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The Effect of Handling or Processing Treatments on Storage Characteristics of Fresh or Frozen Dogfish (*Squalus acanthias*)

by

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Introduction

Except for a small ethnic demand there has never been a real domestic market in the United States for dogfish, Squalus acanthias, (also referred to as spiny dogfish, sandshark, or grayfish) as a food fish. This is probably related to the aversion that the general populace has with sharks, a feeling fostered in large part by writers of fiction who usually portray these fish in evil and menacing roles. Breaded dogfish fillet portions have been served fried at the Gloucester Laboratory to numerous individuals, sometimes without their knowledge of the species being consumed, and the product was usually received very favorably. The species of shark considered to have the best eating qualities are mako, mackerel, soupfin, thresher, white and spiny dogfish (Ronsivalli, 1978). Testmarketing of dogfish in Tidewater, VA supermarkets has shown promising acceptability by the public (Frye, 1982). However, in a taste test sponsored by the Maine Department of Marine Resources the public found dogfish quite acceptable until they found out what they were eating (Anon, 1980). The common name "dogfish" applied to this species does little to enhance its sales appeal. The name "grayfish" has been used in this country and the Food and Drug Administration does sanction its usage provided no attempt is made to deceive the consumer. In Europe dogfish is known under various names including rock salmon.

During the 1940's a commercial market existed for dogfish but solely for the vitamin A which was highly concentrated in their livers. However, with the advent of synthetic vitamin A production, this market soon became extinct in the U. S. and other markets have not developed. Some dogfish meal has been produced for animal feed, but the demand is very limited because of the relatively lower protein content and high urea content

compared to fish meal from teleost fish. As a fertilizer the high nitrogen (urea) content promotes lush foliage development, but with some fruiting plants flowering may be restricted. Besides, chemical fertilizers may be competitively priced and are easier to apply. In the early to mid 1970's a fishery for dogfish did flourish off the northeast U. S. - Canadian Maritimes coast with annual total landings ranging from 17,000 to 24,000 metric tons. However, the greater part of these catches was taken by foreign vessels and with the passage of the Fisheries Management and Conservation Act the total landings by 1978 was down to less than 2,000 metric tons. With the relaxation of fishing pressure on dogfish along the northeast U. S. coastal waters their stocks continued to increase and they have come to be regarded as pests by fishermen because of their damage to nets when inadvertently captured along with other targeted species, and also because of losses in the commercial fishery due to their predation on the fish stocks and on fish caught in gill nets or long lines (Jensen, 1967). Although the size of the dogfish resource in the northwest Atlantic is not exactly known, it has been estimated to be very large to the extent that the dogfish has been considered the largest underutilized fin fishery resource in the northeast.

In European countries such as France, Belgium, Great Britain and Italy, and in some other countries of the world, dogfish is regularly consumed, the European market demand having been spawned by a shortage of protein food during the post World War II years (Tarr, 1958). In Great Britain dogfish fillets have been utilized in the traditional fish and chips, and in the Federal Republic of West Germany smoked dogfish belly flaps, known as

schillerlocken, are regarded a delicacy. Because of a recent decline in the European dogfish resource, U. S. fishermen and processors along the northeast coastal states have been stimulated by the potential of the European dogfish market and an export business has sprung up. Since dogfish have usually been considered and treated as trash fish in this country, some fishermen and processors may not be familiar with the proper methods for handling dogfish in order to maintain the highest quality. Dogfish and other sharks along with skates and rays are members of the subclass elasmobranch, which are characterized by a soft cartilagenous skeleton in contrast to the teleost fish which have a bony skeletal structure. The elasmobranch are known to have a very high concentration of urea. Simidu (1961) reported a urea content in various elasmobranch ranging from 1.4 to 2.1 percent in the flesh. In dogfish the amount was 1.7 percent. Urea is formed in shark by an enzymatic conversion of the amino acid arginine into ornithine and urea. Thus, the sharks have evolved a method of helping to detoxify ammonia which is an end product of nitrogen metabolism in animals. The elasmobranch also have an unusually high content of trimethylamine oxide. Dyer (1952) reported the average trimethylamine oxide nitrogen content in dogfish to range from 190 to 267 mg/100 g flesh. This is much greater than the amount present in teleost fish and is about 2 to 3 times greater than that of gadoid species. About 9 to 18 percent of the total nitrogen in teleost fish consists of non-protein nitrogen, whereas in the elasmobranch the corresponding value is about 33 to 38 percent (Simidu, 1961). In the elasmobranch the non-protein nitrogen is principally urea and trimethylamine oxide. These two compounds serve a very important biological function in the elasmobranch by acting as osmoregulators which permit these fish to exist in a salt water environment

without losing body water through osmosis. However, when the fish dies, these compounds are subject to breakdown due to the action of bacterial enzymes, the rate of the reaction being a function of the temperature. The urea is decomposed to ammonia and the trimethylamine oxide is reduced to trimethylamine. This latter compound is reputed to be responsible for the stale "fishy" odor that develops in spoiling fish. Therefore, because of the very high content of urea and trimethylamine oxide in dogfish, and their predisposition to forming ammonia and trimethylamine, one would expect, a priori, that dogfish would be more susceptible to spoilage at temperatures above freezing compared to the traditional white-fleshed species landed in the northeast, and would therefore require better care in handling. Methods have been devised for removing some of the urea from shark and thus mitigating the ammonia problem. Tishin (1970) proposed treating minced shark meat with urease, neutralizing with an acid and then washing with cold water. He also found that just washing the minced flesh in 4 to 5 changes of cold water was sufficient to remove most of the non-protein nitrogen. Other methods include soaking the meat in a 1 percent salt solution (Ronsivalli, 1978). However, in order to preserve the pink color, which the Europeans consider to indicate high quality in dogfish, it has been recommended that skinned dogfish carcasses or fillets not be washed or placed in contact with ice since this will leach out the pink color.

An impediment to the expansion of an American export business for dogfish to Europe has been the variability in quality of product shipped to that continent which resulted in rejection of shipment in some cases. Therefore, in June 1980 an investigation was initiated at the Gloucester Laboratory

into the problem of ammonia production in the flesh of dogfish, and also, the development of a rapid and reliable test for measuring this ammonia content in the field or at a processing plant without the use of a complicated procedure or equipment. This study was necessitated because of a specification imposed by certain importing countries, notably Belgium and France, on a limit for ammonia in dogfish, and also because at that time a simple rapid method that could be employed by exporters of dogfish for monitoring ammonia content was not available.

Storage studies were conducted in both the frozen and fresh states to determine spoilage patterns, to ascertain storage life, and to establish the reliability of various chemical or physical tests for assessing sensory quality. The investigation, which consisted of seven independent studies, was conducted over a two and one-half year period. The first study was concerned with the storage characteristics of 1 day old gutted dogfish held on ice. This was followed by a similar study except the fish were headed and gutted. Another study compared the iced storage characteristics of either whole, gutted, or headed and gutted dogfish. To determine the effect of temperature abuse, a fourth study compared storage characteristics of whole dogfish held at either 57°F, 46.4°F or 32°F (ice). One final non-frozen study was carried out and its objective was to ascertain the role of autolytic enzymes and microbial enzymes in ammonia production in dogfish.

Two frozen storage studies were conducted at 0°F. One was designed to determine the effect of packaging on storage life, the other was concerned with the use of an antioxidant.

In this report these studies will be reported individually and not necessarily in chronological order.

Methods

Sensory analysis. For organoleptic evaluation samples were enclosed in aluminum foil and baked in an oven at 400°F for 15 to 20 minutes for belly flaps, or for 20 to 30 minutes for fillets. Although this method of culinary preparation is not the best way for savoring the gustatory properties of dogfish, it does enhance the detection of off-flavors. Cooked samples were rated on a scale of 1 to 9 for odor, flavor and texture by a six member taste panel made up from the scientific laboratory staff. In addition to the usual sensory attributes, panelists were instructed to concentrate on their awareness of ammonia. Descriptive terminology and the corresponding numerical ratings were as follows:

excellent	9
very good	8
good	7
fair	6
borderline	5
slightly poor	4
poor	3
very poor	2
inedible	1

Useful shelf life was considered to have expired when the average sensory rating for either odor, flavor or texture reached a value of 6.

Moisture content. This was determined by drying duplicate 10 g samples at 100°C (212°F) to constant weight.

pH. Twenty grams of flesh were blended with 40 ml distilled water for one minute and the pH of the slurry was measured with a Fisher Model 320*

*Mention of trade names or products does not constitute endorsement by NOAA/NMFS.

expanded scale pH meter.

TBA number. Thiobarbituric acid (TBA) number, which is a measure of the degree of oxidative rancidity, was determined by the procedure of Yu and Sinnhuber (1957) modified by the addition of EDTA and propyl gallate to prevent oxidation during sample blending.

Ammonia. The ammonia content of the flesh was determined by the chemical procedure developed by Seligson and Seligson (1951) and later modified by W. Vyncke (1968) of the Fisheries Research Station at Ostend, Belgium. This accelerated microdiffusion technique is very simple and many samples can be analyzed in a single day. Extracts of ammonia from the flesh can be prepared in advance and frozen so that a greater number of samples can be analyzed simultaneously. The only equipment necessary for this procedure is some inexpensive bottles each fitted with a vinyl stopper and footed ground glass rod, a rotator, and a spectrophotometer. Essentially, the method consists of comminuting a sample of dogfish with water in a blender, placing 1 ml of the extract with 1 ml of potassium carbonate solution into a vinyl stoppered bottle having a glass rod fitted in the stopper, and rotating the bottle on its side for 30 minutes. The tip of the glass rod had been wetted with sulfuric acid prior to stoppering, and during the rotation ammonia in the extract is converted to gaseous ammonia which diffuses to the glass rod tip and forms ammonium sulfate. At the end of the rotation period, the rod is dipped into Nessler reagent and the yellow-orange color formed is read in a spectrophotometer. The Belgian specification for ammonia in dogfish (not to exceed 55 mg ammonia nitrogen / 100 g) is predicated on this analytical procedure.

TMA, DMA. The volatile amines, trimethylamine (TMA) and dimethylamine (DMA) were determined by a modification of a gas chromatographic method described by Lundstrom and Racicot (1983). The procedure consists of extraction of the amines by perchloric acid and then extraction in n-amyl alcohol after liberation of the free amine by KOH. The amines are separated on a porous polymer column (Chromosorb 103) and detected by a nitrogen/phosphorous specific flame ionization detector. Quantitation was accomplished using an internal standard, n-propylamine.

TMAO. Trimethylamine oxide (TMAO) was determined as TMA after reduction by $Ti\ Cl_3$ and from this value the TMA content determined on the unreduced sample was subtracted. The concentration of the various amines was based on their nitrogen content and expressed as mg nitrogen per 100 g of samples or mg % N.

TVB. Total volatile bases (TVB) is a measure of the total volatile amine compounds present and it collectively includes ammonia, monomethylamine, dimethylamine and trimethylamine (Simidu, 1961). In this study monomethylamine was not detected in measureable quantity, and TVB was therefore considered to consist of ammonia, di- and trimethylamine. These latter compounds individually or as TVB have been employed as chemical indices of spoilage in fish. There are specific methods for determining TVB, however, we did not employ one of these methods. Instead, ammonia, TMA and DMA were ascertained separately and the contents at any given time were summed to obtain TVB. The concentration was expressed as mg nitrogen per 100 g sample or mg % N.

EPN. Extractable protein nitrogen (EPN) is a measure of the amount of protein nitrogen extracted in a phosphate buffered 0.5 ionic strength KCl solution. This parameter has been used as an index of textural change in frozen fish. It was determined by a slight modification of the method described by Ravesi and Anderson (1969) and was expressed on a percent basis. Protein contents were assayed by a biuret analysis which was modified slightly to remove turbidity from the biuret-extract mixture. The mixture was allowed to stand overnight and then centrifuged to remove the colloidal matter, allowing spectrophotometric measurement of a clear solution.

APC. Aerobic plate count (APC) was made from appropriate dilutions of the flesh on to pour plates of TPE agar (Standard Methods Agar reinforced with 0.5 percent Bacto-peptone and 0.5 percent Na Cl) as recommended by Lee and Pfeifer for seafoods (1974). Duplicate plates were incubated at 20°C (68°F) and colony counts were made after 5 days.

The numbers of urease-positive bacteria were estimated by inoculating half of the colonies from a TPE agar plate containing about 30 to 100 colonies into individual tubes of Bacto-urea broth and observing for a positive reaction following an incubation period. The number of positive cultures was then multiplied by two and also by the dilution factor of the agar plate from which they were taken to obtain the urease-positive count per g.

Fat content. Total lipid was obtained by the methanol-chloroform extraction procedure of Bligh and Dyer (1959).

All chemical, physical or microbial analyses were made on duplicate samples and the average result is reported.

Statistical calculations and analyses were performed on a Hewlett-Packard 97 programmable calculator.

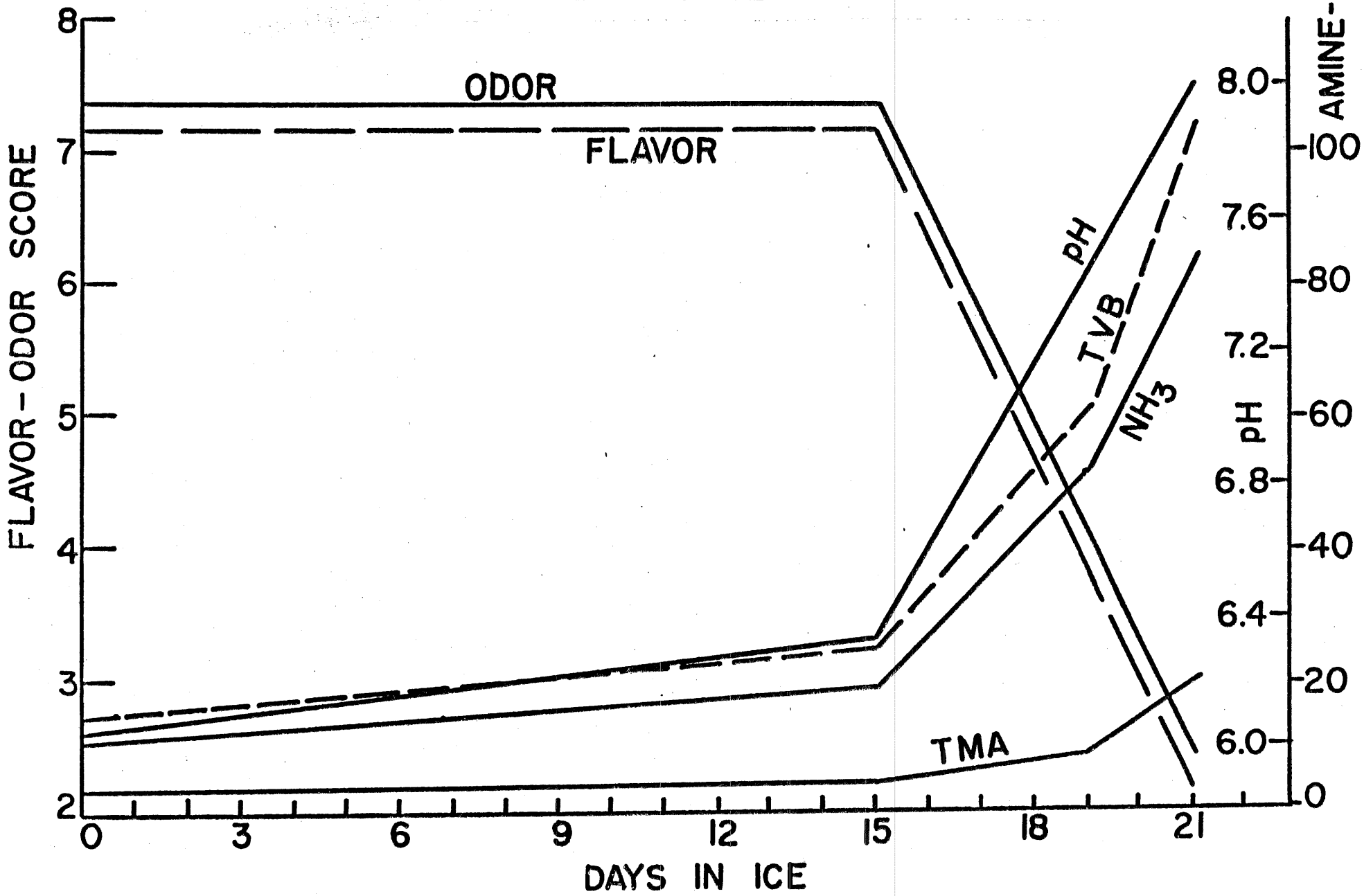
Results

Iced storage study I. To provide information about the spoilage patterns which might be encountered in dogfish, an iced storage study was conducted. Dogfish, received at the Laboratory on the same day that they were caught (July 1980), were immediately gutted, washed and packed in ice. Three fish were taken from storage after intervals of 0, 2, 5, 9, 12, 15, 19 and 21 days for testing. One fillet from each fish was used to monitor changes in ammonia and TMA contents and in pH. The other fillet was used for taste panel evaluation.

The results of these tests are shown in Figure I - 1. Flavor and odor of the fillets remained relatively unchanged during the first 15 days iced storage, but after that, quality deteriorated rapidly and the samples were considered to have lost their shelf life after 16 to 17 days. Ammonia, TMA, TVB and pH increased slightly during the first 15 days, but increased markedly thereafter. Thus, the decrease in quality coincided with the increase in volatile amines and pH. Since we had no previous experience with dogfish, the results of this storage study came somewhat as a surprise. From all the hearsay on keeping quality of dogfish, it was expected that the iced shelf life would be much shorter than what we found, and that an early production of ammonia would limit the shelf life. Instead, the ammonia content remained relatively low for the first two weeks and the spoilage pattern seemed not much different from that which develops in iced gadoid fish.

There was a high correlation between ammonia content and either flavor score ($r = 0.89$) or odor score ($r = 0.92$). From linear regression analysis an ammonia concentration of about 32 mg N/100 g was estimated as being

FIG. I-1 STORAGE CHARACTERISTICS OF GUTTED DOGFISH HELD IN ICE.



indicative of marginal quality dogfish. A high degree of correlation was also observed between flavor score and either pH ($r = 0.94$), TMA content ($r = 0.88$) or TVB content ($r = 0.91$). Thus, from these preliminary results it appears that any of these biochemical tests might be useful for assessing spoilage in dogfish providing there is a sufficient degree of accuracy and reproducibility among different batches of fish.

Ammonia content also correlated very well with pH ($r = 0.97$). Thus, pH measurement also shows potential as a simple rapid method for monitoring ammonia content.

Iced storage study II. In the previous study even though attention had been focused on icing the fish well, toward the very end of the experiment the ice beneath the bottom layer of fish had all melted and the fish, although still well iced above, were in contact with the wood container on their ventral side. Therefore, the iced storage study was repeated in December 1980 to ascertain whether or not an extension of the storage life beyond 16-17 days might be attained under conditions which assured complete icing at all times. Three-day post-mortem iced fish were obtained from the Point Judith, RI Fisherman's Co-operative and after heading, gutting and washing they were stowed in flake ice in standard 125-pound wooden fish boxes, with 9 fish per box, and placed in a cold room at 35 ^{o}F. At various storage intervals three fish were removed for analysis. One fillet and belly flap from each fish was used for sensory evaluation, and the other for bacterial, chemical and physical testing. Practically all the fish were females. Eight fish were randomly selected for yield data. The average whole weight was 9.9 pounds (range 7.5 - 12.9) and the average yield after heading and gutting was 46 percent.

