, or 66.2 percent of the shrimp inside this type of pot escaped.

CONCLUSIONS

Analyses of differences in the average number of shrimp entering or escaping from each type of pots were statistically significant at the 95 percent level of confidence.

The long-tunneled pot was the most efficient pot. Significantly fewer shrimp escaped from this pot than from all other pots tested. Also, significantly more shrimp went into this type of pot than into either the ramp pot or top-loading pot.

The ramp pot was the least efficient pot type. Significantly more shrimp escaped from this pot than from all other pots tested. Also, significantly fewer shrimp entered this pot than pots with conical-web tunnels.

DISCUSSION

The ability of shrimp to escape through cracks and loose web indicates the importance of constructing the pot carefully and of maintaining it properly. As we have just seen, shrimp will escape from pots by forcing themselves, tailfirst, through small cracks and through holes in the web.

The general decrease of shrimp activity during periods of illumination suggests that catches of shrimp might be increased if pots were fished at night instead of during the day, especially in areas of clear water.

Although observations were seriously hampered by the cessation of shrimp activity whenever the lights were turned on to make an observation, information was obtained on the disadvantages of several types of entrance design. After a shrimp was seen swimming out of the ramp entrance, it was obvious that any shrimp swimming along the top of the pot

would be led directly outside. Observing a shrimp walk upside down along the underside of a ramp revealed that shrimp could crawl out of this entrance. Because shrimp tended to approach the pots from the side, circling around them rather than swimming over them, only a few shrimp would find their way into an entrance that is located on the top of a pot with vertical sides. Because shrimp usually approached the pots by crawling along the bottom of the tank, they may be diverted from their path by a vertical wall of web--especially if they are being led directly toward the bait, which was on the bottom of the pot. The gentle sloping web of the long tunnel entrance may tend to "lead" them toward the bait.

Quantitative data obtained from these experiments indicate differences in shrimp-pot entrance efficiencies. The conclusions, however, may or may not reflect the fishing effectiveness of similar pots used to capture the large shrimp taken on commercial grounds. Additional field studies will be needed to determine the effectiveness of these pots under commercial fishing conditions.

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DISTRIBUTION OF ROYAL-RED SHRIMP, *Hymenopenaeus robustus*, ON THREE POTENTIAL COMMERCIAL GROUNDS OFF THE SOUTHEASTERN UNITED STATES

by

Richard B. Roe

ABSTRACT

The royal-red shrimp is an underused species. This paper reports on their distribution on grounds east of St. Augustine, Florida; south-southwest of the Dry Tortugas, Florida; and southeast of the Mississippi River Delta. On these grounds, the shrimp live only on soft bottom types and in water temperatures of 8° to 12° C.; the densities of shrimp vary seasonally on all three grounds; the depth distribution of shrimp also varies seasonally--the shrimp move offshore in summer and inshore in winter.

CONTENTS

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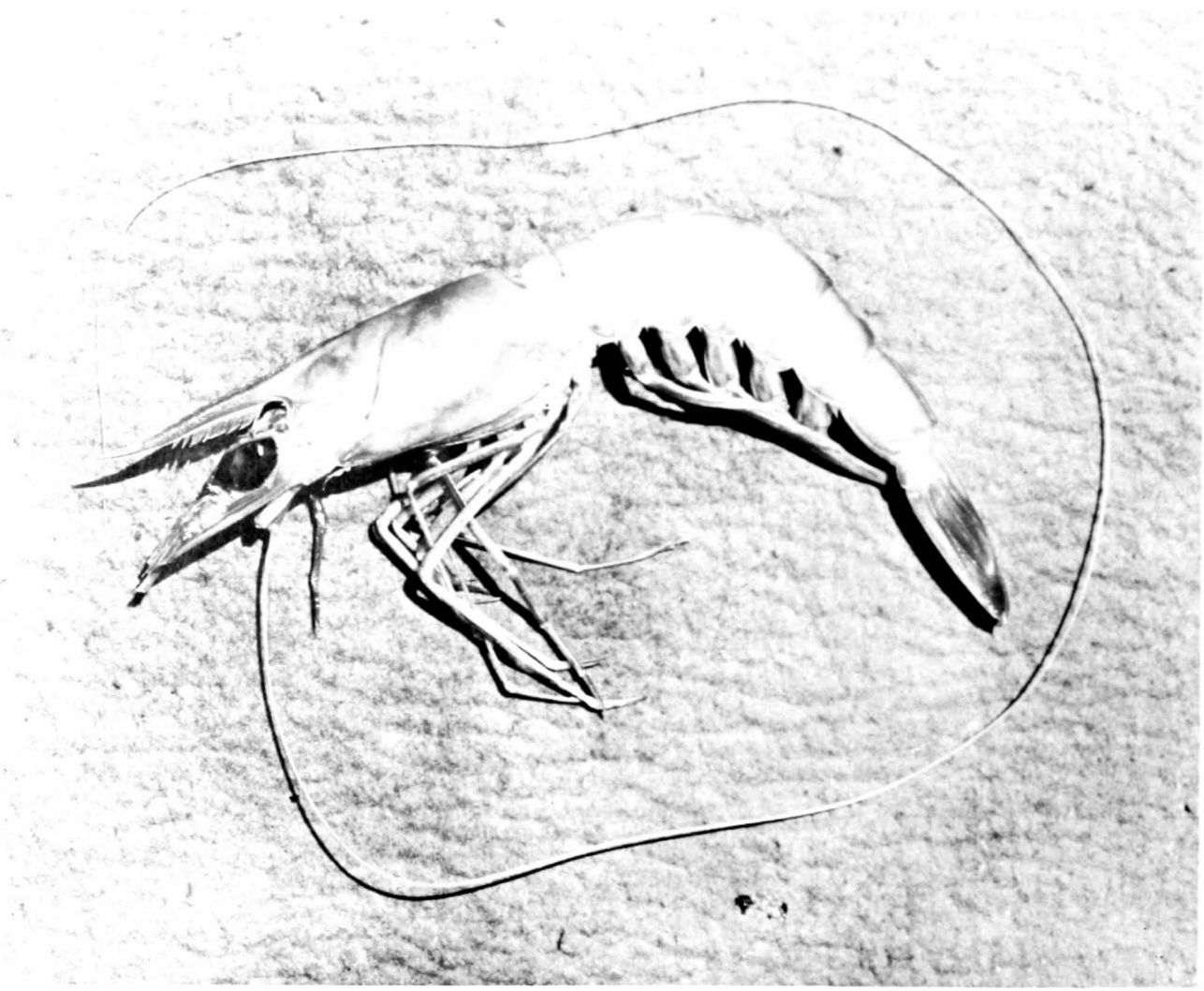

Figure 1.—The royal-red shrimp, *Hymenopenaeus robustus*.

INTRODUCTION

Although the royal-red shrimp is a typical penaeid with well-developed eyes, large pleopods, and exceptionally long antennae (Figure 1), it differs from commercial penaeids of the genus *Penaeus* because it prefers deep, cold water. The species occurs on the upper Continental Slope from as far north as Cape Hatteras, North Carolina, to as far south as the coast of the Guianas, but it is abundant in only a few areas. Little is known of its biology, particularly its reproduction and early life history.

Since the discovery in 1950 of concentrations of royal-red shrimp in the Gulf of Mexico, the Bureau of Commercial Fisheries Exploratory Fishing and Gear Research Base at Pascagoula, Mississippi, has made periodic trawling surveys along the Continental Slope from North Carolina to Brazil to evaluate the commercial potential of this resource (Springer and Bullis, 1952, 1954; Bullis, 1956; Bullis and Rathjen, 1959; Bullis and Thompson, 1959; Bullis and Cummins, 1963; Cummins and Rivers, 1962). Results of these surveys indicate that three grounds off the coast of the United States support commercial quantities of royal-red shrimp. These grounds are located east of St. Augustine, Florida, in the western Atlantic; south-southwest of the Dry Tortugas in the Florida Straits; and southeast of the Mississippi River Delta in the Gulf of Mexico (Figure 2).

The purpose of this paper, which summarizes the data obtained on the exploratory fishing surveys, is to relate certain of the physical characteristics of the individual grounds to their populations of shrimp.

The paper is divided into two main parts. The first reports specific observations on the three potential commercial grounds; the second, general observations.