In Figure II-1 pH value, TBA number and organoleptic scores for flavor, odor and texture have been plotted as a function of iced storage time for fillet portions taken from the stored headed-gutted fish. The same parameters are shown in Figure II-2 for the belly flap sections. During storage there was a progressive deterioration in odor, flavor and texture (softening) and this was accompanied by an increase in pH (due to accumulation of basic amines) and TBA number. Spoilage occurred more rapidly in the belly flap portion compared to the fillet. In fact, the fillets were only on the threshold of spoilage after 18 days whereas the belly flaps were regarded

as marginal at approximately 12-13 days. Rancidity, as measured by TBA number was more evident in belly flaps, however, a few members of the taste panel reported rancid flavors in the fatty strip along the lateral line of the fillet as early as the 16th day, even though the lean muscle tissue was devoid of rancid flavor throughout the entire storage. In general, spoilage of the fillets was due to textural softening and development of ammonia whereas spoilage of belly flaps was caused by rancid flavor, ammonia and textural breakdown. Thus, from the results of these first two studies it would be concluded that the iced shelf life of gutted dogfish, excluding belly flaps, was 16 to 18 days. In the scientific literature there is much variation in the estimated storage life for iced dogfish. James and Olley (1971) show a shelf life for shark of 10 days at 32°F. This data had been cited from a Japanese reference and the species of shark was not mentioned, but more importantly the fish in that study had been stored in air at 32°F and not in melting ice at 32°F. Southcott et al (1960) reported that less ammonia and TMA were produced in dogfish stored in ice compared to air at 32°F. Therefore, this would infer that the iced storage life of the shark referred to in James and Olley's paper would be greater than 10 days. An iced storage life of 16-19 days was determined by Southcott et al (1960) for headed and gutted dogfish. The species was reported as Pacific coast dogfish (Squalus suckleyi) but it has since been established that this is the same fish as Atlantic coast dogfish (Squalus acanthias). Stansby et al (1968) concluded that the storage life of iced headed and gutted Pacific coast dogfish was limited to the development of rancidity and shelf life was estimated as 14 days. In our study storage lives were determined

separately for the belly flap and fillet section with flap removed, and it was conceded that rancidity was probably a significant factor involved in the spoilage of the belly flap, but not of the flap-less fillet. Stansby et al may have been testing the entire fillet with belly flap attached. Even so from the reported shelf lives thus far presented it would appear that the iced shelf life of headed and gutted dogfish would be comparable to that for cod or haddock. The shortest shelf life reported for iced headed-gutted dogfish was 7 days and the basis for this was that ammonia was detected at the end of that time (Jhaveri and Constantinides, 1981). In our study the ammonia content and also, total volatile bases, were relatively stable during the first 16 days storage, but increased thereafter (Figures II-3 and II-4. Stansby et al (1968) similarly found with iced dogfish that the TVB was low (< 12 mg N/100 g) during the first 14 days storage. Moyer et al (1959) reported that the TVB remained at a level of about 10 mg N/100 g throughout the first 12 days of iced storage, and at the end of 3 weeks had only increased to 18 mg N/100 g with no ammonia detected by sensory analysis. The only other reference in the literature to ammonia being detected at an early stage in dogfish stored at 32°F was by Elliot (1952) who noted a slight trace of ammonia after 6 days. However, that study was carried out with minced dogfish stored in jars. It has certainly been established that minced fish is more prone to microbial degradation compared to intact muscle because of the increased surface area, rupture of cells releasing enzymes, nutrients, etc. and it has also been demonstrated that spoilage of dogfish in air at 32°F proceeds faster than in ice at 32°F. Therefore, these factors would account for the early ammonia production

observed by Elliot (1952). However, the short storage life of 7 days on ice reported by Jhaveri and Constantinides is not compatible with our results or those of Moyer et al (1959) and Stansby et al (1968).

In the present study an ammonia content of approximately 20 mg N/100 g was predicted at the estimated spoilage time. A value of about 30 was noted in the first study. These spoilage levels are much less than the value of 60 mg ammonia N/100 g recommended by Vyncke (1968) as the cutoff value for acceptable/unacceptable imported dogfish.

The concentration of DMA remained relatively constant and insignificant ($<$ 1 mg DMA-N/100 g) throughout storage (Figures II-3 and II-4). Dimethylamine has been purported to be formed in small amounts by bacterial action on fish (Beatty and Collins, 1940), and Shewan (cited by Dyer and Mounsey, 1945) has proposed a spoilage test based on DMA content. It would appear from our results that the test would not be reliable with dogfish.

The TMA content increased gradually over the 23 day storage period attaining a final maximum value of about 6 mg TMA-N/100 g. Usually at spoilage, at least for codfish, a TMA-N concentration of about 15 is obtained (Dyer and Dyer, 1949). In their study with iced dogfish, Moyer et al (1959) reported that the TMA content was low throughout 21 days of iced storage, and had only reached a maximum value of 5 mg N/100 g. Similar results were obtained by Southcott et al (1960). These investigators found that the TMA content of iced dogfish remained constant for about the first 14 days and reached an estimated value of 10 mg N/100 g after 20 days. Elliot (1952) likewise encountered a relatively low final TMA concentration in his studies with minced dogfish muscle stored at 32°F. Trimethylamine is formed by the action of a bacterial enzyme, triamine oxidase, on the precursor substance,

trimethylamine oxide, found in marine species. The optimum pH for activity of this enzyme was reported to be 7.2-7.4 in cod (Castell and Snow, 1949). This optimum range was also confirmed in dogfish by Elliot (1952) who speculated that the reason for only a small amount of TMA produced in dogfish (compared to gadoid species) during spoilage is that the urease-positive bacteria become active sooner than the TMA-producing bacteria, thus forming ammonia which soon elevates the pH beyond the optimum for triamine oxidase activity.

In the present study more TMAO was degraded (reduced) than could be accounted for by the production of end products such as TMA or DMA. For example, the TMAO content of fillets decreased by about 58 mg N/100 g over the 23 day period, yet TMA only reached a maximum value of about 6 mg N/100 g. Dyer et al (1946) in studies with iced gutted cod observed a disappearance of TMAO which they attributed to leaching by the melting ice. This same phenomenon may have occurred in our study with iced gutted dogfish. There was a higher initial concentration of TMAO in the fillet portion (263 mg N/100 g) compared to the belly flaps (204 mg N/100 g) and this could be related to the compositional differences between these two anatomical sections.

The fat content plus or minus one standard deviation was found to average 11.2 $\pm$ 2.2 percent for fillets, and 22.6 $\pm$ 3.7 percent for belly flaps. A seasonal variation was reported in the lipid content of dogfish with a mean value and standard deviation of 14.5 $\pm$ 2.2 percent, and highest fat content occurring during the winter months (Jhaveri and Constantinides, 1981). However, these researchers analyzed a composite sample taken from dorsal and ventral areas, therefore our results cannot be compared directly

to theirs. With regard to fat content dogfish must be a unique species among sharks. Sidwell et al (1974) reported a fat content of 0.5 $\pm$ 0.2 percent for shark species. In 19 out of 21 shark species compared, the fat content was less than 2 percent (Gordievskaya, 1971). The two exceptions were Sevengill shark (13 percent fat) and Greenland shark (10 percent fat). Thus, all sharks may be subject to spoilage from ammonia formation, but a few species including dogfish are additionally susceptible to rancidity development because of their high fat content.

We also determined an average moisture content and standard deviation of 71.4 $\pm$ 2.0 percent for fillets and 64.6 $\pm$ 2.4 percent for belly flaps. Very good correlation ($r = 0.93$) was observed between fat content and moisture content (Figure II-5). Thus, a quick estimate of the fat content of dogfish muscle could probably be obtained using a rapid moisture determination method.

Quality loss during iced storage was also monitored with the Torrymeter. Readings were taken at three consecutive positions as depicted in Figure II-6 along the lateral line starting at the first dorsal fin and proceeding to the caudal peduncle. Readings were generally constant in the region between the two dorsal fins, but were lower in the caudal peduncle. The average Torrymeter readings taken at the three different positions are plotted in Figure II-6 as a function of storage time.

The readings dropped steadily from an initial (3 days post-mortem) average reading of 11 to a final reading of about 6-7 at the end of shelf life. A Torrymeter reading of about 6 to 7 is the value we have usually observed in previous studies with marginal quality fish such as cod and haddock. Thus, the Torrymeter could have possible application for measuring quality of iced

dogfish provided the limitations of the instrument are understood and provided the instrument is consistent from one lot of fish to another.

The production of ammonia in fish post-mortem arises from the enzymatic deamination of proteins, amino acids and some other basic nitrogen compounds present in biological tissue. However, in elasmobranch species, which have an unusually high level of urea in the flesh and blood, ammonia formation after death occurs mainly as a result of enzymatic degradation of urea which according to Shewan (1950) may occur in dogfish muscle up to a level of 2 percent. The enzyme responsible for this activity is urease and some microorganisms possess it. During the 23 day iced storage study, the total number of aerobic bacteria (aerobic plate count) and the numbers of microorganisms capable of splitting urea into ammonia (urease-positive) were monitored. The aerobic plate count decreased slightly during the first 12 days, and then rose sharply during the following 11 days to a final average count of about 300 million per gram. (Figure II-7). Moyer et al (1959) reported an aerobic plate count of about 74 million per gram at the time iced dogfish was considered of marginal quality. In the present study the aerobic plate count at the threshold of spoilage was estimated as 15 million per gram. Throughout storage the percentage of urease positive bacteria remained relatively constant ranging from 3 to 20 percent of the total microbial population; however, as the total bacterial count increased, so did the number of urease-positive bacteria which at spoilage was estimated at 2 million per gram. Southcott et al (1960) monitored the aerobic plate count and urease-positive bacterial count of iced headed and gutted dogfish and they concluded that an obligate urea-utilizing population was not established to the exclusion of other types. Our result is consistent with this conclusion.

In Table II-1 are presented linear regression data for flavor score of either fillets or belly flaps as a function of various chemical or physical spoilage indicators for fish. In general there was good correlation between flavor score with either TBA number, TMA, ammonia, TVB, pH, Torrymeter reading and (log) aerobic plate count. The only exception to this was the low correlation between flavor score of fillets and TBA number. It was previously stated that spoilage of fillets was more associated with bacterial activity than with lipid oxidation. Although some of these chemical/physical parameters correlated well with flavor score, they would only be useful as spoilage indicators and not freshness indicators.

The ammonia nitrogen content at spoilage was estimated from the regression equation to be 20-23 mg/100 g. This is well below the specified limit for acceptable exported dogfish and corroborates the results of the first study.

There also was good correlation between ammonia content and pH, which confirms the results of the first study; however, the regression lines computed from the two studies, $Y = 33.9\ X - 194.1$ (study I) and $Y = 24.6\ X - 143.4$ (study II) were sufficiently diverse as to render this predictive parameter (pH) questionable at this time.

Flavor score correlated well with pH ($r = 0.89$) and the relationship is shown in Figure II-8. Although the degree of scatter of the data points precludes the use of pH for accurately assessing flavor score, this paramater might possibly be employed as a rapid screening method to delineate acceptable from non-acceptable fish by establishing some arbitrary pH, such as pH 6.7, as the cutoff value. A rapid measurement on a fresh fillet or skinned fish could be made with a surface electrode.

Table II-1

Regression data for flavor score of either dogfish fillets or belly flaps as a function of various spoilage indicators

	regression	correlation coefficient	intercept	slope	n
fillet	flavor vs. TBA	0.42	8.15	-0.54	21
belly flap		0.77	8.79	-0.79	20
fillet	flavor vs. TMA	0.77	8.61	-0.43	21
belly flap		0.84	9.34	-0.75	21
fillet	flavor vs. ammonia	0.79	8.01	-0.09	21
belly flap		0.91	7.75	-0.09	21
fillet	flavor vs. TVB	0.81	8.22	-0.08	21
belly flap		0.91	8.01	-0.08	21
fillet	flavor vs. pH	0.84	24.46	-2.77	21
belly flap		0.92	26.94	-3.14	21
fillet	flavor vs. log APC	0.93	9.72	-0.49	21
belly flap		0.98	9.82	-0.48	21
fillet	ammonia vs. pH	0.84	-143.4	24.57	21
belly flap		0.90	-206.4	33.87	16
fillet	flavor vs. Torrymeter reading	0.76	3.89	0.39	

FIG. II-1 STORAGE CHARACTERISTICS OF HEADED-GUTTED DOGFISH (FILLETS) HELD IN ICE.

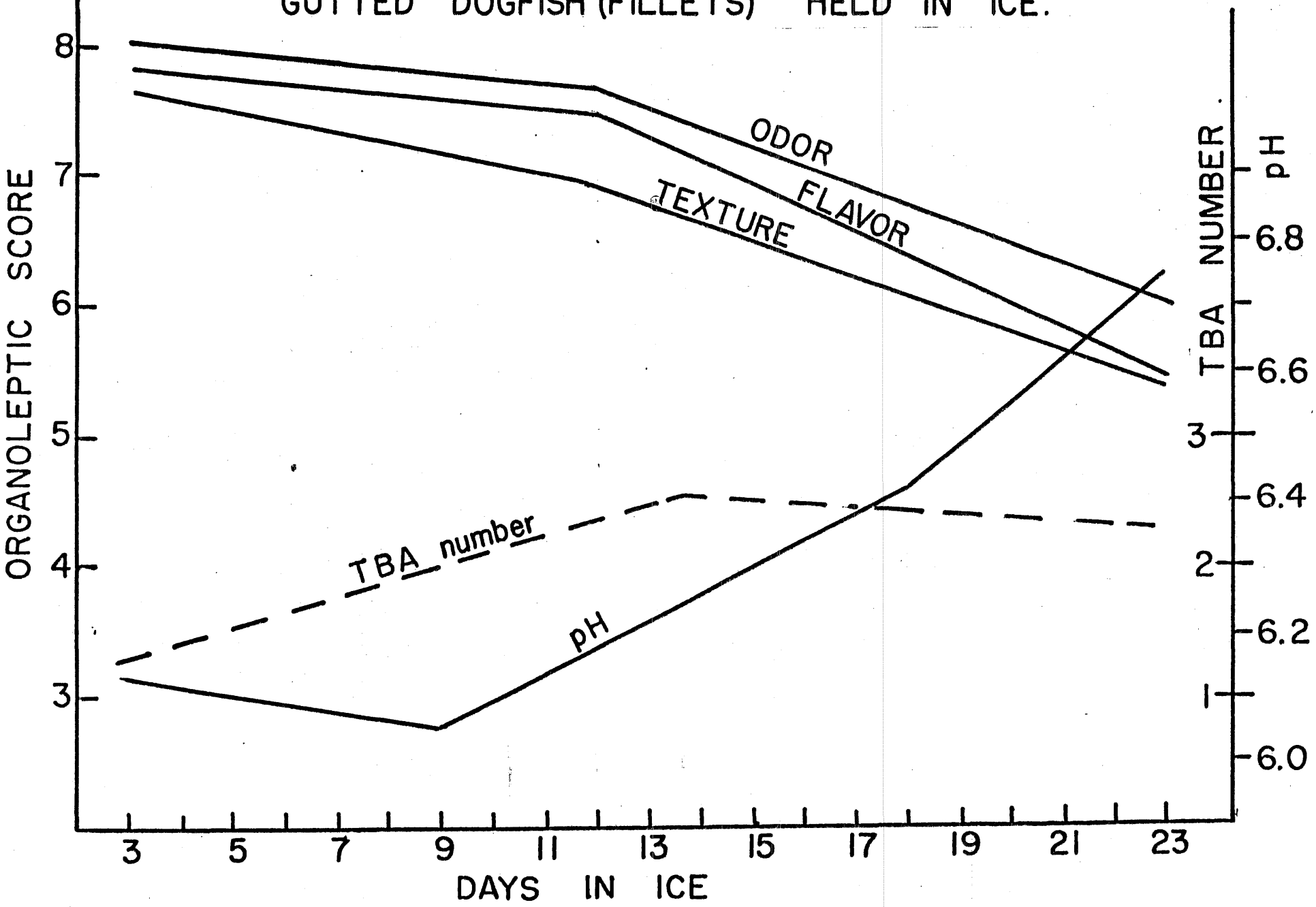


FIG. II-2 STORAGE CHARACTERISTICS OF HEADED-GUTTED DOGFISH (BELLY FLAPS) HELD IN ICE

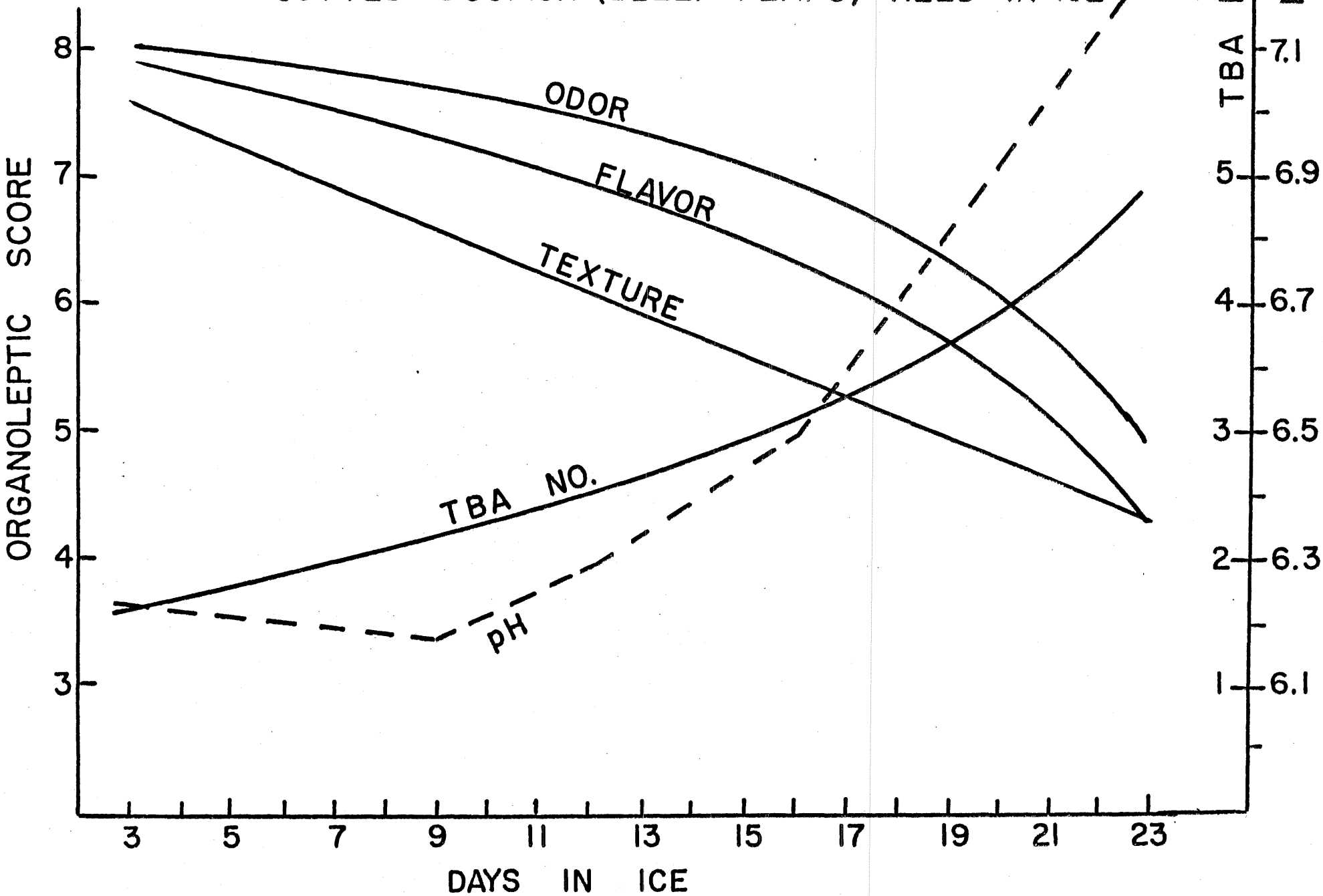


FIG. II-3 EFFECT OF STORAGE TIME AT 32°F ON THE CONTENT OF CERTAIN VOLATILE AMINES IN HEADED-GUTTED DOGFISH (FILLET PORTION).