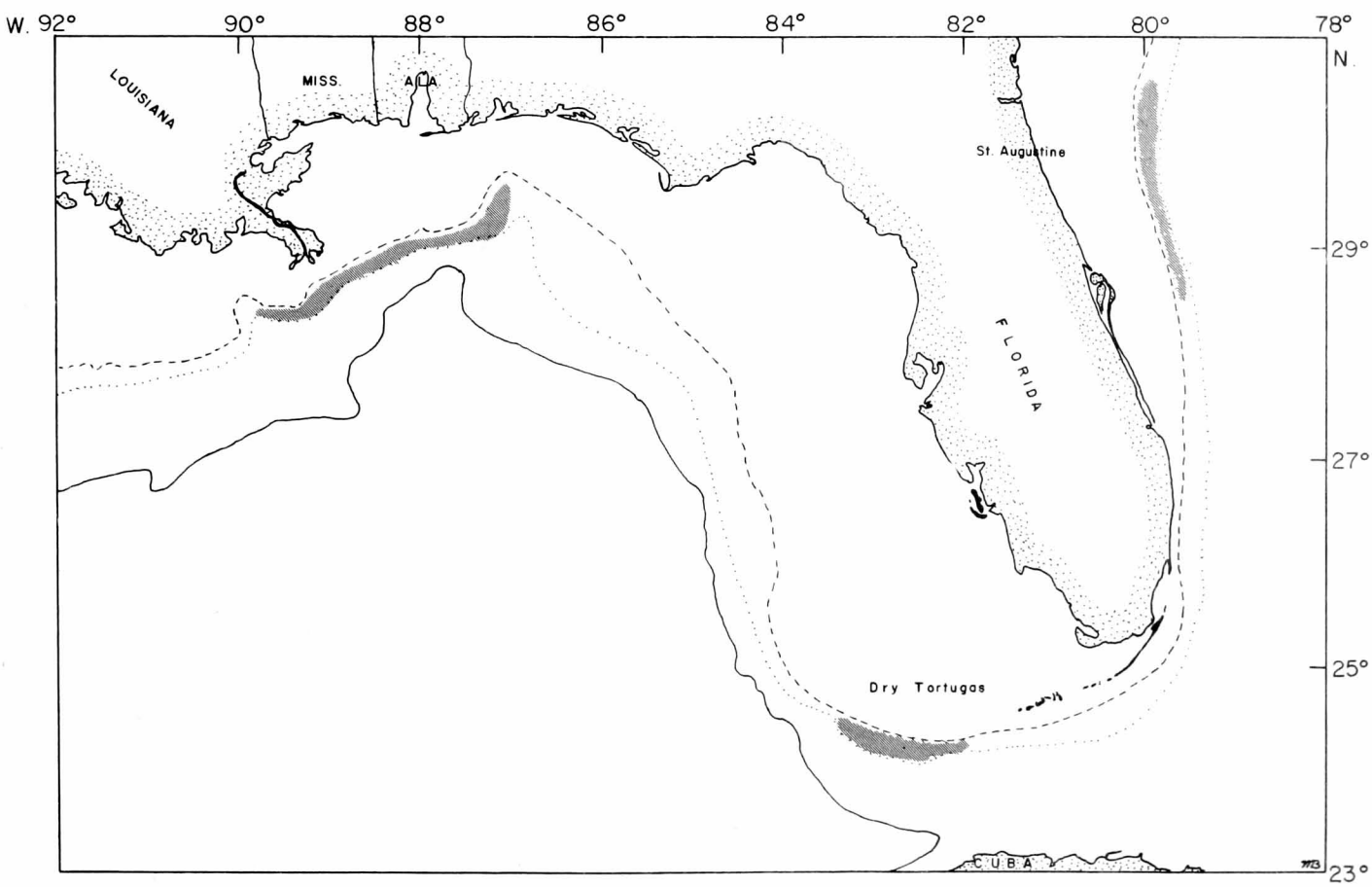

Figure 2.—Geographical boundaries of St. Augustine, Dry Tortugas, and Mississippi River Delta royal-red shrimp grounds.

I. SPECIFIC OBSERVATIONS ON THE THREE POTENTIAL COMMERCIAL GROUNDS

One of the grounds is in the Atlantic Ocean off St. Augustine, Florida; the others are in the Gulf of Mexico.

A. GROUNDS IN THE ATLANTIC OCEAN OFF ST. AUGUSTINE, FLORIDA

In this section, I first describe the physical appearance of the grounds and then report on catch observations..

1. Physical Description of Grounds

The St. Augustine grounds lie between latitudes 27°31' and 31°00' North in 185 to 550 meters along the Continental Slope (Figure 2). The average width of the grounds is 8.3 nautical miles; the angle of the slope is relatively steep (Figure 3).

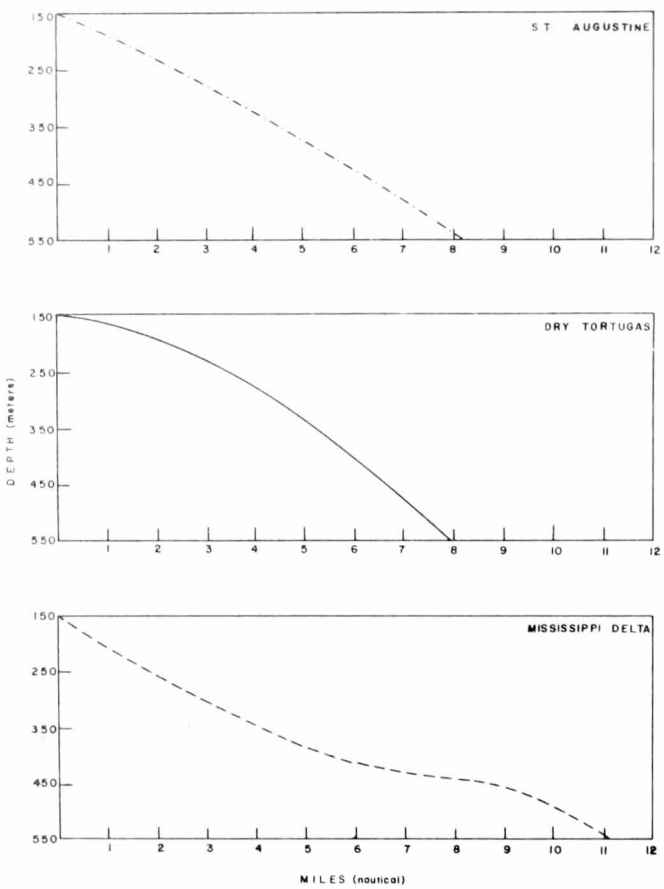

Figure 3.—Slope profiles of the study grounds.

Shrimp trawling is limited to one range of depth for various reasons. The bottom between 185 and 255 meters cannot be trawled because of formations of limestone and dense stands of deep-sea alyconarians (sea fan). From 255 to about 475 meters, the bottom consists of sand or silty-sand sediments (Emery, 1965) commonly called "green mud" by fishermen. The bottom at this range in depth has few obstructions and provides excellent trawling. Beyond 475 meters, the bottom supports large patches of deep-sea coral (*Lophelia prolifera*) and therefore is not trawlable with standard shrimp gear (Bullis and Rathjen, 1959).

Figure 4 shows the average annual temperature profile for the grounds. The 12° C. isotherm lies at about 220 meters; the 11° C. isotherm, at 238 meters; and the 10° C. isotherm, at 256 meters. The 9° and 8° C. isotherms are broad. The 9° C. isotherm covers depths of 275 to 400 meters, and the 8° C. isotherm covers depths of 400 to 475 meters.

2. Catch Observations

Inshore penaeid shrimps (*Penaeus aztecus*, *P. setiferus*, and *P. duorarum*) vary diurnally in availability (Idyll, 1950; Iversen and Idyll, 1959; Fuss and Ogren, 1966; Joyce and Eldred, 1966). This variability dictates the hours of fishing for those species. Because diurnal cycles of availability have not been investigated in royal-red shrimp, day and night catch data were compared for evidence of differences in the availability of the shrimp.

In addition to these observations on the differences, if any, between the day and night catches, data were gathered on the distribution of the shrimp.

a. Comparison of day and night catches.