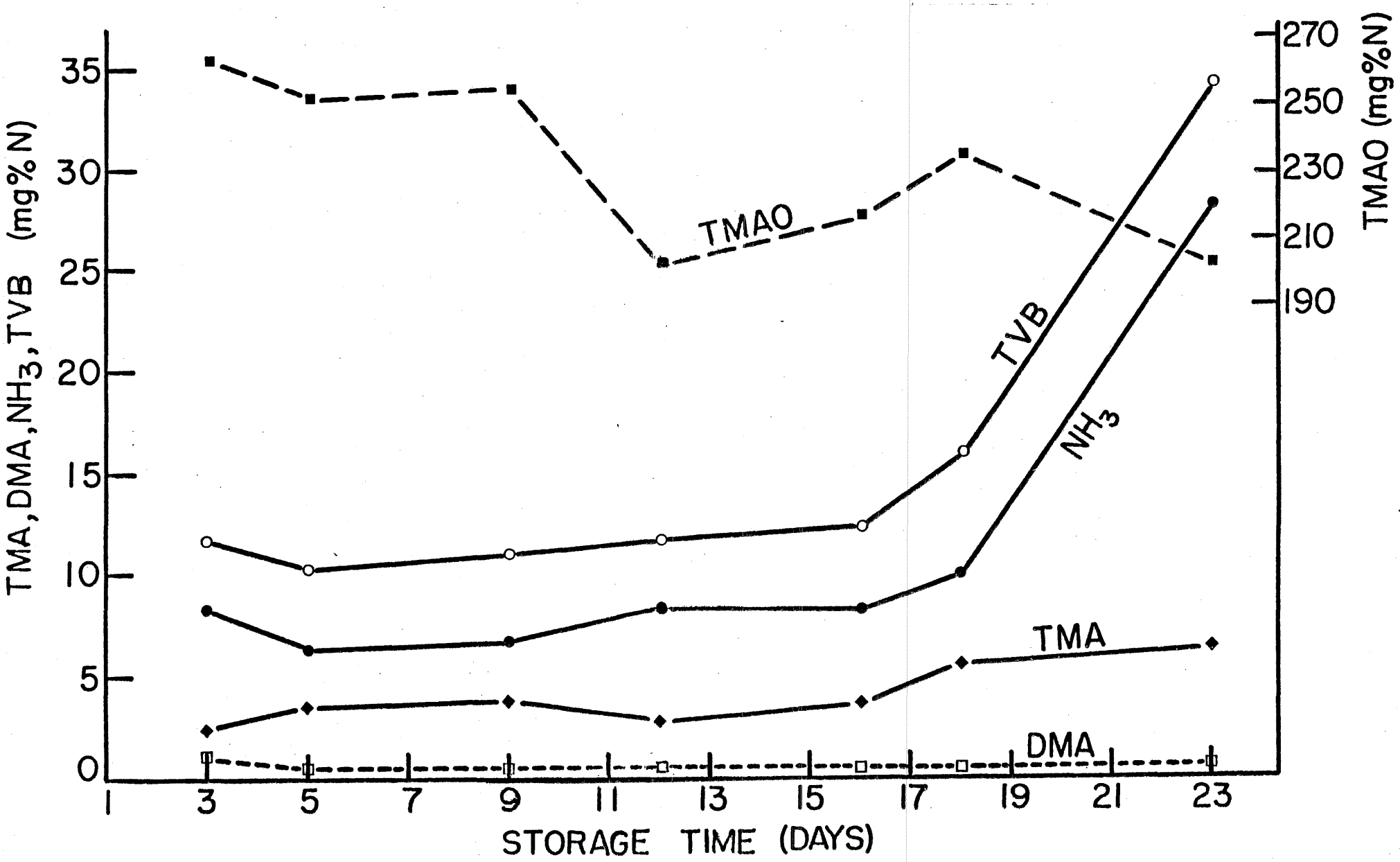


FIG. II-4 EFFECT OF STORAGE TIME AT 32°F ON THE CONTENT OF CERTAIN VOLATILE AMINES IN HEADED-GUTTED DOGFISH (BELLY FLAPS).

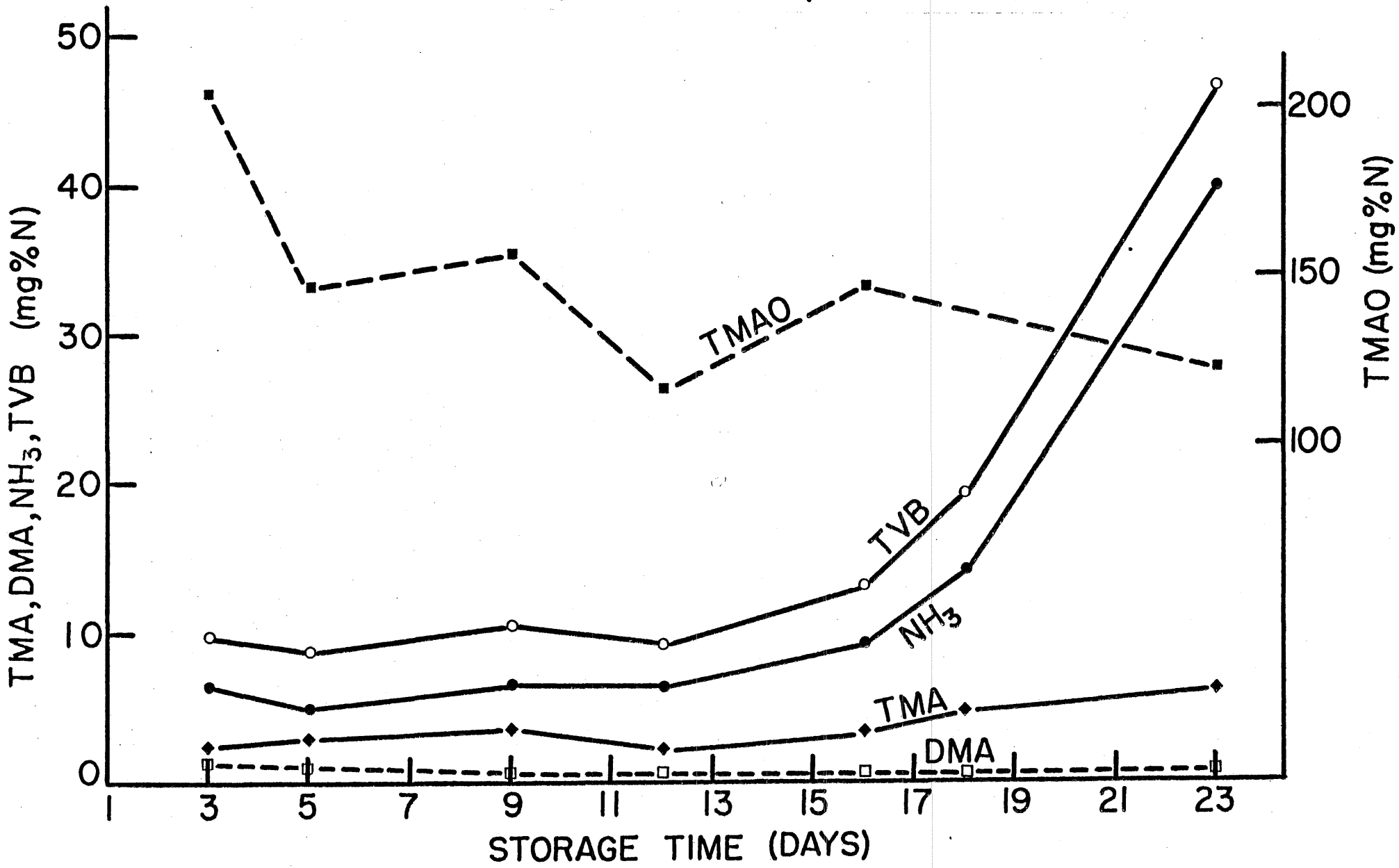


FIG. II-5

FAT CONTENT OF DOGFISH AS A FUNCTION OF MOISTURE CONTENT.

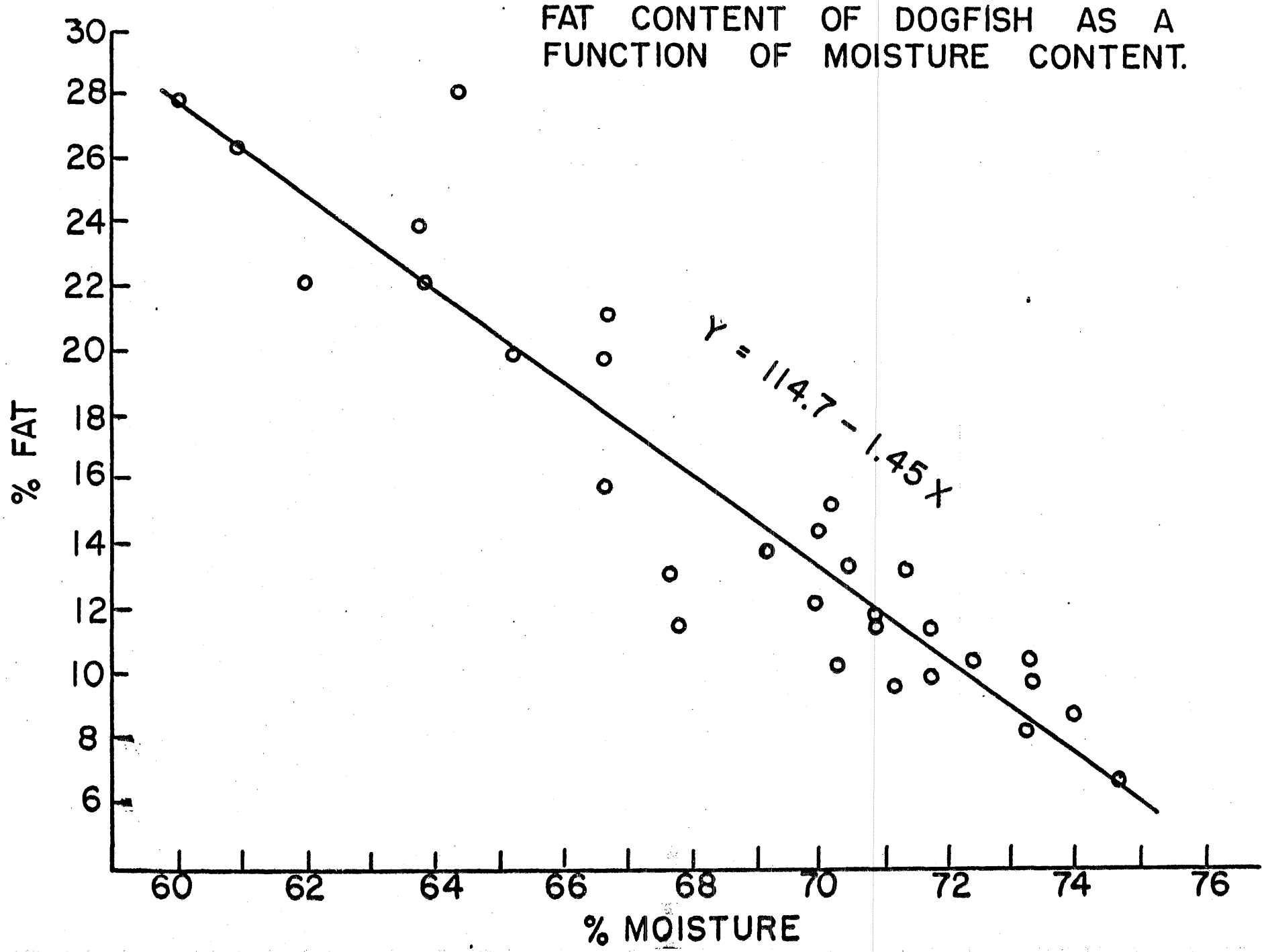


FIG. II-6 EFFECT OF STORAGE TIME AT 32°F ON TORRYMETER READINGS TAKEN AT THREE DIFFERENT POSITIONS ON DOGFISH.

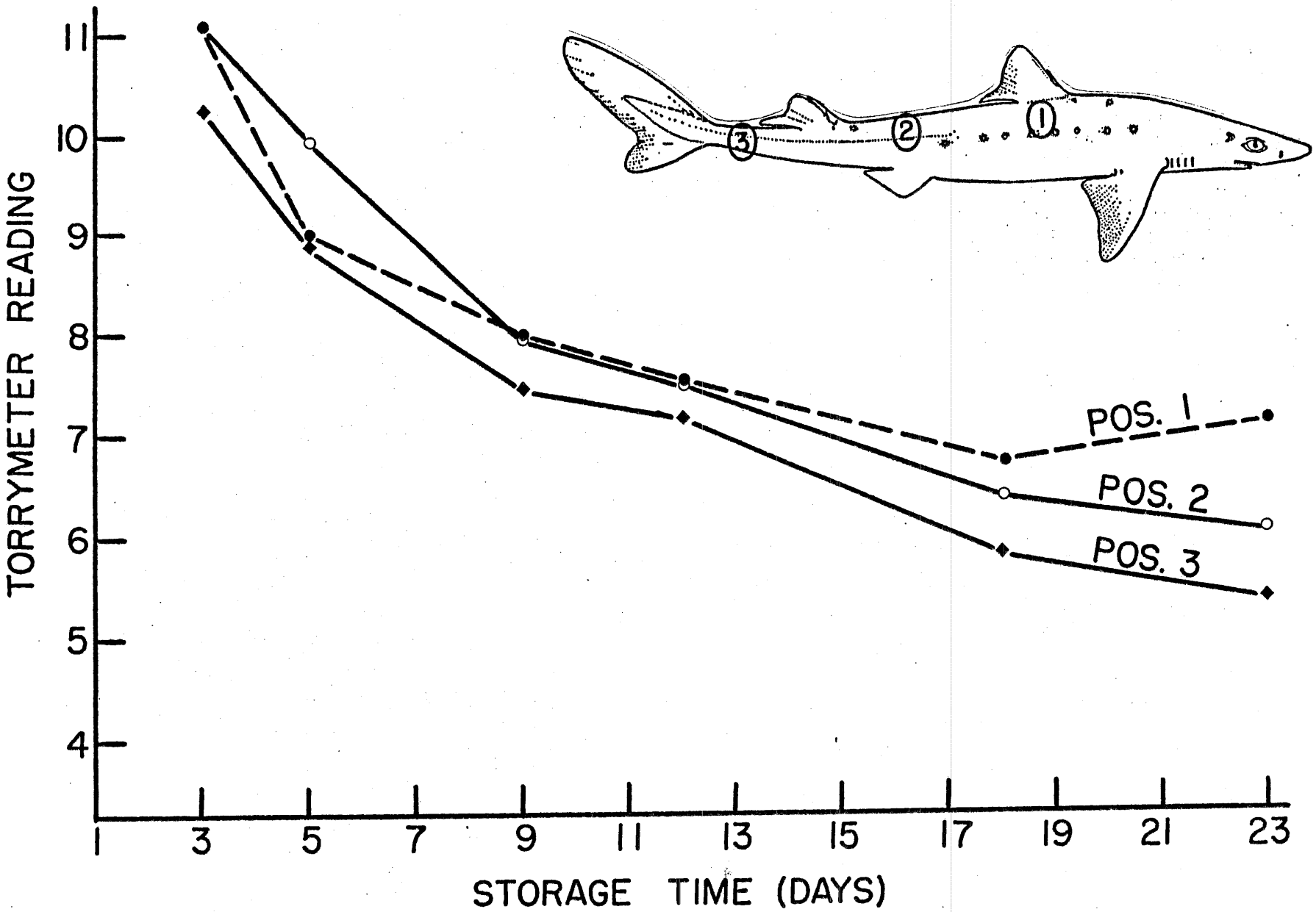


FIG. II-7

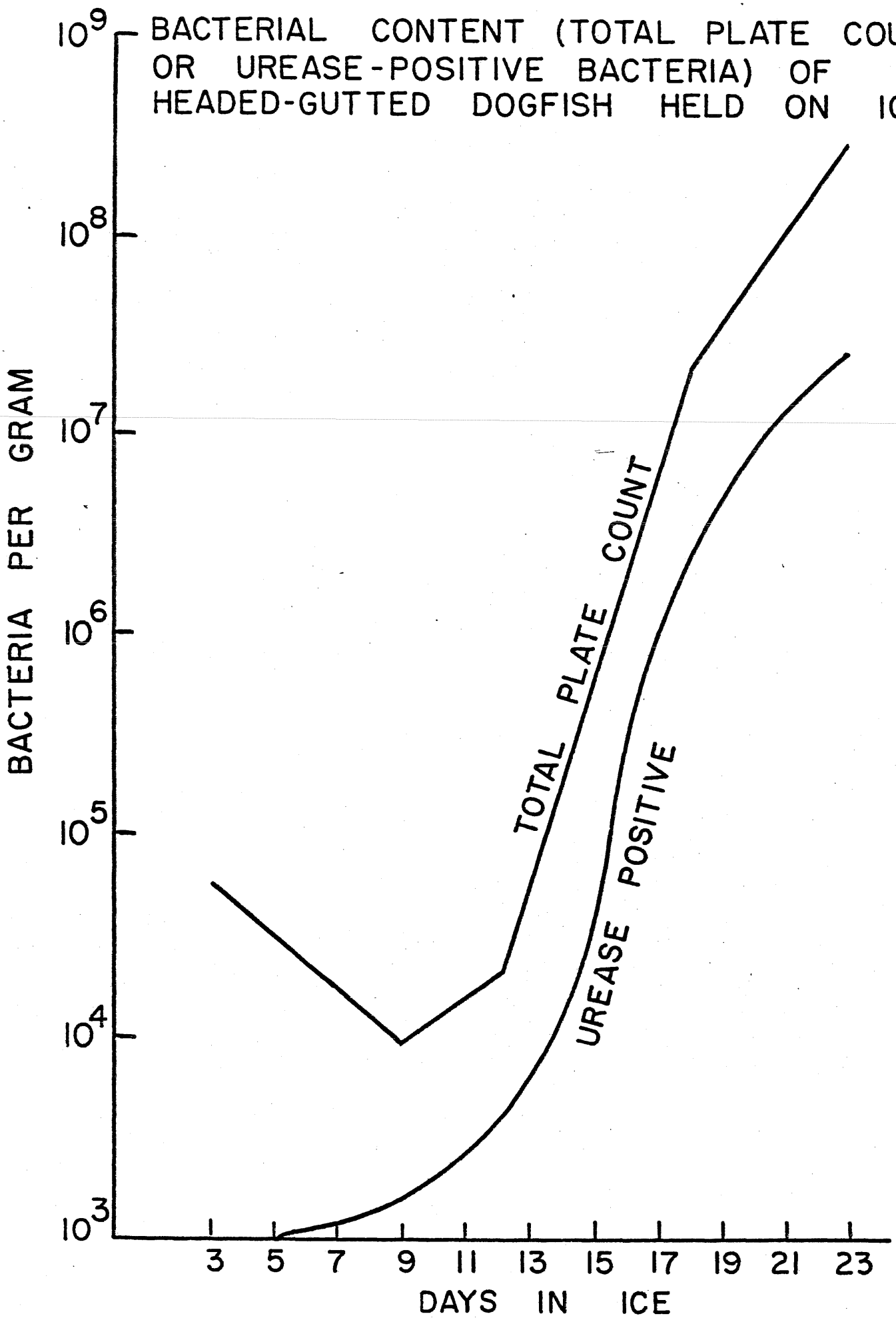
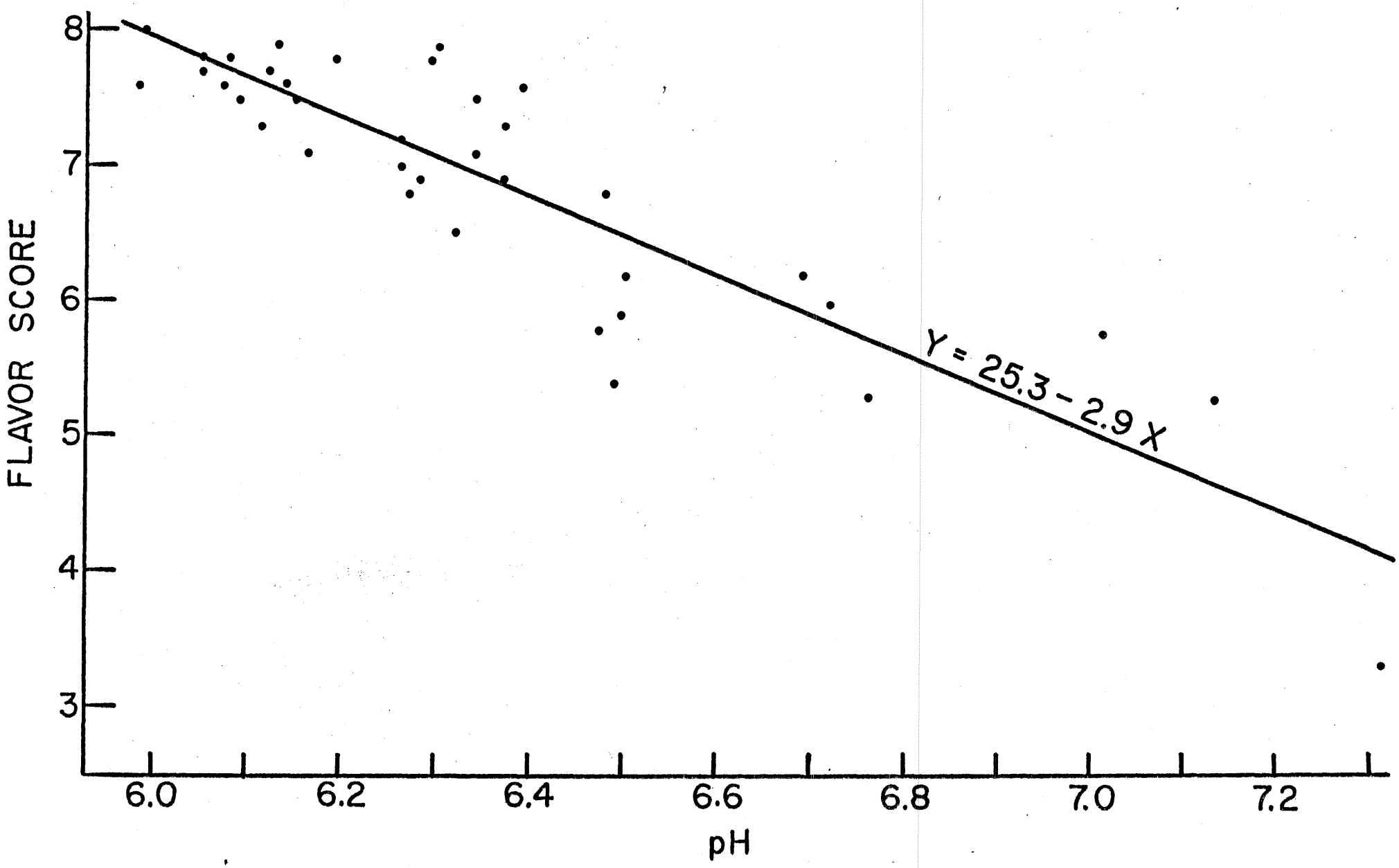

BACTERIAL CONTENT (TOTAL PLATE COUNT OR UREASE-POSITIVE BACTERIA) OF HEADED-GUTTED DOGFISH HELD ON ICE.
BACTERIA PER GRAM
10^9
10^8
10^7
10^6
10^5
10^4
10^3
TOTAL PLATE COUNT
UREASE POSITIVE
3 5 7 9 11 13 15 17 19 21 23
DAYS IN ICE

FIG. II-8 FLAVOR SCORE OF ICED HEADED-GUTTED DOGFISH AS A FUNCTION OF pH.