(1) Methods.—The catch data used in the study were collected from 1950 to 1965 and were obtained with standard Gulf of Mexico flat shrimp trawls having headropes 12.2 to 30.5

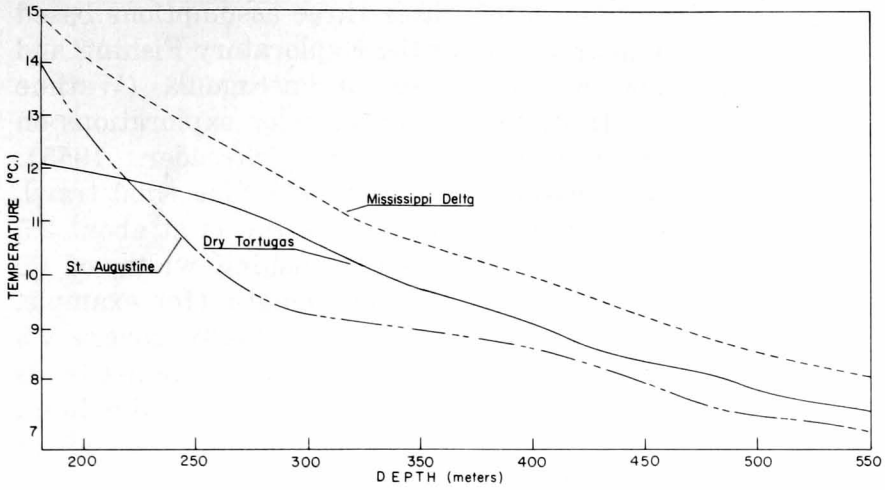

Figure 4.—Bottom temperature profile of the royal-red shrimp grounds off St. Augustine, the Dry Tortugas, and the Mississippi River Delta.

meters long, having 5.1-centimeter stretched-mesh webbing, and having 1.5- to 3.0-meter wooden doors. These trawls were fished 1 to 4 hours. The shrimp caught were sorted and weighed aboard ship. The weights taken were of whole shrimp.

Because of the variety of trawl sizes and fishing times used in the study, we--the staff at the Exploratory Fishing and Gear Research Base at Pascagoula--standardized catch data by converting pounds of shrimp caught per hour to estimates of relative shrimp density expressed in kilograms of shrimp per hectare.

The calculations used in the study may contain errors that are now impossible to eliminate. The sources of these possible errors are the selectivity and limitations of the gear and the distribution of individual animals over the bottom. The shrimp trawl used in this exploratory fishing is selective, owing to a stretched-mesh size of 5.1 centimeters, which allows small individuals to escape through the trawl body and throat. It has further limitations in that (a) burrowed shrimp may be passed over and (b) shrimp near the periphery of the trawl path can avoid capture by moving laterally. Hence a portion of the shrimp population is unavailable to the gear. In addition, the method of standardizing the catch data is based on the assumption that individual shrimp are randomly distributed; however, the distribution may actually be nonrandom.

Owing to these limitations, the densities reported in this paper are intended to be used as estimates of relative densities of shrimp rather than as actual densities.

The method, programmed for the UNIVAC 1004 III, uses towing speed, duration of drag, and gear size to compute hectares by the following formula:

$$\text{Hectares} = \frac{100 \text{ K x M x } 0.6\text{L}}{107640}$$

where, K = speed in knots; M = minutes fished; L = headrope length in feet; and 107640 = a constant.

The final step is to convert catch into kilograms per hectare to provide an estimate of relative density relating to the available portion of the population.

The height of the opening of the net was not used for two reasons: (1) we assumed that royal-red shrimp has the same swimming and jumping abilities as inshore penaeids and hence would not be able to escape the effective vertical net opening of 1.2 to 1.8 meters attained in normal trawling and (2) the introduction of a third dimension, namely height, would create numerous problems in calculating shrimp density, which is primarily a two-dimensional distribution.

For the comparison of day and night catches, data were selected from common time periods and depth intervals in one or more seasons for each of the grounds. The time period for day was selected as being 6 a.m. to

6 p.m. and that for night as being 6 p.m. to 6 a.m.

(2) Results.—The St. Augustine catch data had no distinguishable differences between day and night, because the variations about the means were large.

The same observations were made on the catches from the other two grounds. The data for the St. Augustine grounds, the Dry Tortugas grounds, and the Mississippi Delta grounds all failed to show any decided difference between day and night catches.

b. Shrimp distribution.—This study of shrimp distribution is concerned with the relation of shrimp density to depth distribution and the relation of shrimp density to bottom temperature.

(1) Relation of shrimp density to depth distribution.—The principal aims in calculating shrimp densities on each of the three grounds were to determine the depth distribution of royal-red shrimp and to learn whether or not seasonal variation occurs in this distribution.

(a) *Average depth distribution.*

[1] Method.—The depth distribution was determined by computing shrimp densities for each of the grounds from the total trawling data grouped by 18.3-meter intervals. This distribution is presented in terms of average density.

The method uses three assumptions based on gear studies at the Exploratory Fishing and Gear Research Base at Pascagoula (Wathne and Holt, 1964) and on prior explorations on the Continental Slope (Schroeder, 1955). These assumptions are: (1) that a flat trawl, regardless of size, if it be towed at about 2.5 knots, has an effective fishing width of 60 percent of the headrope length (for example, that a 12.2-meter trawl actually covers 7.3 meters on the bottom); (2) that the net tends bottom continuously over unobstructed bottom; and (3) that the net descends and ascends at constant rates relative to speed of vessel, size of gear, depth of water, and length of warp. This method perhaps oversimplifies the analysis, because some variation is no doubt present; but the method is as exacting as is now feasible.

Bullis (1956) and Bullis and Rathjen (1959) have given detailed accounts of the vessels, gear, and operations used in the collection of the data.

[2] Results.—The most productive area of the St. Augustine grounds lies between latitude 28°30' and 30°20' North. Although shrimp are distributed between 220 and 550 meters, concentrations occur from 275 to 420 meters (Figure 5). Highest densities are found at 290 and 380 meters; lesser densities, from 330 to 348 meters. Shrimp are not abundant in water shallower than 256 meters or deeper than 550.

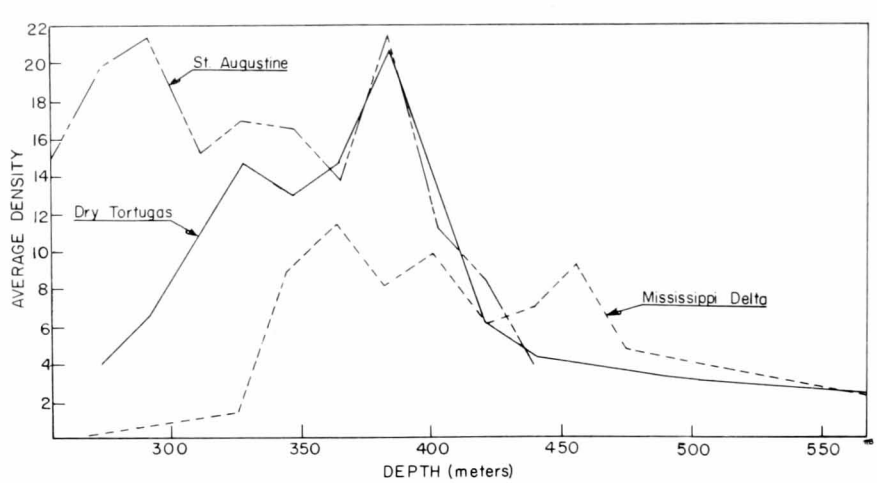

Figure 5.—Average density of shrimp versus depth.

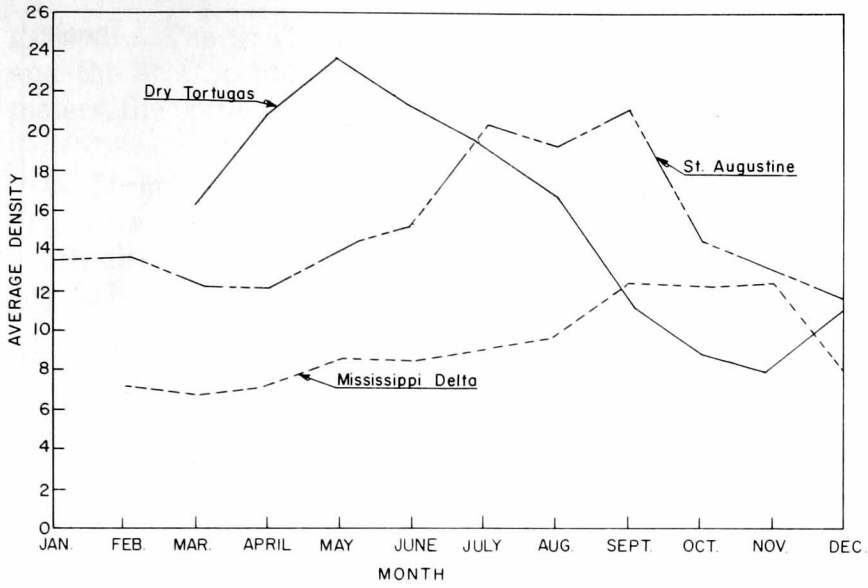

Figure 6.—Seasonal variation in royal-red shrimp density plotted with a moving average of 3.