Iced storage study III

The objective of this study was to determine the effect of heading and gutting, gutting alone, and bleeding on the quality of dogfish during storage in ice. To reiterate, the blood of dogfish contains a high amount of urea which decomposes to ammonia. The elasmobranch also have a high concentration of TMAO, the precursor of TMA, in their blood whereas this compound is absent in the blood of teleost fish (Benoit and Norris, 1945). The visceral contents are also a source for spoilage, particularly when the fish have been feeding, and the end products of bacterial metabolism can diffuse through the stomach/intestinal walls and permeate the flesh. Therefore, it is of importance to determine the role of these potential spoilage factors on shelf life of iced dogfish.

Arrangements were made with a Gloucester fishing captain to either gut, or head and gut some dogfish immediately after boating, and then store in ice along with some fish in the round (whole). It was considered that the gutting or heading and gutting treatment would also serve to bleed the fish. It is conceded, however, that the most effective method of bleeding dogfish is by notching the tail vein or chopping off the caudal peduncle. This permits the heart, if the fish is still alive, to pump out most of the blood. However, fishermen usually disdain grasping live thrashing dogfish to carry out this operation because of the potential harm from the fish's spines and abrasive skin. Also, the large numbers of dogfish usually caught in a directed tow might preclude the practicality of attempting to bleed dogfish in this manner. Bleeding is recommended to preserve flavor and prevent darkening of the flesh due to oxidation of the blood pigments (hemoglobin/myoglobin). However, in the European market a red color of the flesh is

considered an indicator of high quality in fresh dogfish. Therefore, bleeding the fish would appear to be counter productive since this treatment would tend to produce a white or light-colored flesh. Nevertheless, the fish were brought to the laboratory the same day they were caught (9-29-82). Each fish was tagged for identification and an initial Torrymeter reading was taken and recorded. The fish were then re-iced in standard 125 pound capacity wood boxes, with 8 fish per box and gut facing down. The fish were periodically tested over a 24 day period by chemical, microbiological and sensory methods, and only the fillet portion with flap removed was examined.

Results. During the first 10 days of iced storage there was not much difference in (cooked) odor score of fillets cut from fish stored in one of the three different ways. Beyond that time the whole fish (fillets) were scored slightly lower than the fillets from either gutted or headed and gutted fish which were rated approximately the same (Figure III-1). The graphs of flavor score as a function of storage time followed a similar pattern (Figure III-2). On the 15th day some panelists reported a slight rancid flavor in the fillets from the gutted fish and a slight flavor of decomposition in fillets from the whole fish. No ammonia flavor was detected at this time but after 18 days some comments of ammonia flavor was noted. At the end of 21 days all samples were rancid, and in addition fillets from the whole fish had a strong decomposed odor/flavor. The odor of rancidity and putrefaction overpowered any ammonia present. Texture scores during storage have been plotted in Figure III-3 for the three treatments. No textural differences were observed during approximately the first eight days but beyond that the whole fish (fillets) appeared to be rated slightly lower compared to the other two treatments because of a softer texture. The approximate storage times at

which the fish were considered to be of marginal quality due to either an unacceptable odor, flavor or texture were determined by linear regression to be as follows:

	Iced shelf life (days)		
Storage method	odor	flavor	texture
whole	14	11.5	13
gutted	16.5	16	17
headed and gutted	16	16	15

From these results it would appear that flavor deterioration or off-flavor development was the limiting attribute for product acceptability. The advantage of gutting in extending the shelf life is also apparent. From a standpoint of waste disposal, it would be beneficial to eviscerate dogfish at sea. The waste (head and viscera) amount to about 55 percent of the whole weight and current lack of any use for this waste requires that it be dumped under power at sea (off-shore). The cost to the processor for this disposal is estimated at 30 dollars per ton. Storing dogfish in the round for prolonged storage could seriously diminish the shelf life if the belly flaps were to be utilized. After 4 days iced storage a yellow-green discoloration was observed on the belly flaps of some of the whole fish. It is believed that contact with one of the visceral organs was responsible for the discoloration which would be considered undesirable in the trade.

Moisture content did not change significantly throughout the 24 day iced storage period although there was some variability among individual fish. Based on 16 fish per treatment the average moisture contents plus or minus one standard deviation for the three treatments during storage were:

whole, 70.9 ± 3.0; gutted, 69.7 ± 1.9; headed and gutted, 69.7 ± 2.0. The fish stored whole had about a one percent higher moisture content compared to the gutted fish. However, this slightly higher moisture content was not associated with the slightly softer texture of the whole fish since correlation between texture score and moisture content was found to be low. Enzymes in the gut were most probably responsible for the softer texture of the whole fish.

The development of oxidative rancidity as indicated by TBA number is shown for the three different storage treatments in Figure III-4. From the general trend of the data points it appears that over the 24 day storage period rancidity developed to a greater degree in the gutted or headed and gutted fish compared to whole fish. This is perfectly reasonable since gutting the fish exposes the visceral cavity to air which is responsible for the oxidation. Stansby et al (1968) also reported less rancidity (based on peroxide value) in the oil extracted from iced dogfish stored in the round compared to headed and gutted. The rate of increase in TBA number per day (mg malonaldehyde/kg/day) was determined to be: 0.14 for gutted fish, 0.08 for headed and gutted fish, and 0.01 for whole fish.

pH remained relatively constant during the first 10 days and then began to increase thereafter, most rapidly in the whole fish and least rapidly in the gutted fish (Figure III-5).

Trimethylamine nitrogen content remained relatively stable at an average value of about 3 mg/100 g for all treatments over 18 days and then started to rise (Figure III-6). However, by that time the fish were either spoiled or on the verge of spoilage. Therefore, it was concluded that TMA production

did not play a major role in quality deterioration other than contribute to the total volatile bases content.

There was no change in DMA nitrogen content over the entire 24 day storage period for any treatment (Figure III-7). The average contents (and standard deviation) were as follows: whole fish - 0.30 mg % (0.16); gutted fish - 0.23 mg % (0.11); headed and gutted fish - 0.22 mg % (0.10). There was no significant differences among these average values and it was concluded that DMA content did not affect the spoilage process.

Over the 24 day storage the TMAO-nitrogen content decreased linearly from an average initial value for the three treatments of 275 mg % to a final average value of 100 mg % (Figure III-8). The rate of TMAO - N decrease was 9.5 mg % per day for whole fish compared to 5.8 mg % per day for gutted fish or 5.3 mg % per day for headed and gutted fish. During this period there was only about 6-7 mg % TMA-N formed in any of the fish. From these data it is difficult to attribute the unaccountable decline in TMAO content to leaching from melting ice, as has been suggested, since there was a greater rate of loss in whole fish compared to gutted fish which had more leachable surface exposed.

Ammonia content remained unchanged for about the first 10 days of storage and then began to increase, most rapidly in the whole fish and least rapidly in the gutted fish (Figure III-9). The course of ammonia production for the three different treatments seemed to coincide with pH change. The estimated time at which the ammonia nitrogen content reached a value of 55 mg %, which is the limit mandated by one of the importing countries for acceptability, was 20 to 22 days; yet, the taste panel regarded the useful

storage life to have terminated after 11 to 16 days because of quality deterioration due to changes other than an objectionable ammonia content.

The accumulation of total volatile bases followed the same general pattern as ammonia production and this is to be expected since TVB is predominantly ammonia (Figure III-10). In the industry a value of 30 mg N/100 g for TVB is considered to be indicative of spoilage in fish (Farber, 1965). This value is within the range found in the present study at end of useful shelf life.

The percent decrease in Torrymeter reading during storage is shown in Figure III-11. These percentage values were computed by comparing the reading at a particular storage time with the reading initially recorded for that tagged fish. The highest readings, indicative of best quality, were consistently obtained with the headed and gutted fish. The rates of percent decrease in Torrymeter reading per day were determined for the different treatments by linear regression to be: whole fish - 3.3; gutted fish - 3.1; headed and gutted - 2.7. The linear relation between meter reading and storage time would indicate that the instrument might have some application in estimating quality of iced dogfish. The average initial reading based on 48 fish was 11.3 ($\pm$ 1.5). By linear regression analysis it was determined that at spoilage the Torrymeter reading had decreased to 40-55 percent of the initial value. This would indicate a meter reading at end of shelf life of 5-6 which is comparable to the reading of 6 that was determined in study II. There are some precautions to be observed in taking and interpreting Torrymeter readings. For comparison of fish, readings should be made in the same general area. A central location between the two dorsal

fins and along the lateral line is recommended. However, as a check, spot readings should be taken at several other random locations to ensure that the area being monitored did not undergo bruising since this would result in a low reading even though the quality of the fish was high. A low reading will also result from frozen and thawed fish regardless of quality. A single reading will not be reliable. In this study five readings were taken in the same location, that is, a reading was taken, the meter was removed and then repositioned in approximately the same spot, and read again, etc. until five individual readings were recorded. The average standard deviation for five readings taken in this fashion was 0.80. The magnitude of this standard deviation should point out the uncertainty associated with a single reading.

The bacterial content (APC) during storage in ice is plotted in Figure III-12. The APC at any time was greatest in the headed and gutted fish and least in the whole fish. The flesh of freshly caught fish is sterile but bacteria are on the skin, the gills and in the intestines. When the fish is gutted, the visceral cavity is exposed and this predisposes this area to becoming contaminated particularly if the fish have not been properly gutted. Improper gutting, that is, rupture of the intestines or stomach, can cause heavy bacterial contamination of the gut cavity and this can diminish the shelf life of gutted fish. The bacteria growing in the belly cavity of gutted fish will have access to more air compared to bacteria growing in the gut of round fish, and thus may proliferate at a faster rate. Bacteria growing under conditions of restricted air, as in the digestive organs of whole fish, may alter their metabolism (anaerobic) and produce malodorous compounds such as hydrogen sulfide which is typical of "bilgy" fish (McLean and Castell, 1956). The tough hide of dogfish would appear to provide a

good barrier against bacterial penetration, therefore any bacterial invasion of the flesh would probably originate from the belly wall.

Removing the head in addition to gutting creates another cut surface for potential bacterial contamination and a foci for invasion of tissues such as through the vascular system. By not eviscerating, the intestinal bacteria are contained within that organ and do not invade the flesh; however, as was previously stated, bacterial growth within the intestines will produce undesirable end products which will diffuse through the intestinal wall, permeate the flesh and cause spoilage even though the flesh may contain a relatively low count. This appears to be the scene depicted by the curves in Figure III-12.

In Table III-1 are presented the regression data for flavor score and other parameters as a function of various spoilage indicator tests. There was good correlation between flavor score and either ammonia content, total volatile bases, pH, bacterial content or Torrymeter reading. Flavor score correlated fairly well with TBA number in gutted or headed and gutted fish, but poorly in whole fish. There also was a low correlation between flavor score and TMA content. The following spoilage criteria signalling end of useful shelf life were predicted by the regression line for the various parameters:

ammonia nitrogen content	24 - 29 mg %
total volatile bases	28 - 34 mg %
pH	6.2 - 6.3
Torrymeter reading	5 - 7
TBA number	3 - 3.8

There was a high degree of correlation between ammonia content and pH. The regression value for pH corresponding to an ammonia nitrogen content of 55 mg N/100 g was about 6.9.

Table III-1

Regression data for flavor score of dogfish fillets as a function of various spoilage indicators

treatment	regression	correlation coefficient	intercept	slope	n
H & G	flavor score vs. ammonia content	0.92	7.62	-0.06	14
Gutted		0.85	7.61	-0.07	14
Whole		0.84	7.58	-0.06	14
H & G	flavor score vs. TMA content	0.64	8.50	-0.55	14
Gutted		0.33	7.76	-0.35	14
Whole		0.10	6.71	-0.19	14
H & G	flavor score vs. TVB content	0.92	7.79	-0.05	14
Gutted		0.82	7.76	-0.06	14
Whole		0.79	7.76	-0.06	14
H & G	flavor score vs. TBA number	0.69	8.71	-0.88	14
Gutted		0.81	8.81	-0.74	14
Whole		0.22	7.08	-0.40	14
H & G	flavor score vs. pH	0.89	21.44	-2.46	14
Gutted		0.77	23.92	-2.93	14
Whole		0.79	20.44	-2.33	14
H & G	flavor score vs. log APC	0.91	10.92	-0.71	12
Gutted		0.81	10.39	-0.70	12
Whole		0.84	10.78	-0.92	12
H & G	flavor score vs. Torrymeter reading	0.88	2.91	0.43	14
Gutted		0.77	4.73	0.30	14
Whole		0.86	0.42	0.42	14
H & G	ammonia vs. pH	0.97	-234.3	41.91	16
Gutted		0.96	-238.7	42.78	16
Whole		0.96	-219.3	39.57	16
H & G	texture score vs. moisture content	0.28	17.83	-0.16	14
Gutted		0.02	5.99	0.01	14
Whole		0.41	19.03	-0.18	14

FIG. III-1 EFFECT OF STORAGE TIME ON THE ODOR SCORE OF COOKED FILLETS CUT FROM DOGFISH DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

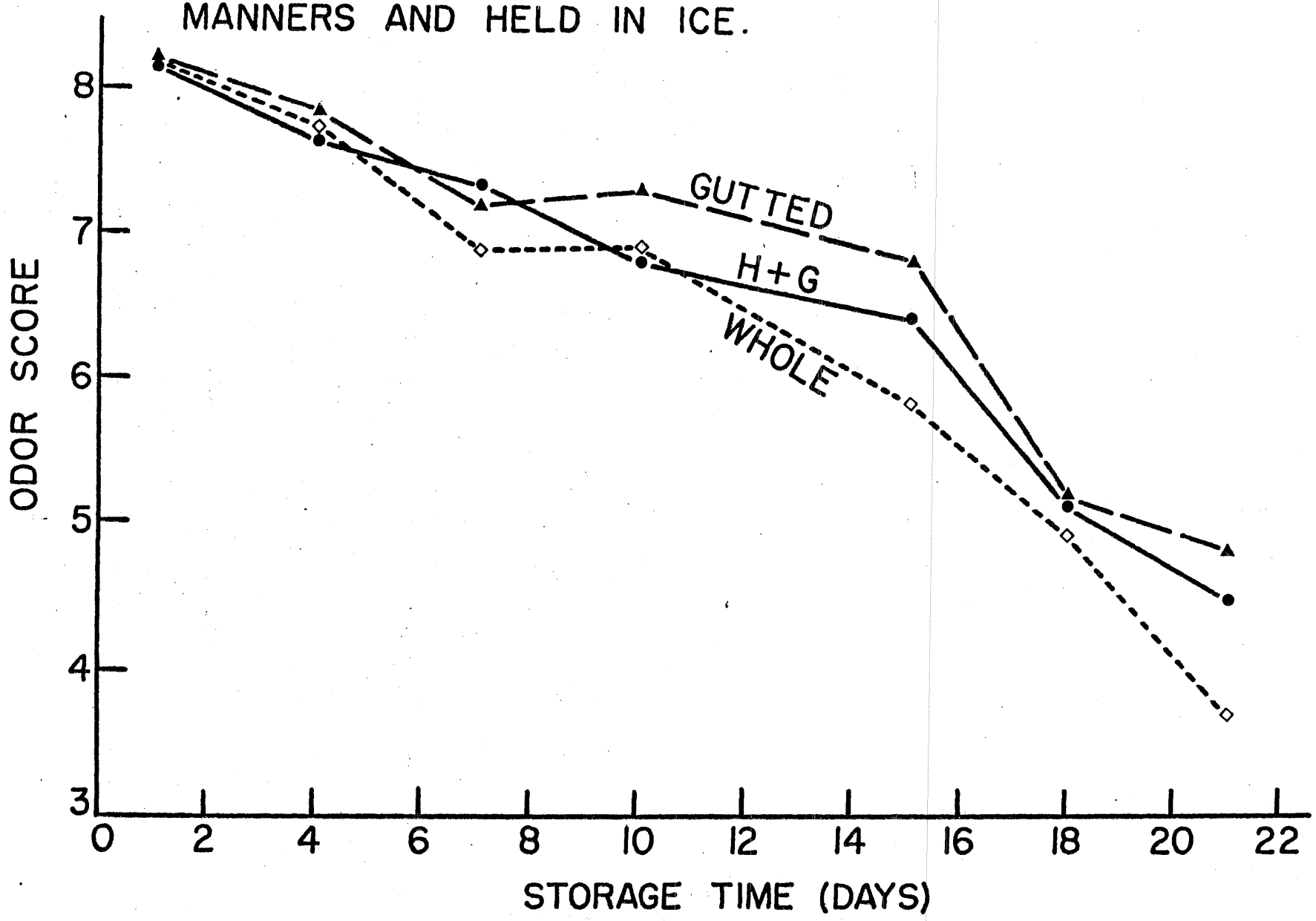


FIG. III-2 EFFECT OF STORAGE TIME ON THE FLAVOR SCORE OF COOKED FILLETS CUT FROM DOGFISH DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

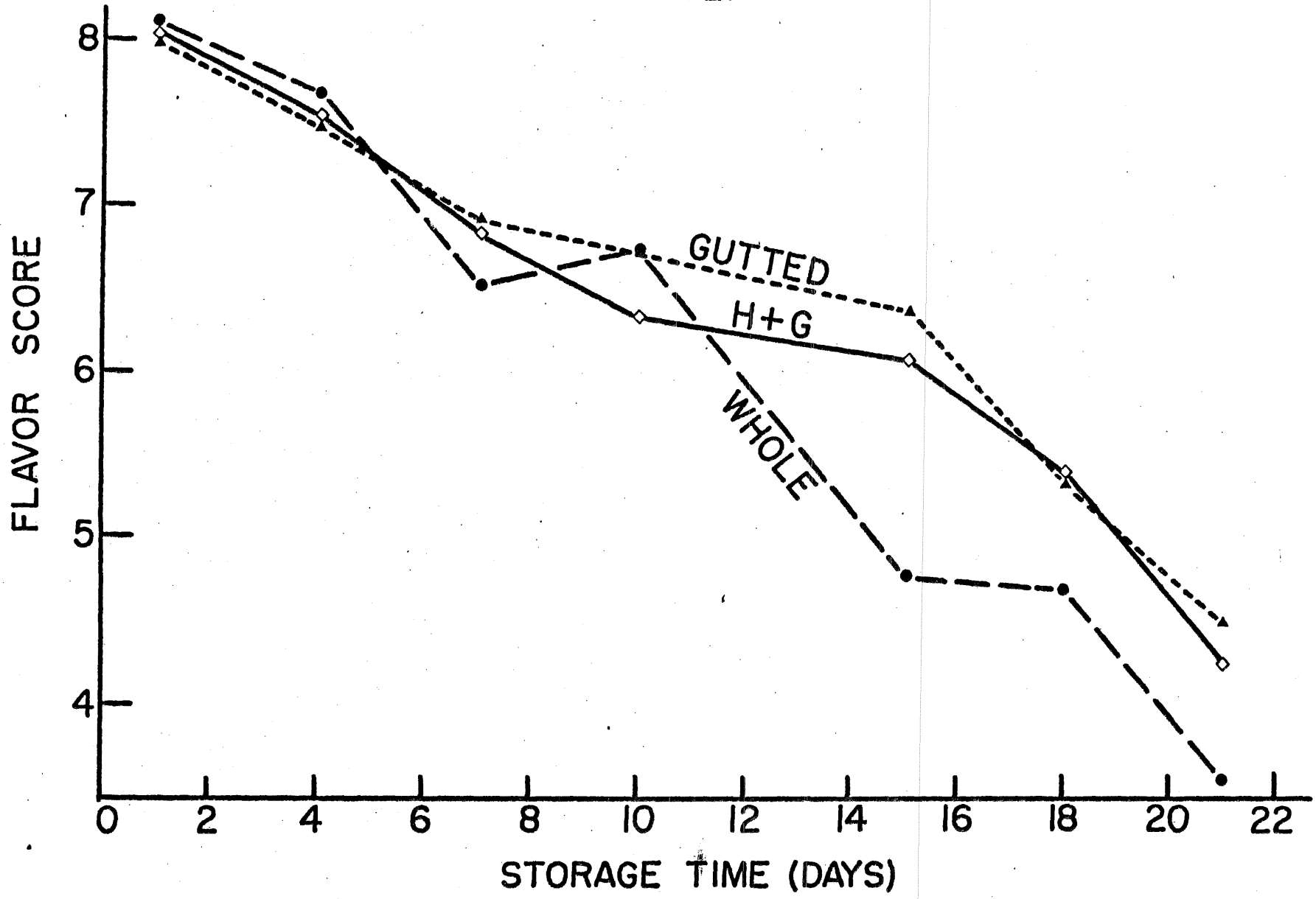


FIG. III-3 EFFECT OF STORAGE TIME ON THE TEXTURE SCORE OF COOKED FILLETS CUT FROM DOGFISH DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

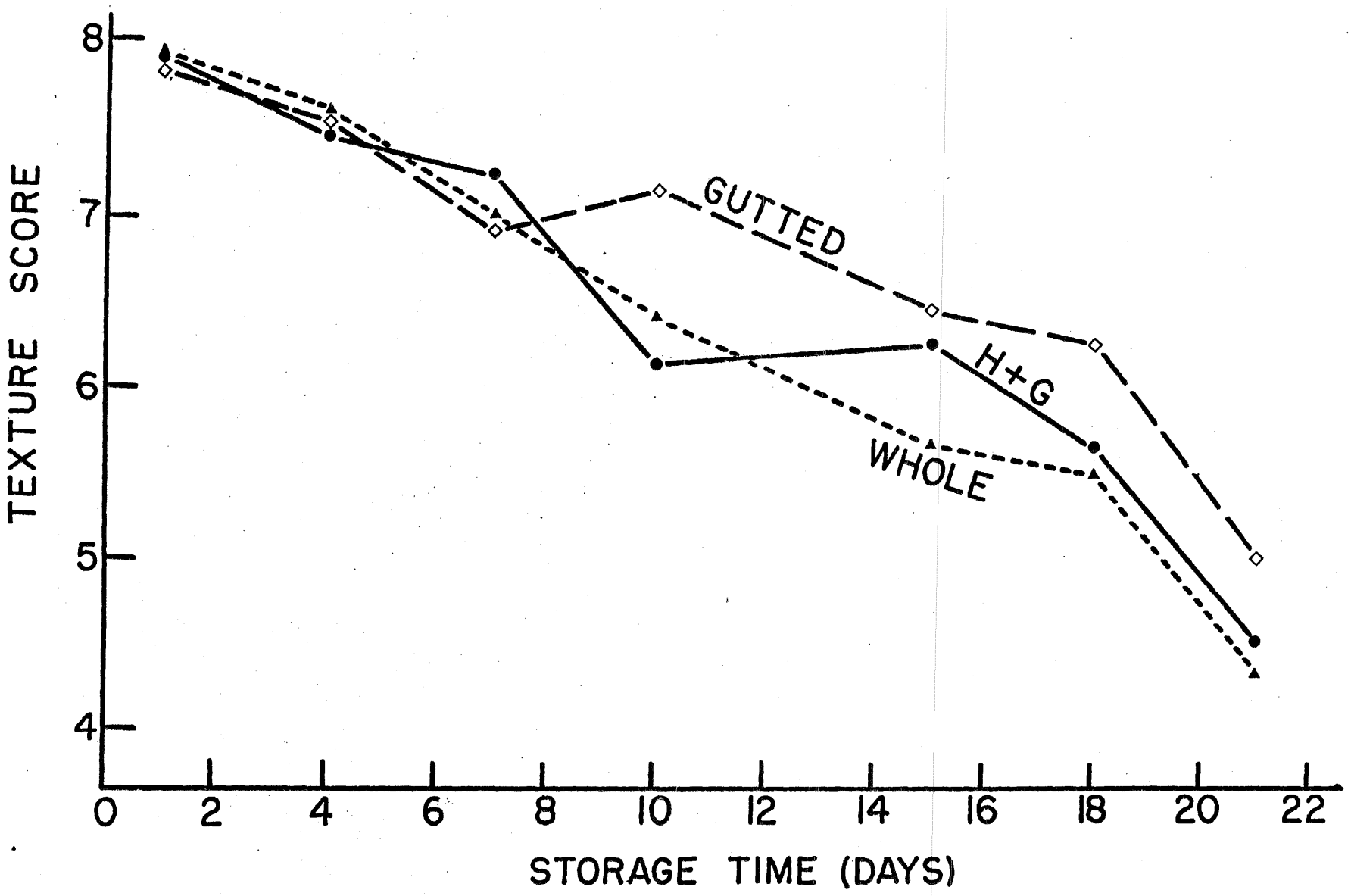


FIG. III-4 EFFECT OF STORAGE TIME ON TBA NUMBER OF FILLETS CUT FROM DOGFISH DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

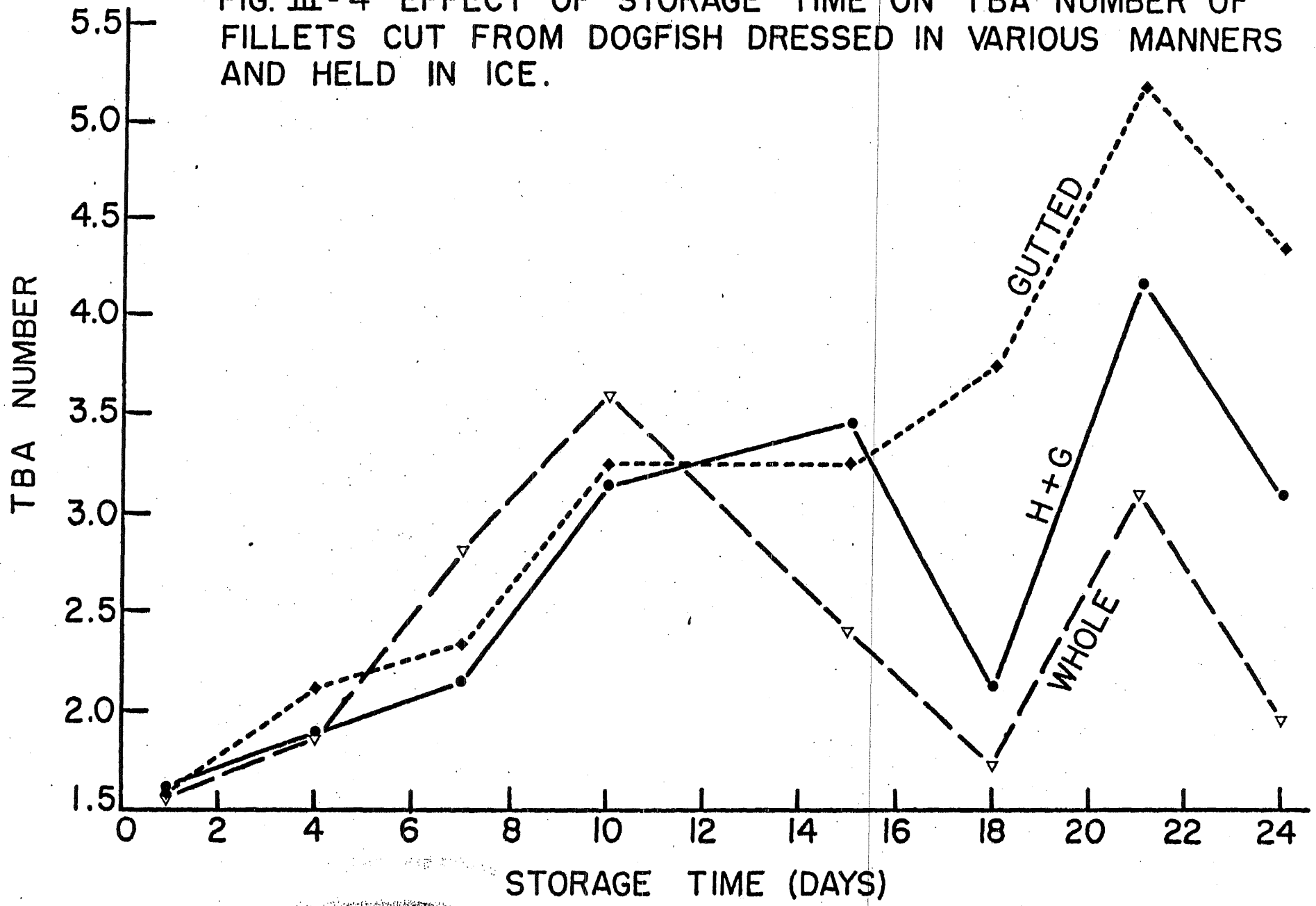


FIG. III-5 EFFECT OF STORAGE TIME ON THE pH OF FILLETS CUT FROM DOGFISH DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

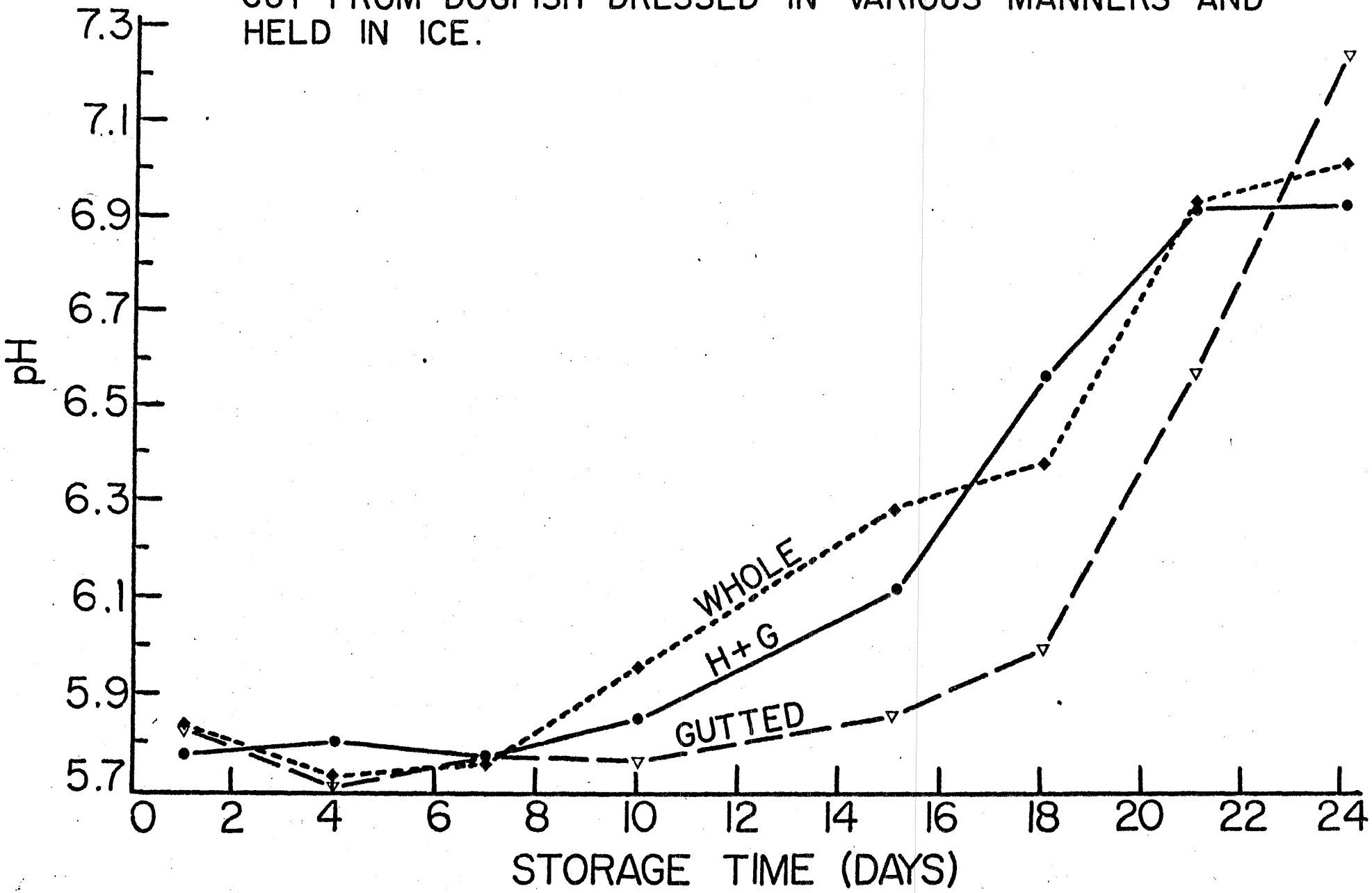


FIG. III-6 EFFECT OF STORAGE TIME ON TRIMETHYLAMINE NITROGEN CONTENT OF DOGFISH (FILLETS) DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

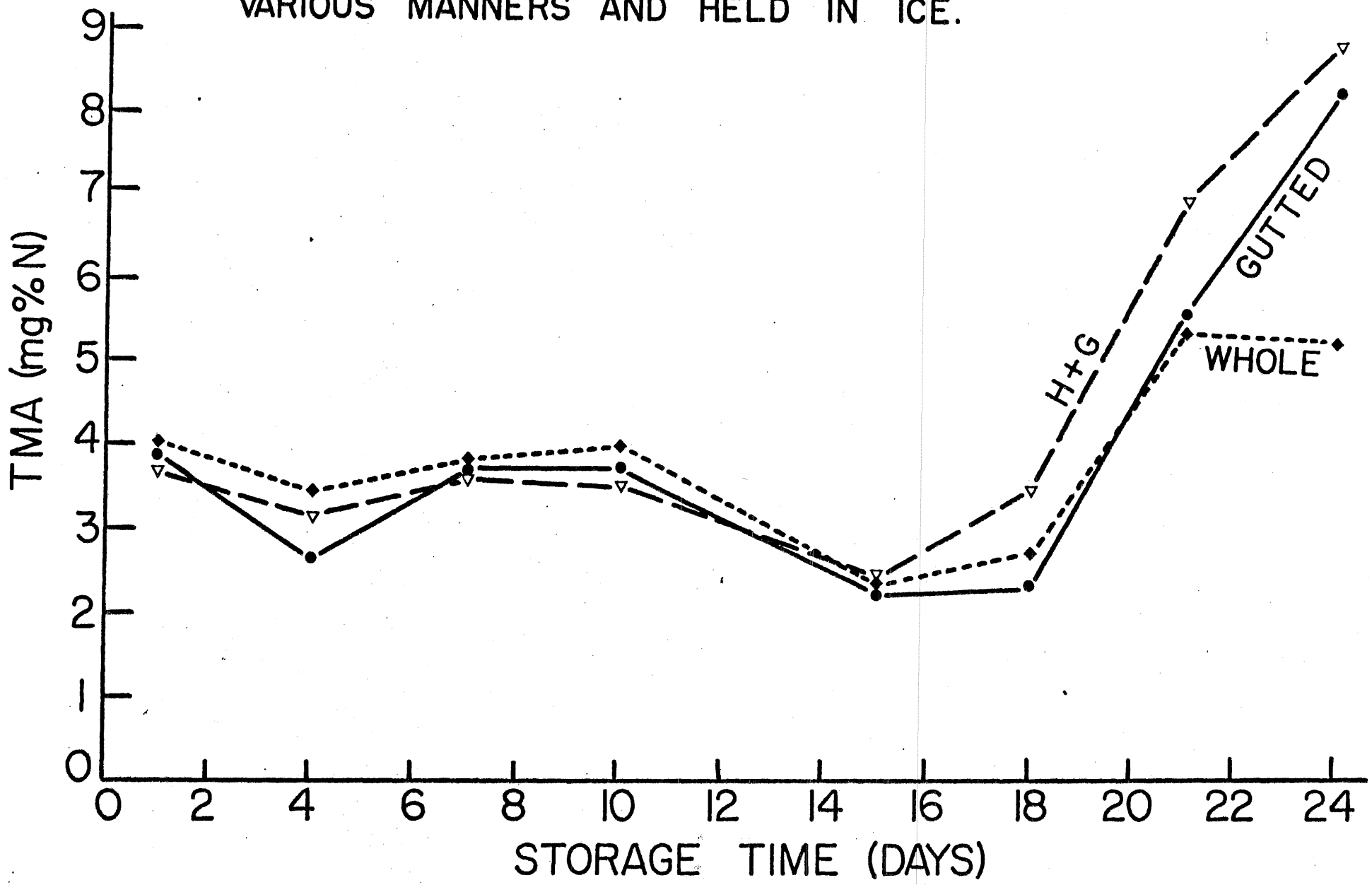


FIG. III-7 EFFECT OF STORAGE TIME ON DIMETHYLAMINE NITROGEN CONTENT OF DOGFISH (FILLETS) DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

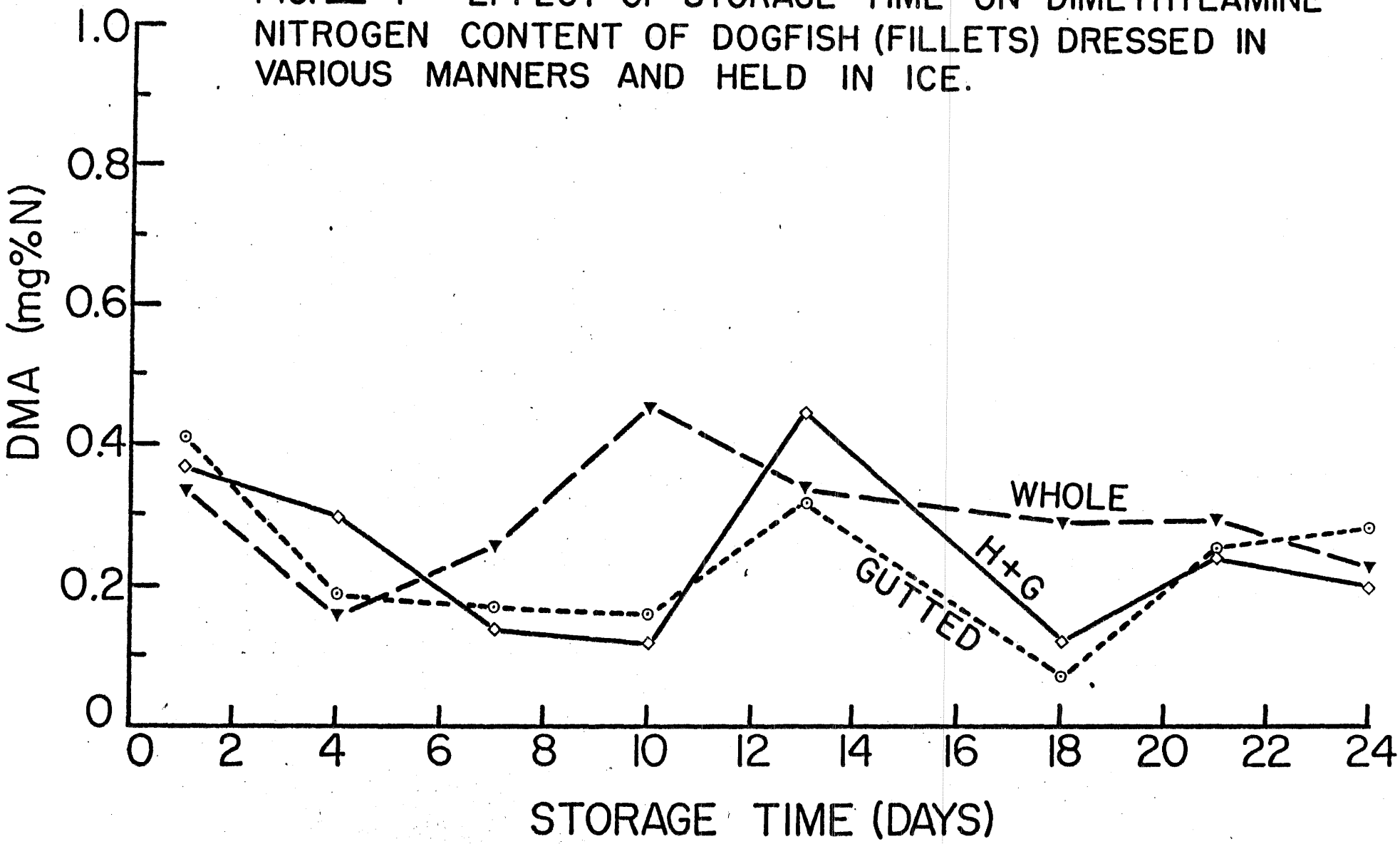


FIG. III-8 EFFECT OF STORAGE TIME ON TRIMETHYLAMINE OXIDE NITROGEN CONTENT OF DOGFISH (FILLETS) DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