(b) *Seasonal depth distribution.*

[1] Method.—Seasonal changes in the average density distribution were determined by grouping the data into 3-month periods (seasons) before calculating densities. The grouped data were then treated in 18.3-meter intervals in the same manner as were those used to obtain the average density distribution. This seasonal grouping is presented in the paper as seasonal density distribution. The months representing the individual seasons were chosen on the basis of climatic similarity --winter (December, January, February); spring (March, April, May); summer (June, July, August); and fall (September, October, November).

[2] Results.—Figure 6 shows the monthly trends in the distribution of the shrimp. Densities are low during December through April. Beginning in late spring, the density of the St. Augustine grounds increases until a plateau is reached between July and September. During the fall, the concentration disperses until an annual minimum density is reached in December.

High densities occur at depths that vary seasonally some 90 meters (Figure 7). In the spring, densities of shrimp are highest at 275 to 340 meters. From spring to summer, shrimp move into deeper water, concentrating between 320 to 430 meters, and are at their greatest density during that time of year. In the fall, densities decrease, and shrimp move into shallower water where the peak density is at 320 meters. The winter population is distributed bimodally between 256 and 420 meters with peaks at 275 and 415 meters.

Though the general movement of shrimp in the fall is toward shallow water, a concentration that seems inconsistent with the excepted seasonal pattern develops during the winter from 355 to 450 meters. The concentrations reached in deeper water and the wide distribution of shrimp over the entire grounds indicate that recruitment may take place from other grounds. A logical source would be the Dry Tortugas, since the general direction of bottom currents away from there could carry larval shrimp eastwardly through the Florida Straits and up the east coast of Florida.

(2) Relation of shrimp density to bottom temperature.—Bottom temperatures were obtained with standard reversing thermometers graduated in degrees Celsius.

Maximum shrimp densities occur in water temperatures of about 9.5° C. (Figure 8). Shrimp are distributed over a temperature range of 7.5° to 10.0° C.; below 8.0° C. shrimp are scattered. No shrimp were taken in 255 meters or less when temperatures were above 10.0° C. Because the bottom is hard inside of the 255-meter contour, it is difficult to say

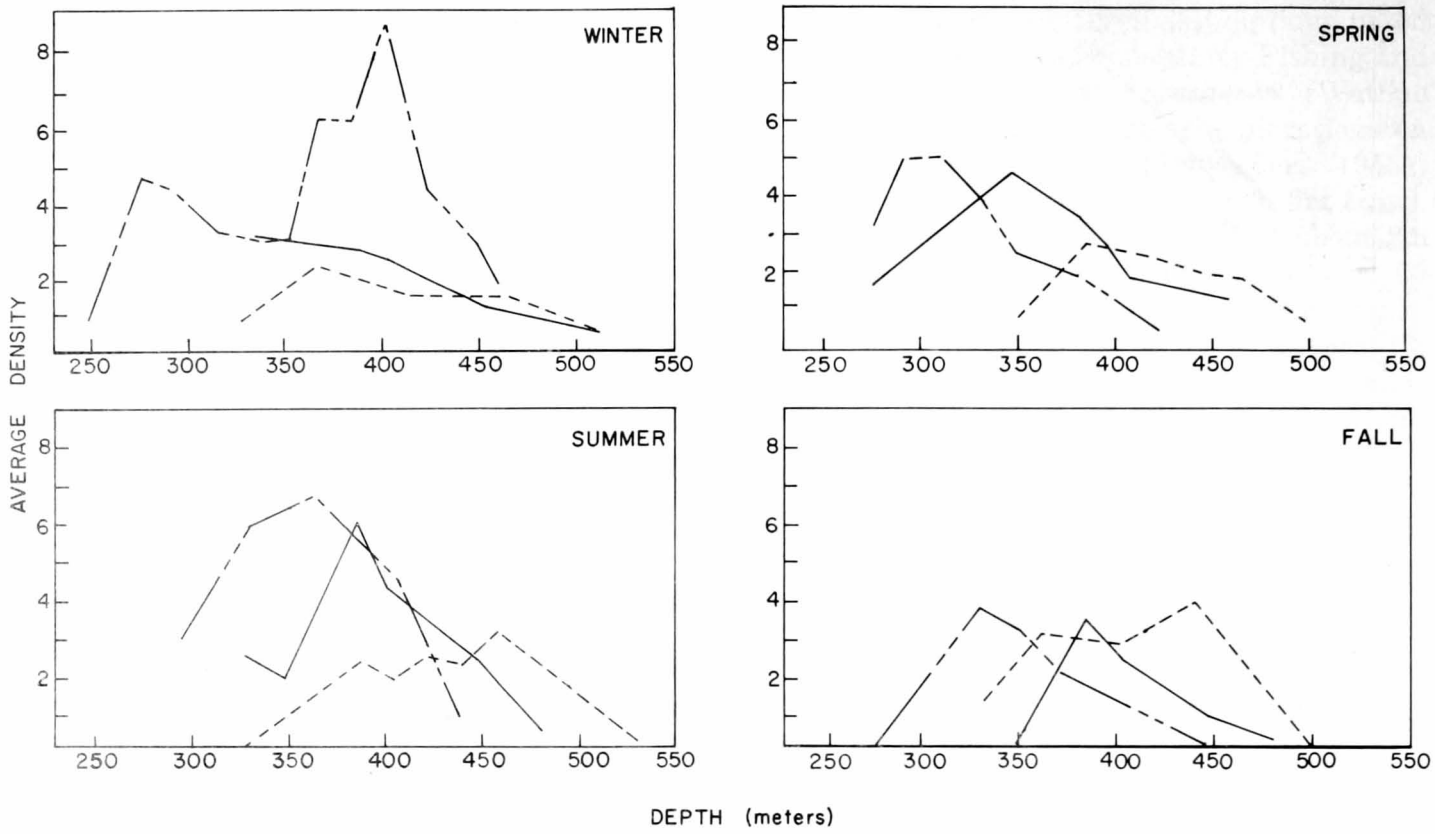

Figure 7.—Seasonal depth distribution of royal-red shrimp density on the study grounds.

which factor, substrate or temperature, is more limiting on the St. Augustine grounds.

The high densities of shrimp occurring in from 275 to 380 meters indicate that the royal-red shrimp on the St. Augustine grounds prefer a water temperature of 9.5° C. Further, the similar depth range (256 to 475 meters) of the 8.0° to 10.0° C. interval and the depth distribution of the shrimp indicate that bottom temperature sets a limitation on their distribution.

B. GROUNDS IN THE GULF OF MEXICO

Two grounds in the Gulf of Mexico support commercial quantities of royal-red shrimp. These grounds are off Dry Tortugas and Mississippi River Delta.

1. Dry Tortugas Grounds

Described in this section are the grounds and the distribution of shrimp.

a. Physical description of grounds. — The grounds described here lie south-southwest of the Dry Tortugas along the Florida Straits between longitudes 82°20' and 84°00' West (Figure 2). The distance between the 185- and 550-meter depth interval averages 5.9 nautical miles and has the steepest angle of slope among the three grounds studied (Figure 3).

Between 185 and 275 meters, the bottom is calcareous, broken with coral formations, and generally unsuitable for trawling. From 275 to beyond 550 meters, the calcareous bottom changes to gray-green coralline mud, which provides excellent trawling (Agassiz, 1888; Bullis, 1956). The eastern and western borders of the grounds are untrawlable because of large patches of the coral *Stylaster*.

Figure 4 shows the annual thermal gradient of the Dry Tortugas grounds. The 12° C. isotherm lies at the depth of about 185 meters, the 11° C. isotherm at a depth of 275 meters, and the 10° C. isotherm at a depth of 330 meters. This separation of whole-degree isotherms by 55 to 95 meters continues throughout the

grounds. The 9° C. contour is at 400 meters, and the 8° C. contour at 475 meters. At 550 meters, the bottom temperature is about 7.5° C.

b. Shrimp distribution.—This section comprises two parts--relation of shrimp density to depth distribution and relation of shrimp density to bottom temperature.

(1) Relation of shrimp density to depth distribution.—Discussed here are the average and seasonal depth distributions of shrimp.

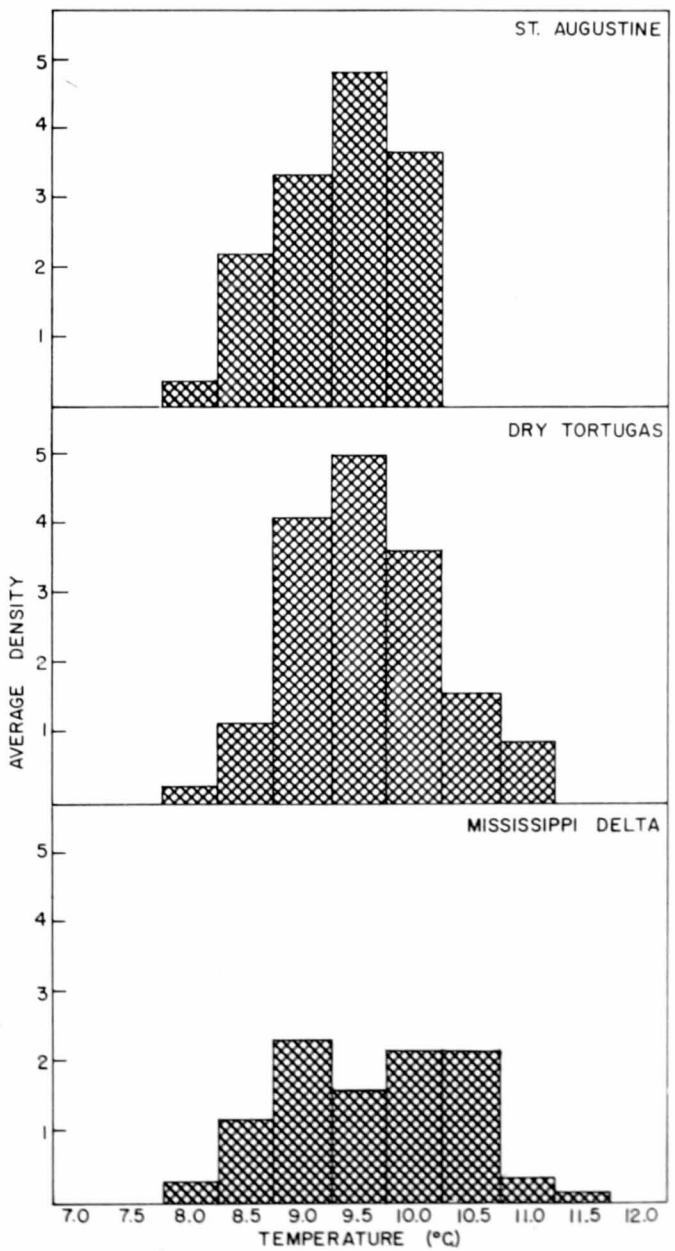

Figure 8.—Relation of shrimp density to bottom temperature.

(a) *Average depth distribution.*—On the Dry Tortugas grounds, shrimp are found at depths from 275 to over 550 meters, but the maximum density occurs between 330 and 410 meters (Figure 5). Shrimp are highly scattered in water deeper than 440 meters and in water shallower than 310 meters. On the upper reaches of the slope, particularly inside of 255 meters, this scarcity of shrimp may be the result of hard bottom.

(b) *Seasonal depth distribution.*—The seasonal variation in shrimp densities on the Dry Tortugas grounds differs markedly from that on the other two grounds (Figure 6). Maximum density is reached in May. From late summer through fall, shrimp disperse or decrease numerically to a minimum density in November. As no trawling was done in January and February, winter trends are difficult to interpret; however, the December portion of the graph can be extrapolated through March to show a gradual increase in shrimp density.

The depth of maximum density shows little seasonal variation (Figure 7). In the spring, shrimp are concentrated in depths from 330 to 400 meters. During the summer, shrimp reach maximum density in depths from 365 to 390 meters. Although shrimp concentrations are dense in depths from 365 to 390 meters during the fall, the maximum density of shrimp at those depths is considerably lower than is that in summer, indicating that the population disperses in the fall to cause the wide depth distribution (330 to 505 meters) evident during the winter. Maximum densities in winter occur at depths of 320 to 356 meters.

With respect to density and changes in depth distribution, the population of shrimp on these grounds appears to be more stable than that on the St. Augustine grounds. Density is at its lowest in winter, probably as a result of scatter rather than as the result of an actual decline in the population.

(2) Relation of shrimp density to bottom temperature.—As on the St. Augustine grounds, a relation exists between the temperature of the water and the distribution of the shrimp. The shrimp are concentrated where

bottom temperatures approach 9.5° C. (Figure 8). Densities are lower in water of 8.5° to 9.0° C. and 10.0° to 10.5° C., beyond which shrimp are not abundant. Warmer waters lie in the shallow portion of the grounds where, as on the St. Augustine grounds, the bottom is hard and is not inhabited by shrimp. Therefore, an upper-thermal limit cannot be determined accurately. As is evident from Figure 7, the lower-thermal limit is about 8.0° C.

The 9.5° C. isotherm is between 365 and 385 meters on the Tortugas grounds. The depth range of shrimp, 275 to over 550 meters, corresponds to a range in temperature of 7.5° to 11.0° C.

2. Mississippi Delta Grounds

This section describes the grounds and distribution of shrimp.

a. Physical description of grounds. — The Mississippi Delta grounds are in the north central Gulf of Mexico southeast of the Mississippi River Delta between longitudes 87°15' and 89°50' West. The average width of the grounds between the 185- and 550-meter depth interval is 10.4 nautical miles (Figure 3). The angle of slope is quite gradual.

The bottom topography over the entire grounds is relatively smooth except for the eastern and western boundaries, which contain patches of the coral *Lophelia* (Moore and Bullis, 1960). The sediments of these grounds are of terrigenous origin consisting of a fine silt-clay mixture that is extremely cohesive and that often contains small quantities of shell fragments. Both the nature of the bottom sediments and the occurrence of occasional topographical disturbances (Bullis, 1956) indicate the influence of the Mississippi River on the grounds. Trawling conditions are generally excellent over the grounds except on the east and west fringes in water deeper than 550 meters, where the bottom is precipitous and hard.

Figure 4 shows an annual temperature profile that is deeper and more gradual than that of the other grounds. For example, the 12° C. isotherm, lying in 275 meters, is 55 meters deeper than that of the St. Augustine grounds and 90 meters deeper than that of the Dry Tortugas grounds. At 400 meters, the bottom temperature is 10° C. on the Delta grounds as compared with 8.5° C. on the St. Augustine grounds and 9° C. on the Dry Tortugas grounds. Conversely, the 9° C. isotherm is at 455 meters; the 8° C. isotherm, at 550 meters.

b. Shrimp distribution. — Presented in this section are the relation of shrimp density to depth distribution and relation of shrimp density to bottom temperature.

(1) Relation of shrimp density to depth distribution.—I discuss first average depth distribution and then seasonal depth distribution.

(a) *Average depth distribution.*— Shrimp on the Mississippi Delta grounds are distributed primarily between longitudes 87° 30' and 89°30' West in water 275 to over 550 meters deep; however, density is highest at 330 to 475 meters with peaks at 365, 410, and 460 meters (Figure 5). Shrimp are less concentrated on these grounds than on the St. Augustine or Dry Tortugas grounds despite the larger geographical area of the Delta grounds and the broad depth distribution of the species. The substrate limitations in this area do not appear to be as extensive as are those on the St. Augustine or Dry Tortugas grounds.

(b) *Seasonal depth distribution.* — The levels of density are less seasonally differentiated on these grounds than are those on the other grounds, and the transition between minimum and maximum levels is generally several months in duration (Figure 6). The lowest density occurs during January through March after a rapid decline from the plateau of maximum concentration maintained from September to November.

The main seasonal effect on the royal-red shrimp is a change in the depth distribution of maximum density (Figure 7). In winter and spring the high densities occur at 370 and 390 meters, whereas in summer and fall the high densities occur at 460 and 440 meters. This seasonal movement indicates that the temperatures of water at those depths undergo normal

cycles of heating and cooling. Unfortunately, data on seasonal bottom temperature are not available to test this hypothesis.

(2) Relation of shrimp density to bottom temperatures. — The relation between shrimp density and water temperature in the Mississippi Delta area is similar to that on the other grounds (Figure 8). Shrimp are most concentrated at 9.0° to 10.5° C., but they do not show a preference for a temperature of 9.5° C. as do those on the other grounds. The absence of a 9.5° C. peak, however, may reflect the oscillating movement of shrimp between 365 and 455 meters and the broad depth distribution of the 9.0° to 10.5° C. temperature range. Shrimp become increasingly scattered on either end of the 9.0° to 10.5° C. range, and the 8° C. isotherm appears to set a lower-thermal limit. The 12.0° C. isotherm, which lies in 275 meters where the bottom is soft and apparently well-suited for royal-red shrimp, is apparently approaching the upper-thermal limit for the species.