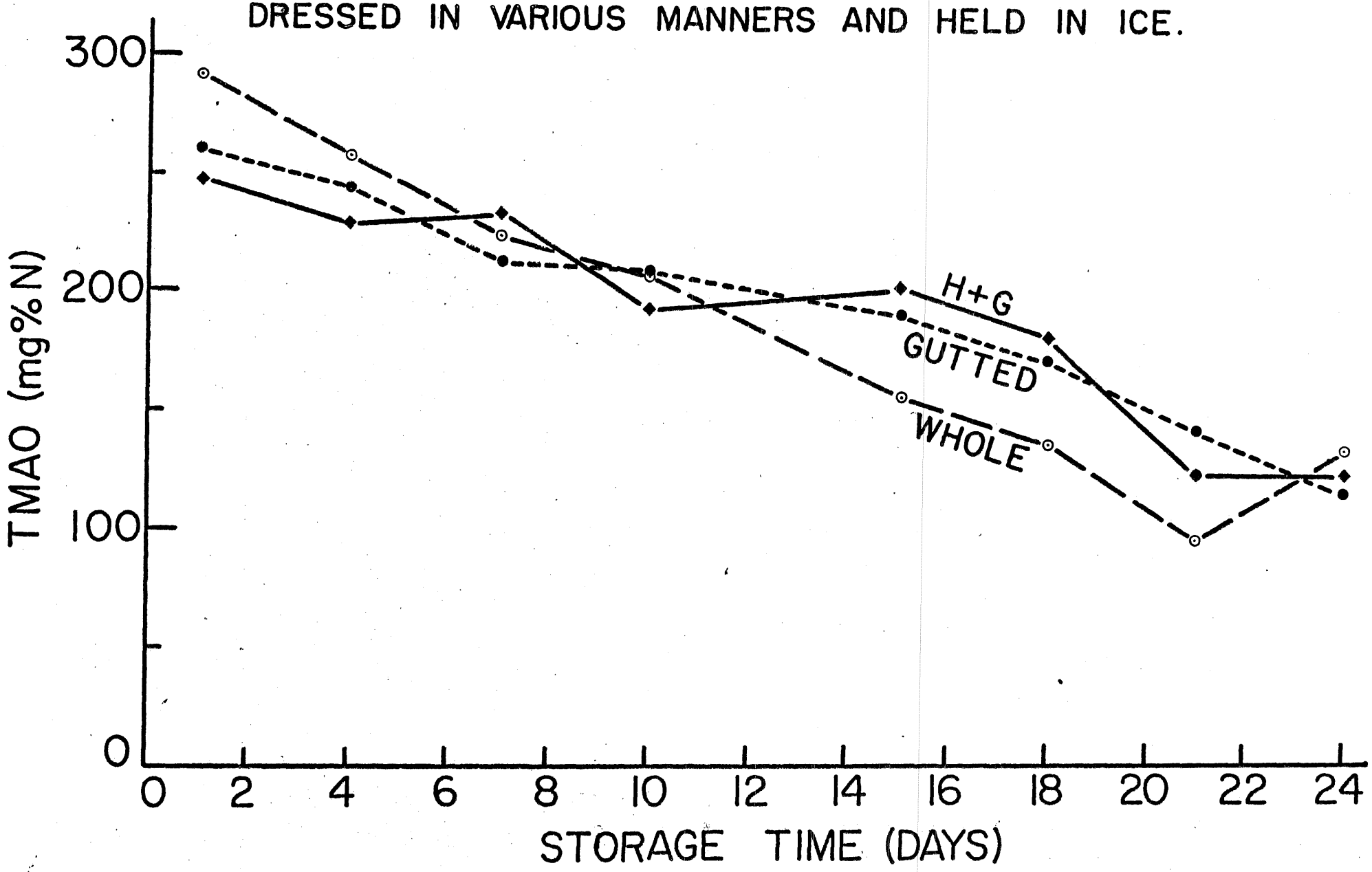


FIG. III-9 EFFECT OF STORAGE TIME ON AMMONIA NITROGEN CONTENT OF DOGFISH (FILLETS) DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

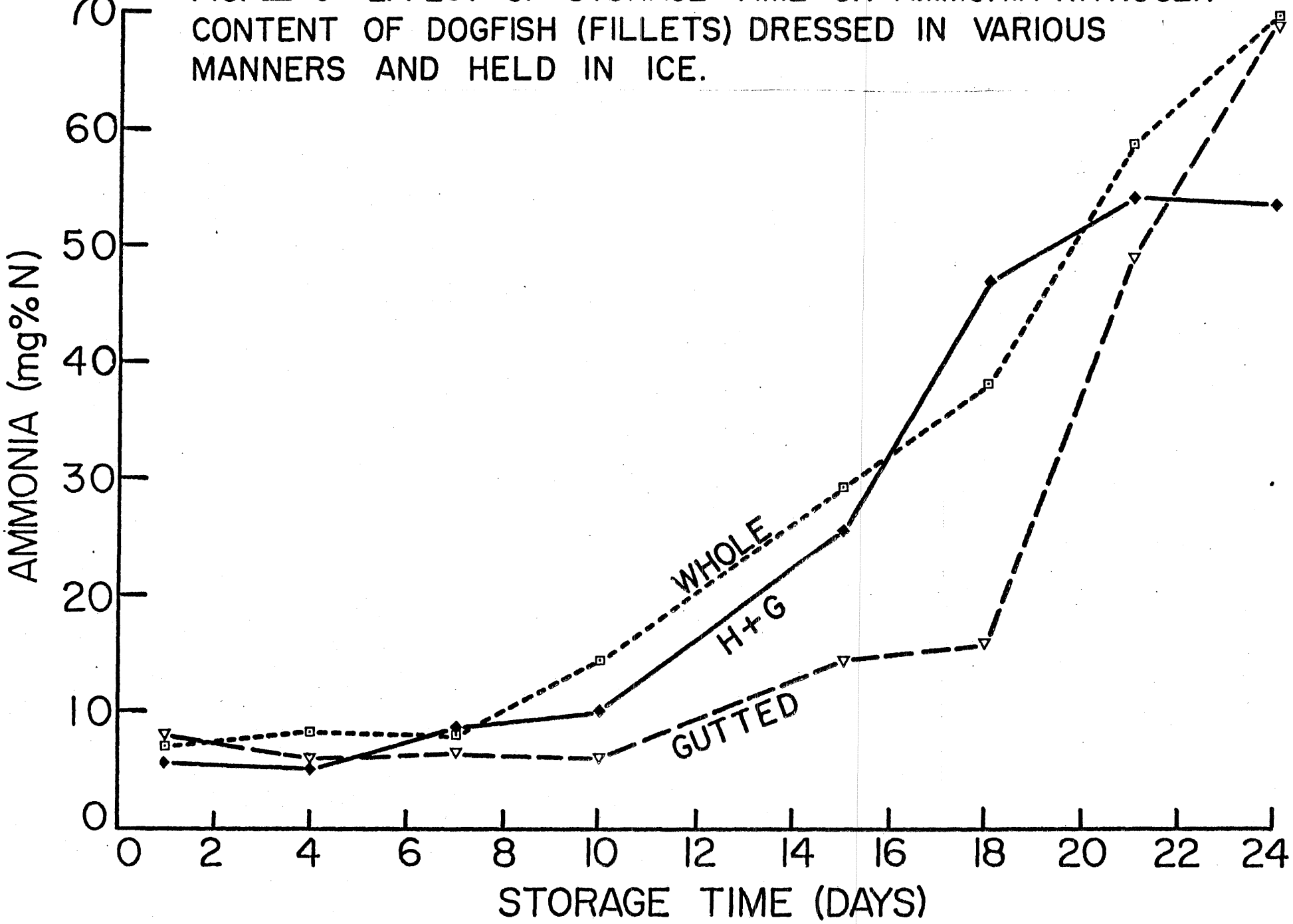
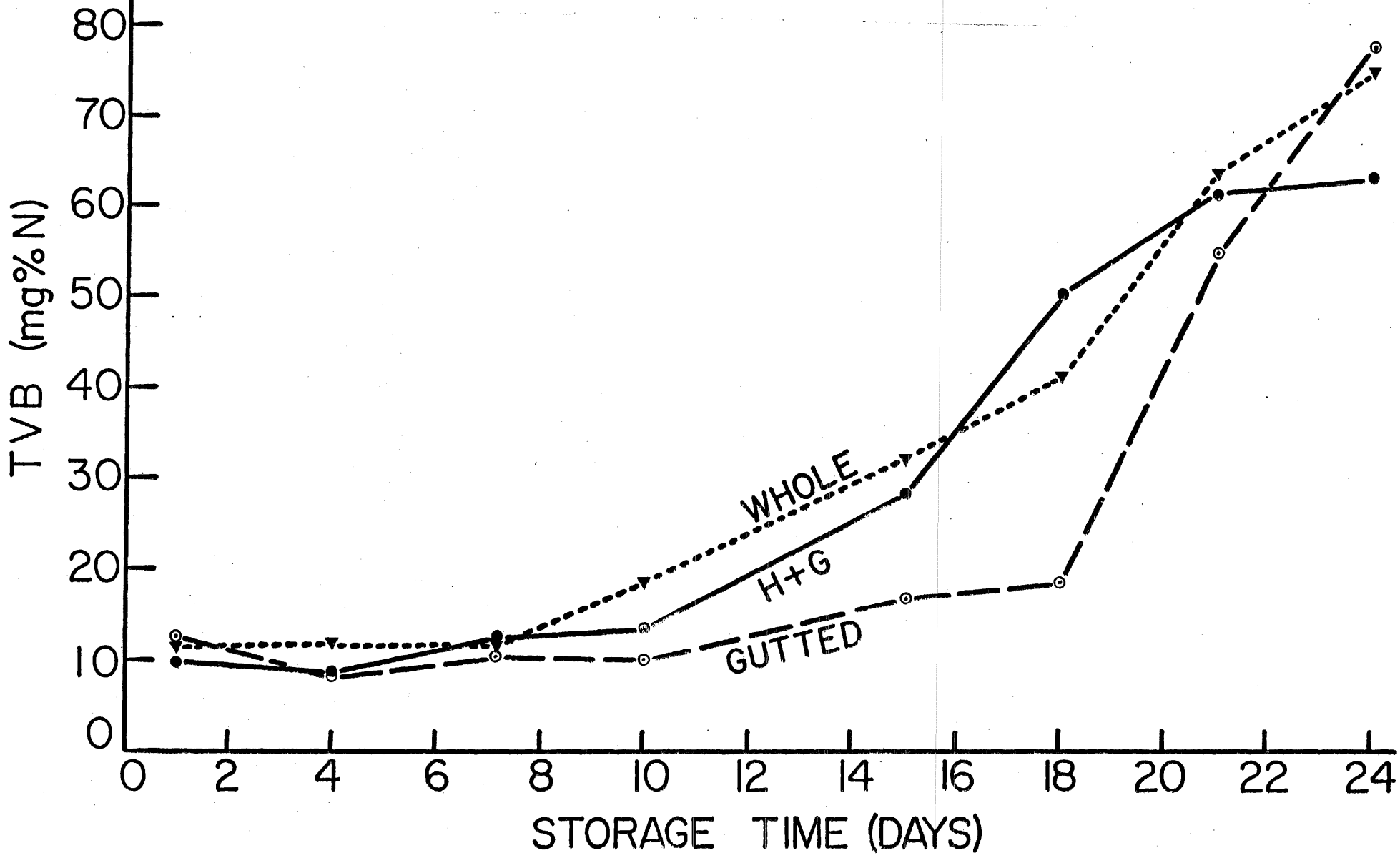


FIG. III-10 EFFECT OF STORAGE TIME ON TOTAL VOLATILE BASE NITROGEN CONTENT OF DOGFISH (FILLETS) DRESSED IN VARIOUS MANNERS AND HELD IN ICE.



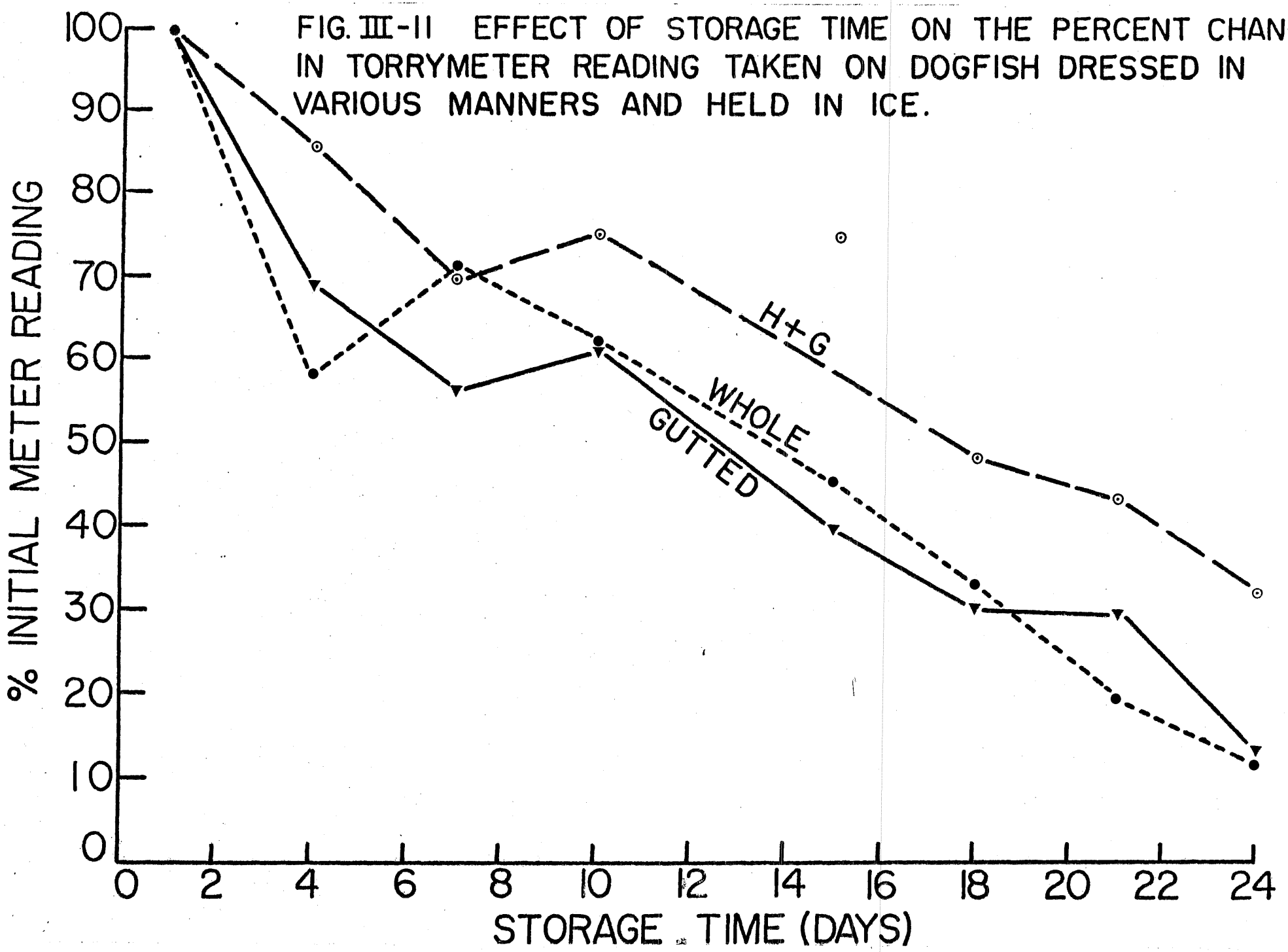
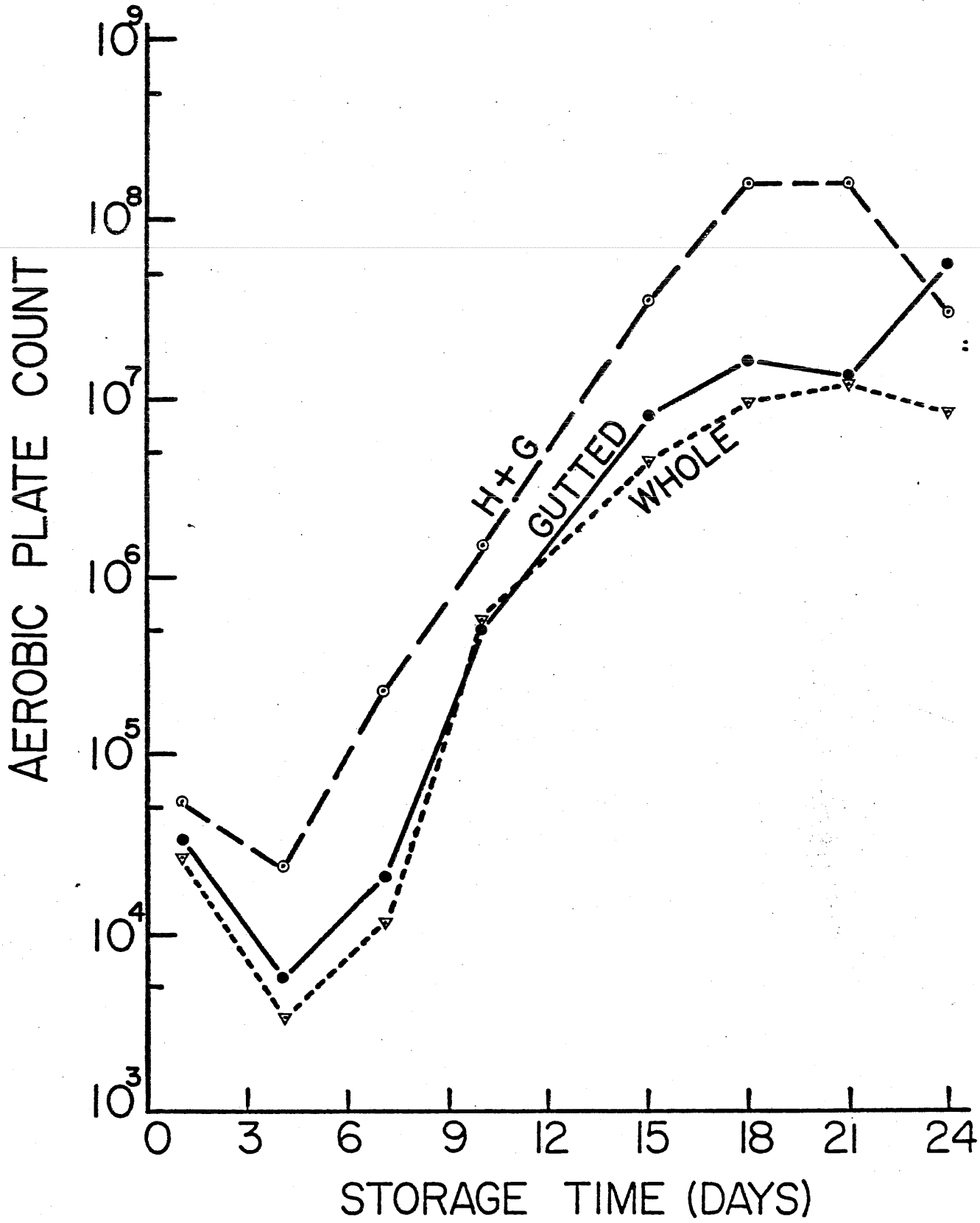


FIG. III-11 EFFECT OF STORAGE TIME ON THE PERCENT CHANGE IN TORRYMETER READING TAKEN ON DOGFISH DRESSED IN VARIOUS MANNERS AND HELD IN ICE.