The 275- to 550-meter depth range of shrimp on these grounds coincides with an 8.0° to 12.5° C. temperature range, which is comparable to the depth-temperature relation found on the other grounds studied.

II. GENERAL OBSERVATIONS ON THE THREE POTENTIAL COMMERCIAL GROUNDS

In this section, the role of the environment, the substrate as a limiting factor, and the effect of bottom temperature are considered.

A. ROLE OF THE ENVIRONMENT

Shrimp occurred in higher densities on some grounds than on others. Although this variability is difficult to explain completely, the distribution of shrimp on the grounds can be related in part to the amount of environmental disturbances on the study grounds.

The Mississippi Delta grounds have the lowest densities of royal-red shrimp among the grounds studied. A reason for these low densities can be inferred from the observations of van Andel and Curray (1960), who found that active sedimentation and deposition around the Mississippi Delta produce severe topographical changes on the outer shelf and upper slope. The constant deposition of fluvial material also causes a gradual expansion of the Continental Shelf; which, with sedimentation and deposition, produce mud slides, erosion, and other substrate disturbances that interfere with the permanent establishment of faunal populations.

These environmental disturbances apparently do not exist elsewhere in the Gulf of Mexico; hence, the Dry Tortugas grounds offer a more stable substrate than does the Mississippi Delta grounds. This difference is reflected in the higher densities and limited vertical movement of the royal-red shrimp in the Dry Tortugas grounds. Undoubtedly, these grounds are affected by the Florida Current, which bathes the area with a gentle eastwardly flow, but its effects do not appear to be detrimental.

Although little is known of the slope environment of the east coast of Florida, it can be presumed to be influenced primarily by the Gulf Stream. Current velocities of the Gulf Stream have not been measured on the St. Augustine grounds, but studies off Cape Hatteras, North Carolina, showed that a current of 1.0 knot occurred at a depth of 400 meters (Stommel, 1965). If this speed approximates current velocities off Florida, and nothing indicates that it does not, then the Gulf Stream certainly does not harmfully affect the slope community. The high concentrations of shrimp on the St. Augustine grounds indicate a relatively stable environment in contrast to that of the Delta grounds.

B. SUBSTRATE AS A LIMITING FACTOR

The composition of the bottom has been shown to limit the geographical and bathy-

metric distribution of royal-red shrimp. This relation is best illustrated by the 1,500 exploratory fishing stations made on the Continental Slope of the southern United States from Cape Hatteras to Brownsville, Texas, the great majority of which produced royal-red shrimp only on sand, silty-sand, and other soft sediments. On rare occasions, small numbers of shrimp were taken over limestone or coralline bottom contiguous to soft-bottom areas. The transition from soft to hard substrate on the upper reaches of the St. Augustine and Dry Tortugas grounds seems to present a barrier to extensive shrimp distribution and can be considered as being a possible example of the limiting characteristics of certain types of bottom.

Royal red shrimp occur on sand, silty-sand, terrigenous, and calcareous sediments and show no apparent preference for a particular sediment. The species is widely distributed on the Continental Slope wherever soft bottoms occur; however, large concentrations have not been found outside the study areas.

C. EFFECTS OF BOTTOM TEMPERATURE

Large aggregations of royal-red shrimp occur on the grounds only within the narrow temperature range at 9° to 10° C., and densities are highest at about 9.5° C. Below 8° and above 12° C., royal-red shrimp are highly scattered.

The depth distribution of royal-red shrimp is related to the location of the 9° to 10° C. range. Because of the location of the optimum temperature range, shrimp on the St. Augustine grounds are concentrated at shallower depths than are those on the Mississippi Delta grounds.

Since complete data are not available, the effects of bottom temperature on seasonal shrimp distributions are speculative. Some influence can be shown by a phenomenon observed in winter on the Dry Tortugas when shrimp were evenly distributed from 365 to 550 meters. At that time, the 9° C. isotherm was found to lie in 365 to 495 meters. The broad configuration of this isotherm would seem to permit a wide dispersion of shrimp in contrast to the dispersion in other seasons when the optimum temperature range occurs at a relatively narrow depth zone.

Changes in the temperature structure, such as was noted above, undoubtedly contribute to the annual and seasonal variations in faunal distribution; however, other environmental and biological factors, which are now poorly understood, may directly influence the distribution of royal-red shrimp.

CONCLUSIONS

1. The distribution of royal-red shrimp is restricted to soft-bottom types and to water temperatures of 8° to 12° C. The highest concentration of the shrimp is in water temperatures of 9° to 10° C. The variations in the depths at which the 9° to 10° C. range occurs among the grounds result in shrimp being found in shallow water on the St. Augustine grounds, intermediate water on the Dry Tortugas grounds, and deep water on the Mississippi Delta grounds.

2. The densities of shrimp vary seasonally on all three grounds. Late summer and fall are periods of high density on the St. Augustine and Mississippi Delta grounds; whereas, late spring and summer are periods of high density on the Dry Tortugas grounds.

3. The depth distribution of shrimp also varies seasonally, for the shrimp move offshore in summer and inshore in winter. Shrimp occur at depths of 255 to 550 meters on the St. Augustine grounds, at depths of 275 to over 550 meters on the Dry Tortugas, and at depths of 275 to over 550 meters on the Mississippi Delta area. Within each of these ranges, the seasonal variation in concentration is considerable.

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MS. #1913

EFFECT OF VARYING THE EXTRACTION PROCEDURE ON THE PROTEIN EXTRACTABILITY OF FROZEN-STORED FISH MUSCLE

by

Elinor M. Ravesi and Margaret L. Anderson

ABSTRACT

The amount of extractable protein in frozen-stored fish muscle is often used as a criterion of its textural quality. An assessment of the texture of fish muscle by an organoleptic test panel, however, often shows poor correlation with the amount of protein that is extracted from the muscle.

Because we hypothesized that the amount of protein that can be extracted from frozen fish muscle depends, in large part, upon the technique of extraction used, we studied the effects of varying the solubility-test procedure, using one lot of frozen-stored cod muscle. Depending on the length of time that the sample was blended and the concentration of the neutral-salt extractant used, the amount of extractable protein varied between (1) values considered to represent minimum extractability in frozen cod muscle that has undergone extensive textural deterioration and (2) values considered to be typical of recently frozen cod.

These contradictory results indicate a need for standardizing the extraction procedure. We believe that such standardization will minimize the lack of correlation now found in the literature between the content of soluble protein and the extent that the texture of frozen fish muscle has deteriorated as evaluated organoleptically.

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INTRODUCTION

How to assess the amount of deterioration in the texture of fishery products has been an enduring problem in the technology of frozen fish. Since Reay (1933) first detected a relation between extractability of protein in frozen haddock muscle and its history of frozen storage, investigators have attempted to correlate the content of extractable protein and the deterioration of the texture of fish muscle as evaluated by a panel of organoleptic testers. These investigators have reported varying degrees of correlation.

Dyer and his associates have worked extensively on this problem. He reported (1951) that when cod stored at −12° C. and −23° C. was extracted with 5-percent NaCl, the amount of actomyosin that went into solution followed a pattern that correlated with that of the test-panel assessment of the same fish. Dyer and Morton (1956) obtained similar results using 5-percent NaCl in their study of frozen plaice fillets stored at −12° C. Luijpen (1957) found good correlation between protein solubility in cold 5-percent sodium chloride solution and test-panel scores with cod stored at −10° C., but at storage temperatures of −20° and −30° C., protein solubility failed to decline as rapidly as the test-panel scores on texture did. Work by Dyer, Morton, Fraser, and Bligh (1956) with rosefish stored at −10° C. also showed poor correlation, as did work by Cowie and Little (1966) with cod stored at −20° C.

Dyer, French, and Snow (1950) studied the effect of a number of variables on the amount of protein extracted from fresh fish muscle--such as, ratio of flesh to extractant, pH of extractant, blending technique, composition and concentration of salt, temperature of extraction, and centrifugation time and force. From the results, they set up a general extraction procedure that they felt would yield maximum extractable protein.

In establishing the conditions of the extraction procedure, Dyer and his associates allowed considerable latitude. The blending time ranged from 3 to 5 minutes, the pH of the extractant varied from 7.0 to 7.5; and although they chose an extractant of 5 percent NaCl (0.86M) and 0.02M $NaHCO_3$, Dyer, Brockerhoff, Hoyle, and Fraser (1964), in a later study, used a 0.6M NaCl and 0.003M $NaHCO_3$ buffered solution. They concluded that one of the most important variables in the extraction of proteins is the degree of subdivision of the muscle fibrils and that the type of salt used for the extraction is not critical. They found, however, that the efficacy of extraction by the different salts used ranged from 77 to 91 percent.

The procedure suggested by Dyer and his coworkers has been modified by many investigators in their attempts to follow the development of toughness in fish. Ironside and Love (1958), for example, macerated about 1 gram of flesh with only 15 milliliters of ice-cold 5-percent NaCl solution for 1 minute at 2,200 revolutions per minute in a Marsh-Snow Homogenizer.[1] They recovered the extractable protein by washing the homogenate with more cold NaCl solution and centrifuging the homogenate at 4,000 revolutions per minute. In a later study, Love (1962) macerated the flesh in a "modified" Marsh-Snow Homogenizer at 8,750 revolutions per minute. Kelly (1967) used an Ultra Turrax homogenizer to macerate from 0.5 to 1.0 gram of muscle with 100 milliliters of 5-percent NaCl solution (adjusted to pH 7.0 with $NaHCO_3$) for 5 seconds. The mixture was then centrifuged for 30 minutes at 2,000 times gravity. In studies on frozen fish, Dyer and associates (1964) extracted the protein from unthawed myotome samples, whereas in our laboratory, we thaw the samples at a room temperature of about 26° C. immediately before we blend them. Ironside and Love (1958) have also used thawed samples in their extraction procedure.

Methods for determining the content of protein in the supernates of centrifuged extracts of fresh-fish muscle homogenized for brief periods of time in neutral salt solutions have been assumed to be equally efficacious, because most of the techniques used extract as much as 90 to 95 percent of the protein.

[1] Trade names referred to in this publication do not imply endorsement of any commercial product but are cited to promote full understanding of the type of equipment used.

Recent studies by us, however, on cod muscle aged in ice showed that, during the extraction procedure, complexes of protein with free fatty acid are dissociated and that exposure to the salt solution breaks down the resistance of the muscle to fragmentation (Anderson and Ravesi, 1968). This finding suggests that the concentration of salt and the length of time that the muscle is blended are important factors in the solubility test and that they may markedly influence the amount of protein extracted, especially from frozen-stored fish.

The purpose of the present study was to test this possibility by using some of the variations of the solubility test found in the literature in order to assess the extractable protein content of a single lot of frozen-stored cod muscle. Because length of blending time, concentration of salt, and physical state of the tissue are the variables most likely to differ in the various extraction procedures, we have considered these variables in our present study.

I. MATERIALS AND METHODS

In the general extraction procedure, samples of myotomes were blended with the extractants to produce homogenates that were subsequently analyzed for their content of soluble protein.

A. MATERIALS

Our choice of materials for this study was based on results of extraction studies made at both our laboratory and other laboratories. The history of the myotome samples is not in itself critical inasmuch as all the samples used had identical histories. The extractants used in the study are those most commonly used in extraction studies of this nature.

1. Myotome Samples

About 15 gutted cod (*Gadus morhua*) weighing about 15 to 25 pounds (6.8 to 11.3 kilograms) each and obtained commercially were stored on ice until they were filleted within 24 hours after they were caught. Myotomes were obtained from them by the method described by Ironside and Love (1958). The myotomes from all the fish were mixed together thoroughly, and 10-gram portions were weighed into aluminum weighing dishes. These portions were vacuum packaged in polyethylene bags in sets of three to provide triplicate samples for analysis. The myotome samples were immediately frozen at −29° C. for 1 day, were stored for 1 month at −18° C. (on one occasion, the temperature rose to −12° C. for about 3 days), and then were kept for an additional 7 months at −29° C.

2. Extractants

Three different extractant solutions were used. Their compositions were: (1) 5-percent NaCl (0.86M), 0.02M $NaHCO_3$ (Dyer, French, and Snow, 1950); (2) 3.5-percent NaCl (0.6M), 0.003M $NaHCO_3$ (Dyer, Brockerhoff, Hoyle, and Fraser, 1964); and (3) 3.35-percent KCl (0.45M), 0.0157M Na_2HPO_4, 0.0031M KH_2PO_4 (Connell, 1958). The pH of these solutions fell within the range 7.0 to 7.5.

B. METHODS OF ANALYSIS

As was just indicated, all samples were analyzed in triplicate. Protein was extracted from myotomes in the frozen state and in the thawed state.

When myotomes were extracted in the frozen state, the samples were allowed to soften in the salt solution for 2½ minutes before blending was started.

When myotomes were extracted in the thawed state, frozen samples were kept at an ambient temperature of about 26° C. until they thawed, whereupon they were blended immediately.

For each analysis, a 10-gram portion of myotomes was blended with 390 milliliters of extractant solution. The samples were blended in a pint-size (.47-liter size) Waring Blendor jar that had been fitted with a plexiglass baffle that, in turn, was submerged about 1 inch (2.54 centimeters) below the surface of the extractant to prevent the extractant from foam-

ing. The extraction was carried out in a refrigerated laboratory at 1° C. with all equipment and solutions equilibrated to this temperature. The blending time shown in Table 1 was made up of 10-second intervals of blending separated by 5-second intervals of stirring. A portion of the muscle-extractant homogenate was centrifuged at 1,100 times gravity for 20 minutes in a refrigerated centrifuge at 1° C. The protein contents of the centrifuged solutions were assayed by the automated technique described by Failing, Buckley, and Zak (1960).

Table 1.—Effect of physical state of the sample, blending time, and concentration of extractant on the amount of protein extracted from the sample

Physical state	Blending time	Amount of protein that was extracted from the sample when it was blended in:		
		3.35% KCl, 0.0157M Na_2HPO_4, 0.0031M KH_2PO_4	3.5% NaCl, 0.003M $NaHCO_3$	5% NaCl, 0.02M $NaHCO_3$
	Minutes	*Percent*	*Percent*	*Percent*
Thawed	1.5	23.4	30.1	34.9
Frozen		28.9	28.1	30.8
Thawed	2.5	33.8	41.3	48.5
Thawed	3.0	43.3	46.8	55.3
Frozen		41.3	51.5	51.8
Thawed	4.0	52.3	55.9	59.3
Thawed	5.0	58.4	64.3	70.2
Frozen		62.5	67.3	70.6

Note: The data are the averages of triplicate samples.

II. RESULTS AND DISCUSSION

Table 1 shows the results of this extraction study.

The soluble-protein contents of the extraction mixtures made from the three different salt solutions show the horizontal and vertical trends that our experience had led us to expect --that is, in both the frozen and thawed samples, the amount of extracted protein increased markedly with increasing blending time and increasing concentration of salt.

Considering the lengthy period that these samples were in frozen storage, we can assume that much denaturation of protein occurred. Depending upon the treatment used, however, anywhere from 23.4 to 70.6 percent of protein was extractable. A sample of cod in which the concentration of extractable protein was only 23.4 percent would be expected to be quite dry, tough, and unacceptable to a test panel; conversely, a sample in which the concentration of extractable protein was 70.6 percent would be expected to have recently been frozen and to be highly acceptable.

Because one sample cannot simultaneously have both sets of properties, the test obviously lacks precision. Depending upon the conditions of the solubility test used, the amount of extractable protein in this sample would show varying degrees of correlation with an organoleptic evaluation of its textural quality. This variability would explain the different degrees of correlation found by individual investigators using even slightly different techniques of extraction.

The frozen samples, although allowed to sit in the salt solution for 2½ minutes before being blended, were still partially frozen at the time that blending was commenced, so that the amount of exposure of the tissue to the action of the blades was actually decreased, but only briefly. On the other hand, investigators using this technique state that it has the advantage of lessening the build up of heat during the blending process. (Such a build up, of course, would lower the amount of protein extracted.)

From our results, drawing any conclusions concerning the overall effect of the physical state of the sample on the amount of protein that is extractable is difficult, because the differences between the comparable frozen and thawed samples often were small, and the differences varied inconsistently.

These inconsistencies were probably due, in large part, to the different operational efficiencies of the individual Waring Blendor jars used and to variations in the samples. Two samples cannot be treated identically when different blendor jars are used. In fact, the same jar may not perform with equal efficiency during two different blending operations. Constant maintenance of the jars by adjustment and replacement of bearings, washers, and blades can minimize this variation.

Table 2 shows the variation in results obtained when triplicate samples of muscle were treated identically except that different Waring Blendor jars were used.