FIG. III-12 EFFECT OF STORAGE TIME ON THE BACTERIAL CONTENT OF FILLETS CUT FROM DOGFISH DRESSED IN VARIOUS WAYS AND HELD IN ICE.



Fresh storage study IV

The objective of this study was to determine the effect on quality of mishandling dogfish due to temperature abuse that could occur either aboard the fishing vessel or in the processing plant.

One day old dogfish obtained from a local processor (10-20-82) were briefly sprayed with water and tagged for identification. Average weight and standard deviation was 7.7 $\pm$ 2.1 pounds. After recording initial Torrymeter readings on each fish, eight whole fish were placed in storage at either 57°F (14°C) or 46.4^{o}F (8°C) to be compared to fish stored on ice (32°F). At periodic intervals two fish were removed from storage and the fillets were examined by sensory, chemical, physical and microbiological tests for quality.

Results. During storage the rate of decrease in odor, flavor and texture scores of fillets removed from the whole fish was commensurate with the storage temperature (Figures IV-1, 2, 3). It was surprising to learn that during the first two days of storage the difference in quality due to the temperatures used was not as great as had been anticipated. After the first day a slight bitter flavor was detected in fillets from fish held at either 46.4 or 57°F, but no ammonia or decomposition odors were noted. It is believed that the slight bitter flavor may have been indicative of the initial stages of oxidative rancidity. By the end of 4 days some stale flavor was present but ammonia was slightly detectable in the cooked samples; however, a strong ammonia odor prevailed after 5 days at 57°F or after 7 days at 46.4^{o}F. Shelf life was estimated from the curves in Fig. IV - 1, 2, 3 to be as follows:

storage temperature	shelf life (days) based on: odor	flavor	texture
32°F (ice)	14	12	13
46.4°F	5	5	5
57°F	4	3.5	4

As in the previous study flavor change was the most important sensory factor governing shelf life.

It might have been reasoned beforehand that at the two elevated storage temperatures the fish would lose some moisture and this could affect texture. During the latter part of the storage period the fish held at either 46.4°F or 57°F did have a dry skin and slightly shrivelled appearance, but the moisture content did not decrease. It would seem that the skin or hide of dogfish is a good barrier to moisture loss.

In Figure IV-4 shelf life (days) based on flavor deterioration has been plotted as a function of storage temperature over the range of 32 to 57°F. The relationship appears to be a first order reaction. The sharp change in slope over the temperature span of 32 to 46°F compared to the span of 46 to 57°F illustrates the beneficial effect on shelf life of storing product to as close to 32°F as possible. The reciprocals of these shelf lives were considered to represent the spoilage rates for the reaction at the corresponding temperatures, and from these data an Arrhenius plot was constructed (Figure IV-5). From the slope b of the regression line the activation energy, E_A, for the reaction was determined in accordance with the following equation:

$$\text{slope } b = -\frac{E_A}{(2.303)(R)}$$

where R = molar gas constant

= 1.987

The activation energy was computed to be 14,700 cal/mole and this is just in accord with the activation energy values of 15000-18000 reported by Torry Research Station scientists for various spoilage tests on wet fish in the temperature range of 34 to 59°F (cited by James and Olley, 1971). A slightly higher activation energy of 17000 cal/mole was calculated by James and Olley (1971) for spoilage of uniced shark (species not revealed) based on the time required for the ammonia nitrogen content to reach 30 mg/100 g. Their raw data were taken from published results of various researches and may have been derived from studies on mixed species of shark. In general, the spoilage times cited by James and Olley were lesser by a factor of about 0.75 compared to those determined by us.

There was no significant increase in TBA number at any of the storage temperatures to signify a major development of oxidative rancidity (Figure IV-6). In the previous study it was found that rancidity was not a problem in iced whole fish, and the results of this study indicated there was no serious problem even at temperatures up to 57°F although some rancidity did eventually develop.

The rate of increase in pH during storage was also temperature dependent (Fig. IV-7). The initial pH of the fish in this and also in the previous study was approximately 5.8. The ultime pH for most teleost fish at the resolution of rigor has been stated to be 6.2-6.5. Gordievskaya (1971) reported that shark meat is rather acidic compared to other fish. One would expect this acidic nature of dogfish flesh to enhance its keeping qualities.

The effect of storage temperature on production of basic volatile amines--ammonia, TMA, DMA--is shown in Figures IV-8, 9, 10. Whereas in previous studies where it was seen that TMA or DMA did not increase significantly during storage on ice, these amine compounds did increase substantially during

storage at 46.4 ^{o}F or 57 ^{o}F. Since these amines are produced as a result of microbial activity, one would expect the bacterial growth to reflect a similar temperature dependency. That they did can be seen in Figure IV-11, however, one should note the lag in bacterial increase during the first 8 days at 32 ^{o}F. This lag contributed to the extended shelf life at this lower temperature.

The decrease in Torrymeter readings made on the dogfish during storage can be seen in Figure IV-12. By graphical interopolation of the curves it was determined that a meter reading of 4-6 would be indicative of end of shelf life, based on shelf lives of 12 days at 32 ^{o}F, 5 days at 46.4 ^{o}F and 3.5 days at 57 ^{o}F as estimated from sensory evaluation. During storage at the two higher temperatures, the skin of the fish began to dry out and the Torrymeter would not register a read out unless the skin was slightly moistened.

In Figure IV-13 rates of TMA, DMA and ammonia production, and bacterial growth have been plotted as a function of temperature. These rates are the regression slopes of the curves shown in the previous four figures. The figure illustrates the rapid increase in rate of either bacterial growth or volatile amine production that resulted at storage temperatures above approximately 50 ^{o}F. Therefore, it behooves the fisherman or processor to maintain low storage temperatures for dogfish in order to retard the production of the volatile amines and other decomposition products of bacteria which are strongly associated with spoilage. With proper iced storage dogfish have been shown to have good keeping quality, comparable to cod and haddock stored under similar conditions.

In Table IV-1 are presented some regression data for flavor score of dogfish, stored whole at either 32, 46.4 or 57 ^{o}F, as a function of some chemical or physical spoilage indicators. There was good correlation

between flavor and pH at each of the three storage temperatures, however, the regression lines predicted slightly different pH values at end of shelf life. These were: pH 6.2 (32°F), pH 6.5 (46.4°F) and pH 6.7 (57°F). When regression analysis was performed on the combined data from the three temperatures, a pH value of 6.4 was determined at end of useful shelf life (flavor score = 6). In general this pH value is lower than the value reached in teleost fish when shelf life has expired.

Similarly there was fairly good correlation between flavor score and ammonia content at each of the storage temperatures, however, at the expiration of shelf life the ammonia nitrogen content varied from 26 mg % at 32°F storage to 74 mg % at 57°F storage. The main interest in this study was to ascertain the length of time dogfish could be stored under different conditions and still be acceptable after cooking. Since some ammonia is volatilized during cooking, spoilage based on odor of the raw fish would probably have been judged to occur earlier than the values we are reporting. Nevertheless, it is difficult to accept raw fish with an ammonia-N content of 74 mg % as representative of marginal quality and there is no rational explanation as to why the taste panel did not react more strongly to this high level of ammonia if it were indeed present.

Flavor score and TMA content correlated poorly with fish held at 32°F, but very well with fish stored at 46.4 or 57°F. At 32°F TMA content did not begin to show any significant increase until the product was on the verge of spoilage, whereas at the other two temperatures TMA production increased rapidly after a one day lag. There was good correlation between flavor score and bacterial count at each of the three temperatures and the average count at end of shelf life was shown to be about 2 million per gram. One should be warned, however, about the risks in using absolute bacterial numbers to

predict shelf life since spoilage in fish is associated more closely with the numbers of certain spoilage types of bacteria rather than with the total number of bacteria. The principal spoilage bacteria in marine fish are the pseudomonads and these normally constitute just a small percentage of the microflora of freshly caught fish; however, depending upon the sanitary conditions in the fishing boat or in the processing plant, their concentration on fish can be markedly increased through contact with contaminated surfaces and this would affect the length of shelf life and also the total number of bacteria (APC) at spoilage. It would be conservative to state that at the threshold of spoilage the aerobic plate count would be in the millions, but one cannot state with certainty that it would be 2, 10 or 50 million.

Flavor score correlated fairly well ($r = 0.79-0.86$) with Torrymeter readings of dogfish stored at the three different temperatures. The meter reading at end of shelf life was predicted by the regression lines to be 6-7.

Table 4-1

Regression data for flavor score of dogfish stored whole at various temperatures as a function of certain spoilage indicators

Storage Temperature	Regression	Correlation coefficient	Intercept	Slope	n
32°F	flavor score vs. pH	0.81	27.12	-3.45	14
46.4		0.77	24.57	-2.88	8
57		0.97	21.30	-2.28	8
32	flavor score vs. ammonia content	0.79	7.54	-0.06	14
46.4		0.61	7.77	-0.05	8
57		0.98	8.24	-0.03	8
32	flavor score vs. TMA content	0.10	6.69	-0.18	14
46.4		0.96	8.32	-0.42	9
57		0.93	8.38	-0.24	8
32	flavor score vs. log bacterial count (APC)	0.80	10.80	-0.89	14
46.4		0.89	18.18	-2.33	10
57		0.80	15.83	-1.71	8
32	flavor score vs. Torrymeter reading	0.86	3.41	0.42	14
46.4		0.86	1.11	0.64	10
57		0.79	2.85	0.53	8
32	ammonia content vs. pH	0.95	-237.6	42.59	15
46.4		0.95	-246.9	43.66	8
57		0.98	-482.9	84.22	10

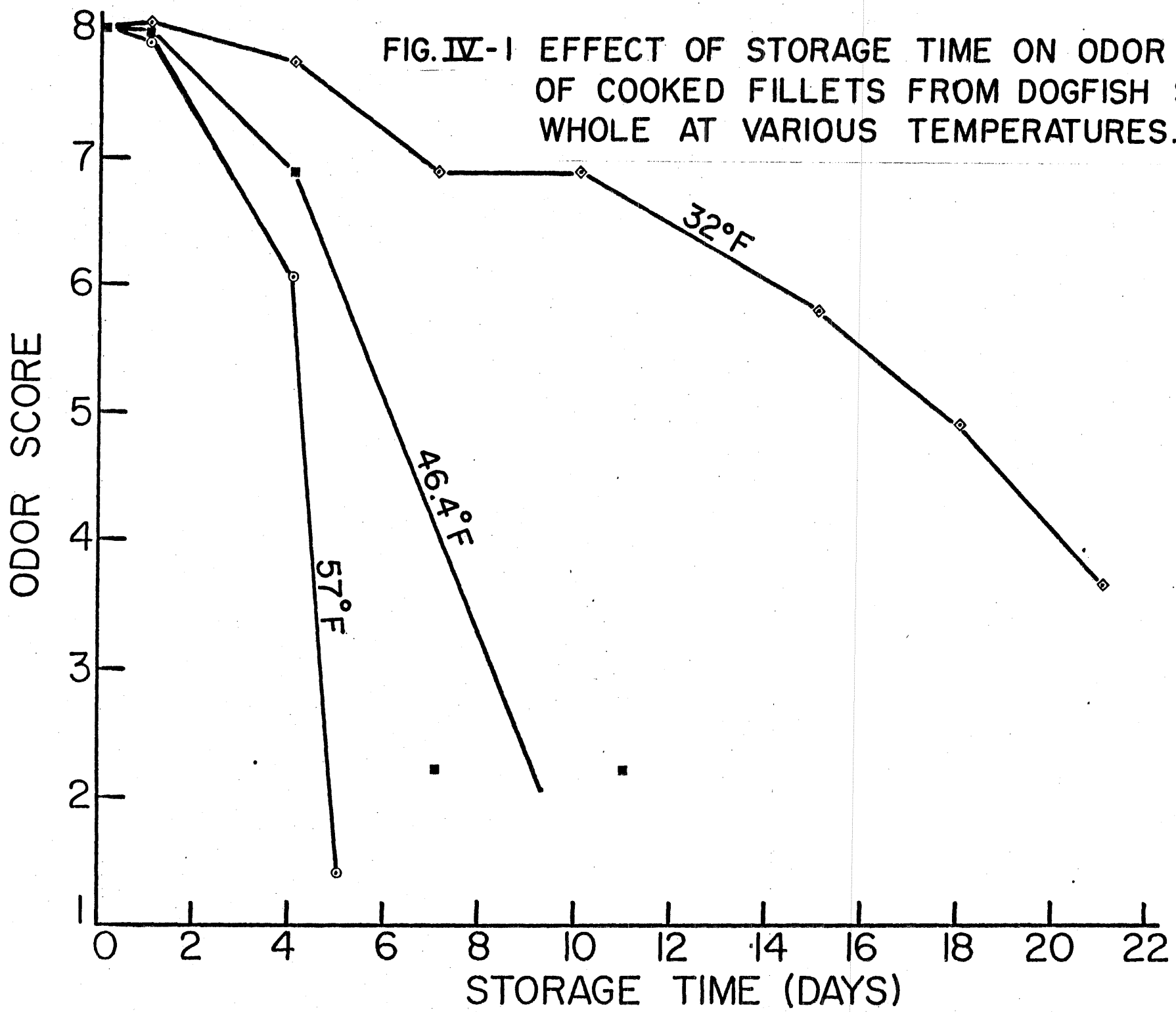


FIG. IV-1 EFFECT OF STORAGE TIME ON ODOR SCORE OF COOKED FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

FIG. IV-2 EFFECT OF STORAGE TIME ON FLAVOR SCORE OF FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

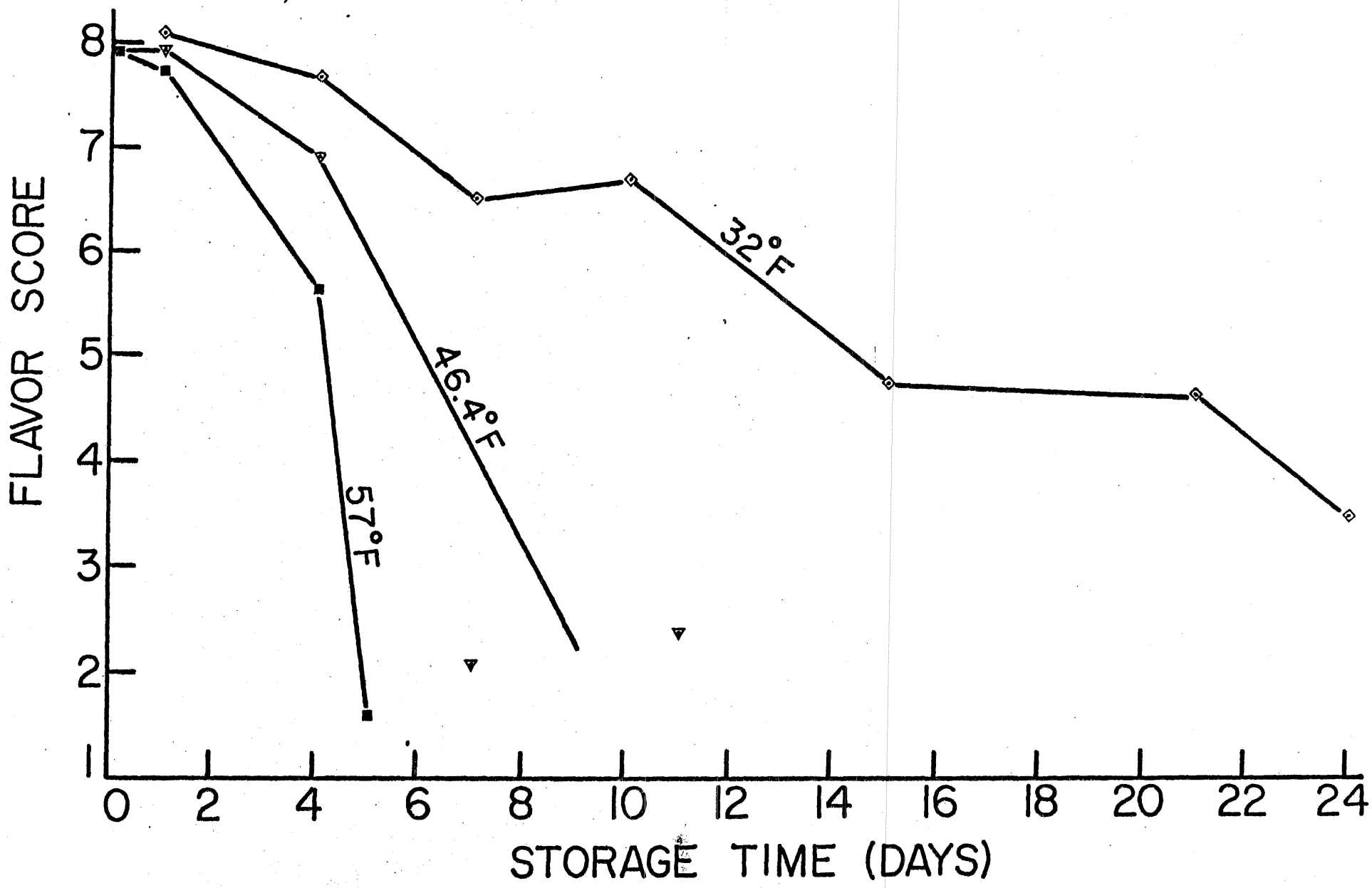


FIG. IV-3 EFFECT OF STORAGE TIME ON TEXTURE SCORE OF FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

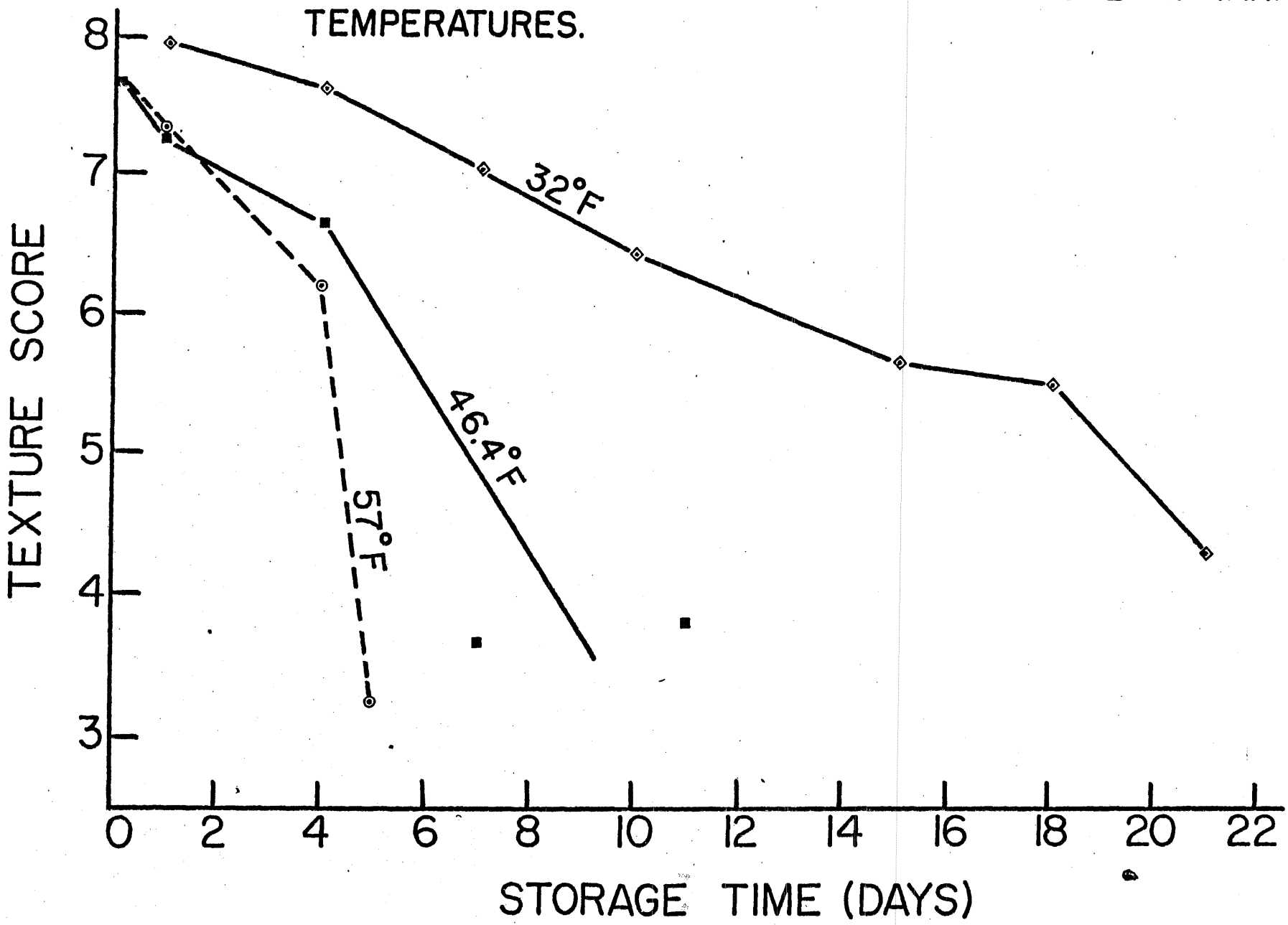


FIG. IV-4 SHELF LIFE OF WHOLE DOGFISH AS A FUNCTION OF STORAGE TEMPERATURE.