Table 2.—Variation in the amount of protein extracted from triplicate samples blended with 5-percent NaCl (0.02M $NaHCO_3$) solution

Blending time	Amount of protein extracted from:		
	Sample I	Sample II	Sample III
Minutes	*Percent*	*Percent*	*Percent*
1.5	31.1	36.6	37.1
2.5	54.2	44.8	46.3
3.0	54.9	55.6	55.6
4.0	58.8	62.6	56.7
5.0	69.9	67.7	72.8

SUMMARY AND CONCLUSIONS

Depending upon the concentration of salt of the extractant used and the length of time that the myotomes were blended, the amount of protein extracted from a single sample of frozen-stored fish muscle varied markedly. Accordingly, investigators using different techniques of extraction will find varying degrees of correlation between the concentration of extractable protein in a sample and its texture as assessed by a panel of organoleptic testers.

The lack of validity of the solubility test thus is due, in large part, to the fact that the conditions of the test have not been rigidly defined. Thus, because a wide variety of results can be obtained with different techniques of extraction, researchers in this field should try to devise a standardized procedure that will show good correlation between the concentration of extractable protein and texture as determined by an organoleptic panel.

More than one set of conditions, however, may be needed. That is, different species of fish may require different sets of conditions. Furthermore, fish stored, say, at 0° C. may respond differently from fish stored at −30° C.

We believe that standardization of the solubility test would not only increase its value as an objective test of quality but that it would also make comparisons of the work of different investigators easier.

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To help a reader be selective, we present him with a three-sentence abstract at the beginning of each report. The first sentence states the problem that needed solution, the second sentence tells what was done about the problem, and the third sentence reveals the payoff of the research. With that information, the reader can quickly decide whether reading the report will be worth his time.

To help him read rapidly, we use several techniques. The most important of these is to reveal the structure, or plan, of the research to him in the opening part of the report, because to get a quick comprehensive grasp of the report, he must know what the plan of the research was.

We therefore recommend that, in writing a report for *Fishery Industrial Research*, you reveal the plan of your research early in the report and, furtehrmore, that you thereafter keep the plan prominently before the reader, so that he cannot become confused about it. To accomplish these ends, you will need to use the same plan, or outline, for your report that you used for your research. Assuming, for example, that your overall research involved two closely related experiments -- Experiments I and II -- we recommend that you use the following outline:

TITLE OF THE OVERALL RESEARCH

Introduction (states the need for, and precise purpose of, the overall research and reveals its main divisions)

I. Title of Experiment I

  A. Materials and methods used in Experiment I

  B. Results from Experiment I and discussion of the data

II. Title of Experiment II

  A. Materials and methods used in Experiment II

  B. Results from Experiment II and discussion of the data

Discussion of, and conclusions from, the overall research
Introduction (states the need for, and precise purpose of, the overall research and reveals its main divisions)

Thus, we do not recommend the following outline, though it is commonly used:

TITLE OF THE OVERALL RESEARCH

I. Procedure

  A. Materials

  B. Methods

II. Results and discussion

  A. Results of Experiment I (plus any information needed on materials and methods to make the results clear)

  B. Results of Experiment II (plus any information needed on materials and methods to make the results clear)

Note that if you were to use the second outline, you would not reveal the plan of the research to the reader until he reads your results and discussion. Even then, you may not reveal the plan unless you present the results of each experiment separately, as indicated in the second outline. If you present them as a unit, as often is done, you may not reveal the plan of the research at any point in your report. In that event, the reader will have to figure backward from your tables and graphs and perhaps reread your report several times in order to infer the plan. Such inferences take time that he cannot afford to spend. So, to give the reader a quick grasp of your report, use the same plan for your report that you used for your research, as indicated in the outline recommended.

For further information, see Circular 272: *Organizing the Research Report to Reveal the Units of Research*, by F. Bruce Sanford (for sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, Price 15 cents).

by F. Bruce Sanford, Lena Baldwin, and Mary S. Fukuyama

A. APPROACH

Write your paper for a reader who has had advanced scientific training. Organize and write it in such a way that he can read it rapidly, yet understand it the first time through.

B. COMPONENTS OF THE PAPER

1. Title

Select a title that reveals the overall purpose of your research. When appropriate, include scientific names of species.

2. Abstract

Make the abstract semidescriptive: tell what the report is about, and end with a statement of your overall conclusion. (This conclusion will answer the question stated, or implied, by your overall purpose.) Keep the abstract short, but do not use the title of the paper as the assumed antecedent of otherwise irreferable pronouns.

3. Contents

Include a table of contents.

4. Introduction

In the introduction, (1) orient the reader to your overall purpose, (2) state the purpose explicitly, (3) orient the reader to the subpurposes, and (4) end with a listing of the subpurposes.

Include in each orienting discussion all the important words that will occur in the subsequent statement of purpose. Avoid unnecessary reviews and economic data.

When stating the overall purpose, include a word such as "purpose" so that the reader can quickly identify the statement for what it is.

5. Main Divisions

Do not use such generalized divisions as "Experimental." Instead, be specific by making the main divisions of the paper correspond to the main divisions of your research—Experiment I, Experiment II, and so on. Give each experiment a specific title so that the reader will gain immediate insight into the scope of the experiment.

For main divisions, do not use "Materials," "Procedures," and "Results" (except when, as is rare, your paper reports only a single unit of research, such as Experiment I); instead, use these headings for minor divisions. When you use them, consider the following suggestions:

a. Materials and methods.—Describe in detail the materials and the methods used in your first experiment. If the materials and methods used in succeeding experiments are similar to those in the first, merely describe the differences when you report the succeeding experiments.

If a method includes several closely consecutive steps, number them and write out the steps; use the active voice—for example, "In the separation of acids from the aqueous phase, the analyst:

1. Neutralized a 1-milliliter portion of the aqueous layer to a pH of 10 with 0.1 N NaOH.
2. Transferred the neutralized solution to Flask A.
3. Placed Flask A in a bath . . . ."

b. Results.—Report all numerical data in tables and graphs—avoid cluttering the text with numbers. In the discussion of results, do not repeat the data that are contained in the tables and graphs. Instead, analyze the data by pointing out significances and implications. Use summary tables; do not overwhelm the reader with unnecessary tables of raw data.

6. Conclusions

Draw conclusions from your results. Make sure that the overall conclusion and the subconclusions correspond with your overall purpose and subpurposes. Present the conclusions in logical sequence.

7. Summary

End the report with a summary. Make the summary quantitative, not merely descriptive. If the report is short, end it with "Summary and Conclusions." If it is long, separate the two.

8. Acknowledgment

Avoid titles of individuals—such as mister, doctor, or professor. Simply acknowledge the assistance received.

9. Literature Cited

Make your citations complete and accurate so the reader can find the original with ease. Follow the format used in *Fishery Industrial Research.*

C. MECHANICS

1. Abbreviations

Avoid abbreviations unless you have compelling reason to use them—for example, if you lack space in your tables. If you use abbreviations, use the ones standard in your discipline. End the abbreviation with a period. See the latest issue of *Fishery Industrial Research.*

2. English Usage, Punctuation, and Capitalization

Meticulously follow established practice in grammar, punctuation, and capitalization. For precise, forceful statements, use personal pronouns where appropriate and thereby avoid illogical constructions or ambiguities.

3. Headings

Use the system of headings shown in the latest edition of *Fishery Industrial Research.*

4. Numbers

Use Arabic numbers unless you have a compelling reason to use Roman numbers or to write the numbers out. See the latest issue of *Fishery Industrial Research.*

5. Tables and Graphs

a. Tables.—Number each table and give it a title. (The title, placed at the top of the table, is a brief statement of such applicable referents as the nature, classification, or chronology of the information presented, and the political division, geographical area, or physical plant to which the data refer. These points are sometimes referred to as the "what," "how classified," "when," and "where" of the table.) Do not place a period at the end of the title. When headings apply to information in more than 1 column, word them so that they reveal the meaning of the data in all columns covered. Place all units of measurement over figure columns, and underline. Separate all columns with vertical lines, but use horizontal lines and footnotes sparingly. Place each table on a separate page. See the latest issue of *Fishery Industrial Research.*

b. Graphs.—Number each graph. Place the title at the bottom of the graph, and end it with a period. In wording the title, follow the suggestions for titles of tables. Frame all 4 sides of the graph. Place tick marks on the inside of the frame at only the left and bottom sides unless you have compelling reason to do otherwise. Identify ordinate and abscissa; capitalize all letters in the identification. Place units of measurement in parentheses, and print them in lower case. Unless it clutters the graph, label each curve directly instead of using a legend or a key. Place each graph on a separate page. See the latest issue of *Fishery Industrial Research.*

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