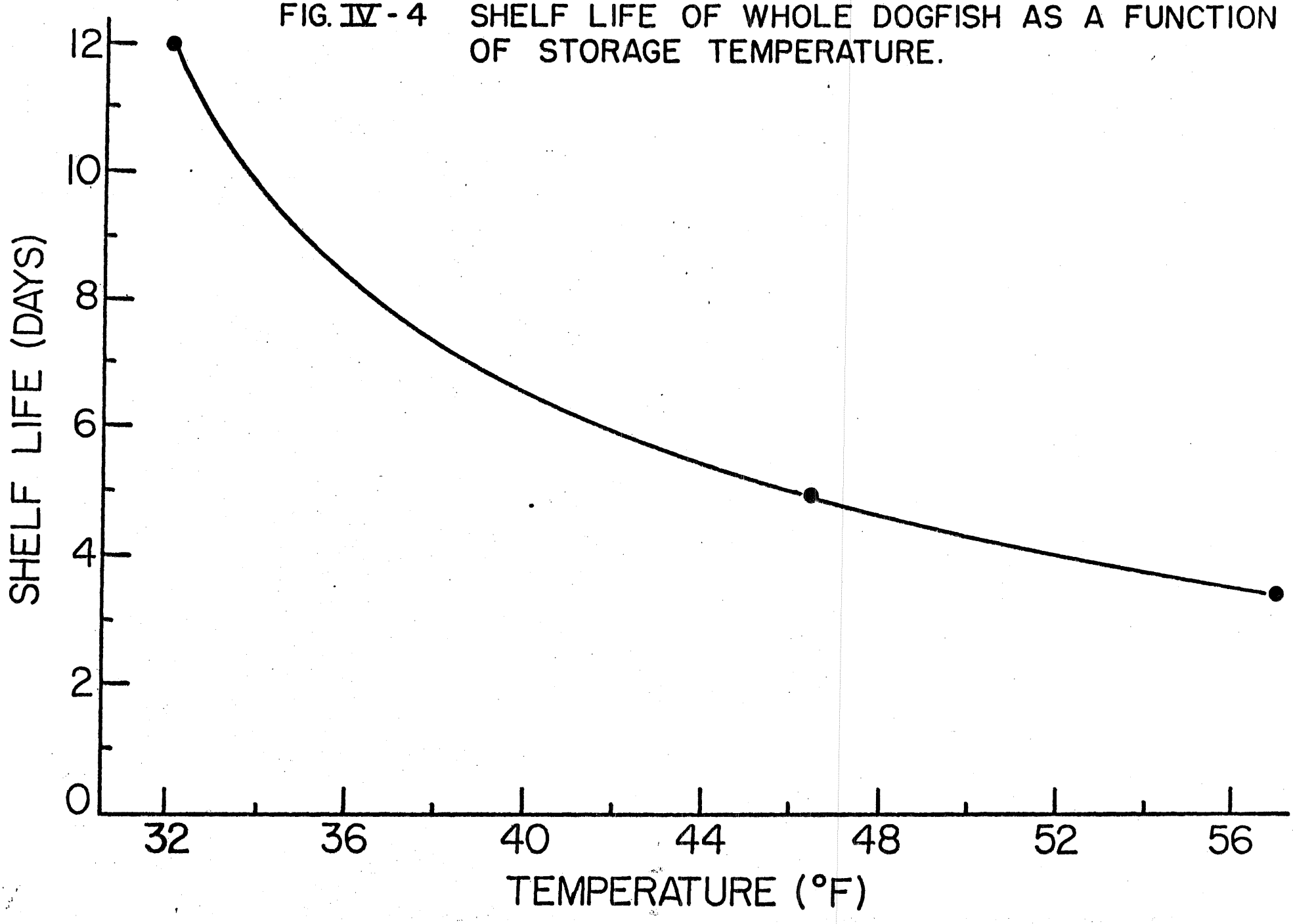


FIG. IV-5 SPOILAGE RATE OF WHOLE DOGFISH AS A FUNCTION OF TEMPERATURE.

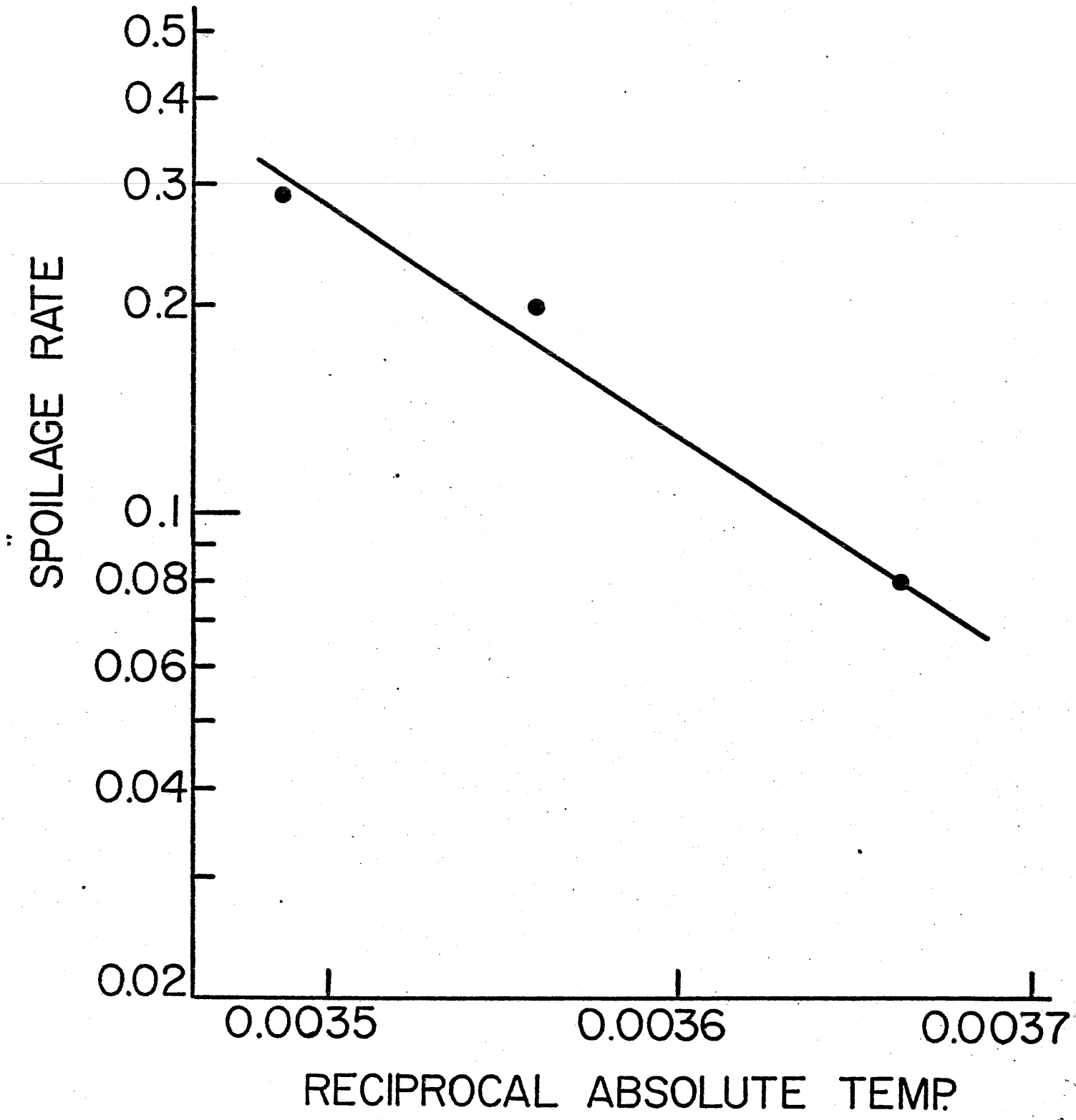


FIG. IV-6 EFFECT OF STORAGE TIME ON TBA NUMBER OF FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

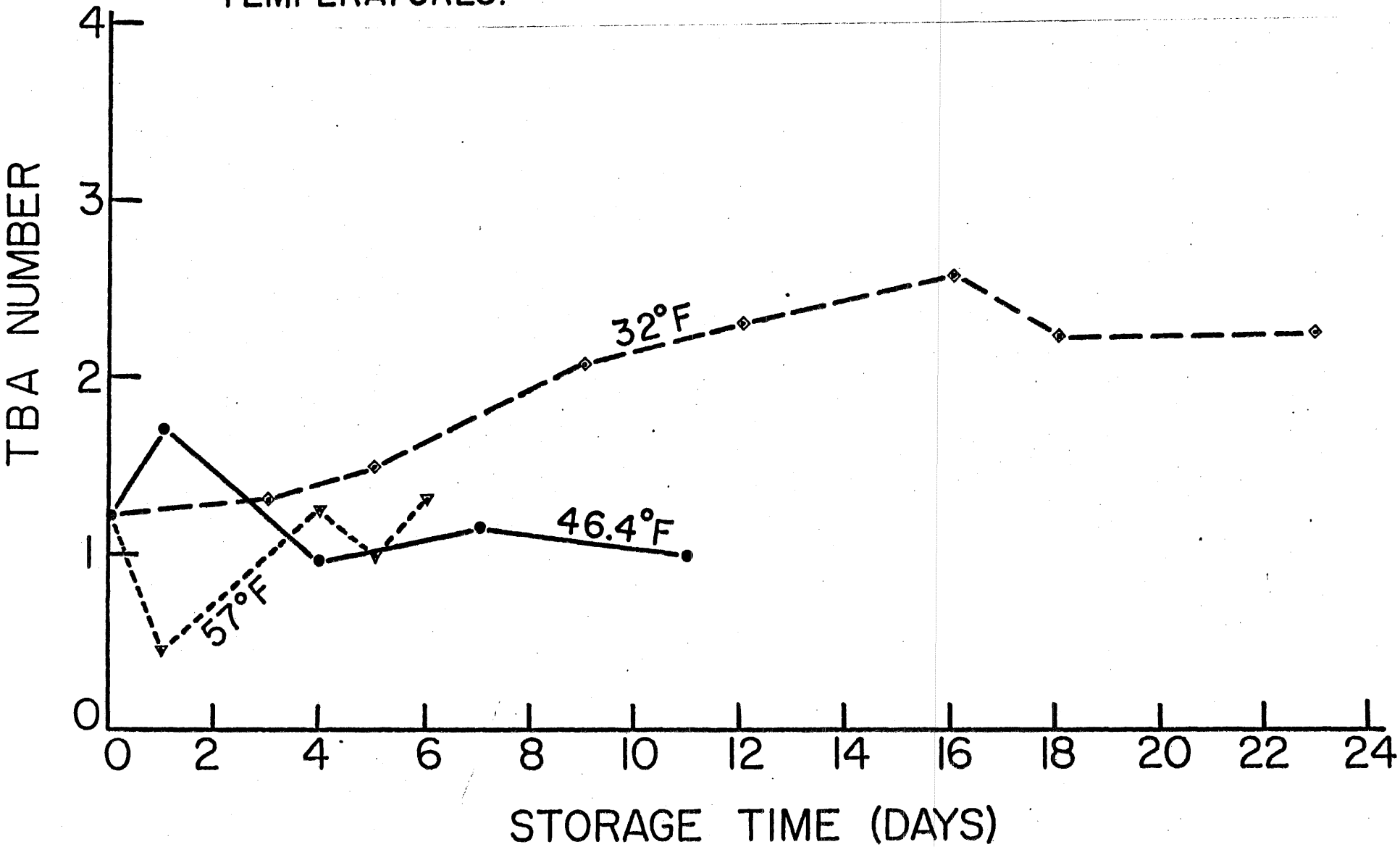


FIG. IV-7 EFFECT OF STORAGE TIME ON pH OF FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

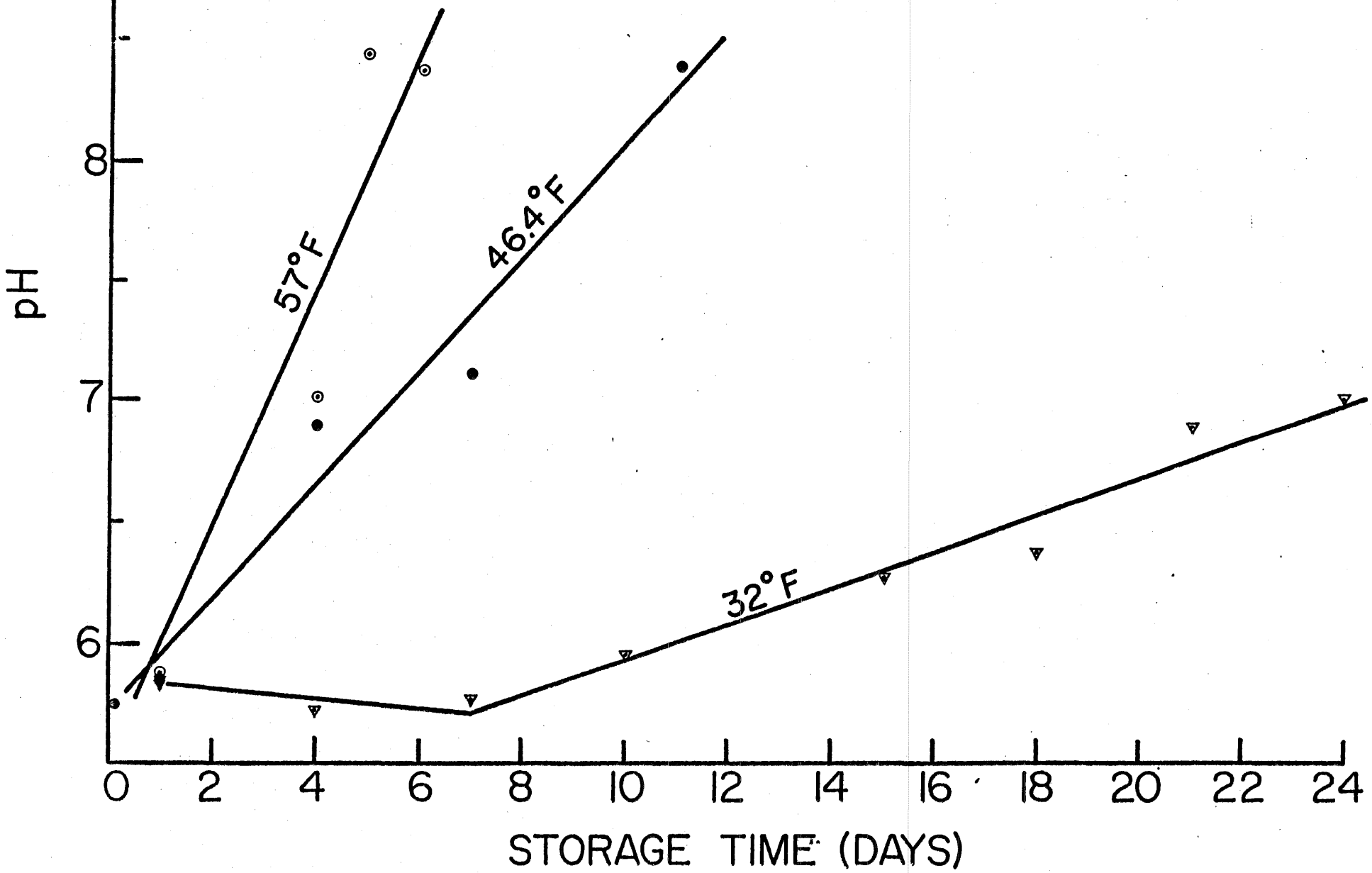


FIG. IV-8

EFFECT OF STORAGE TIME ON AMMONIA NITROGEN CONTENT OF FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

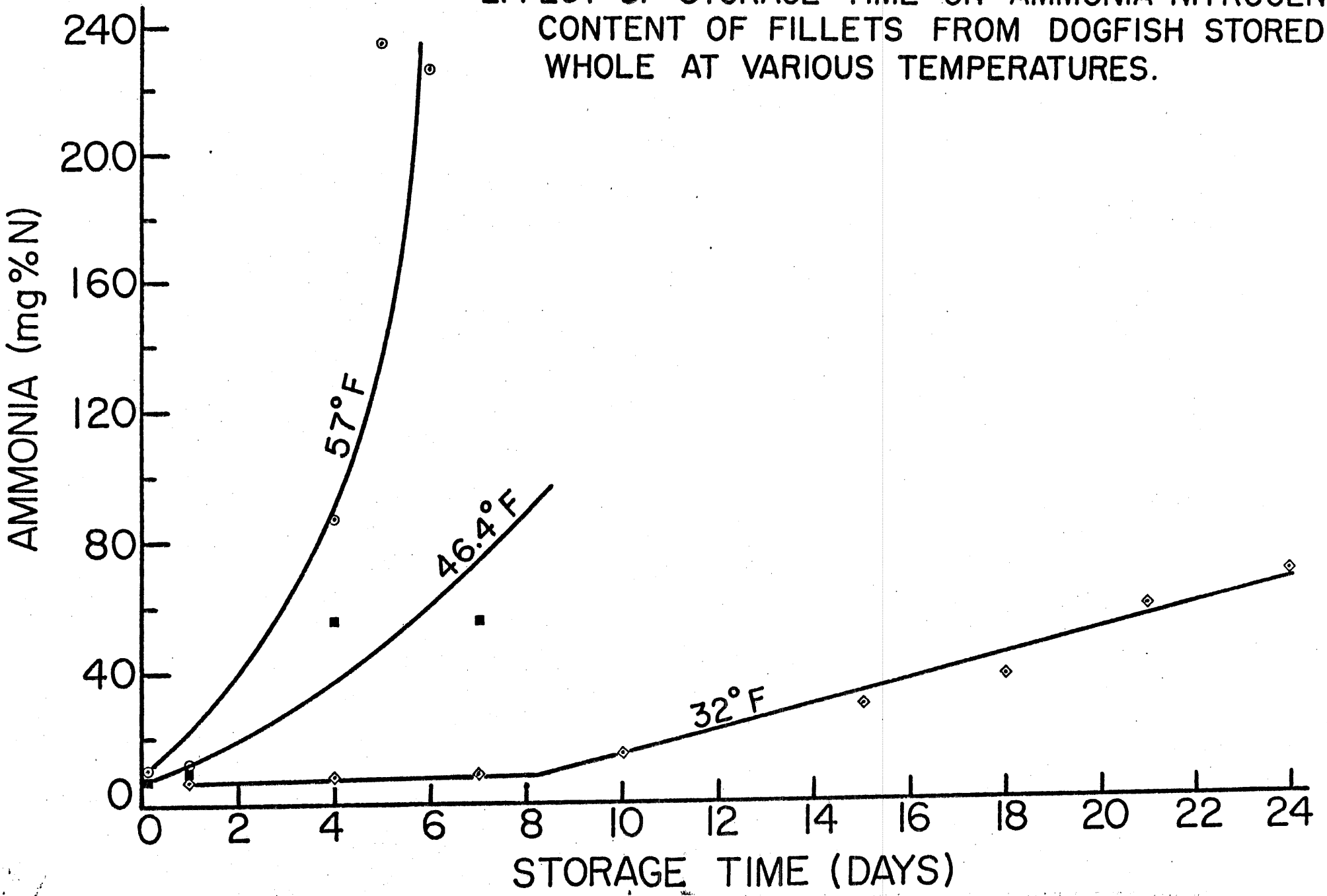


FIG. IV-9

EFFECT OF STORAGE TIME ON TRIMETHYLAMINE NITROGEN CONTENT OF FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

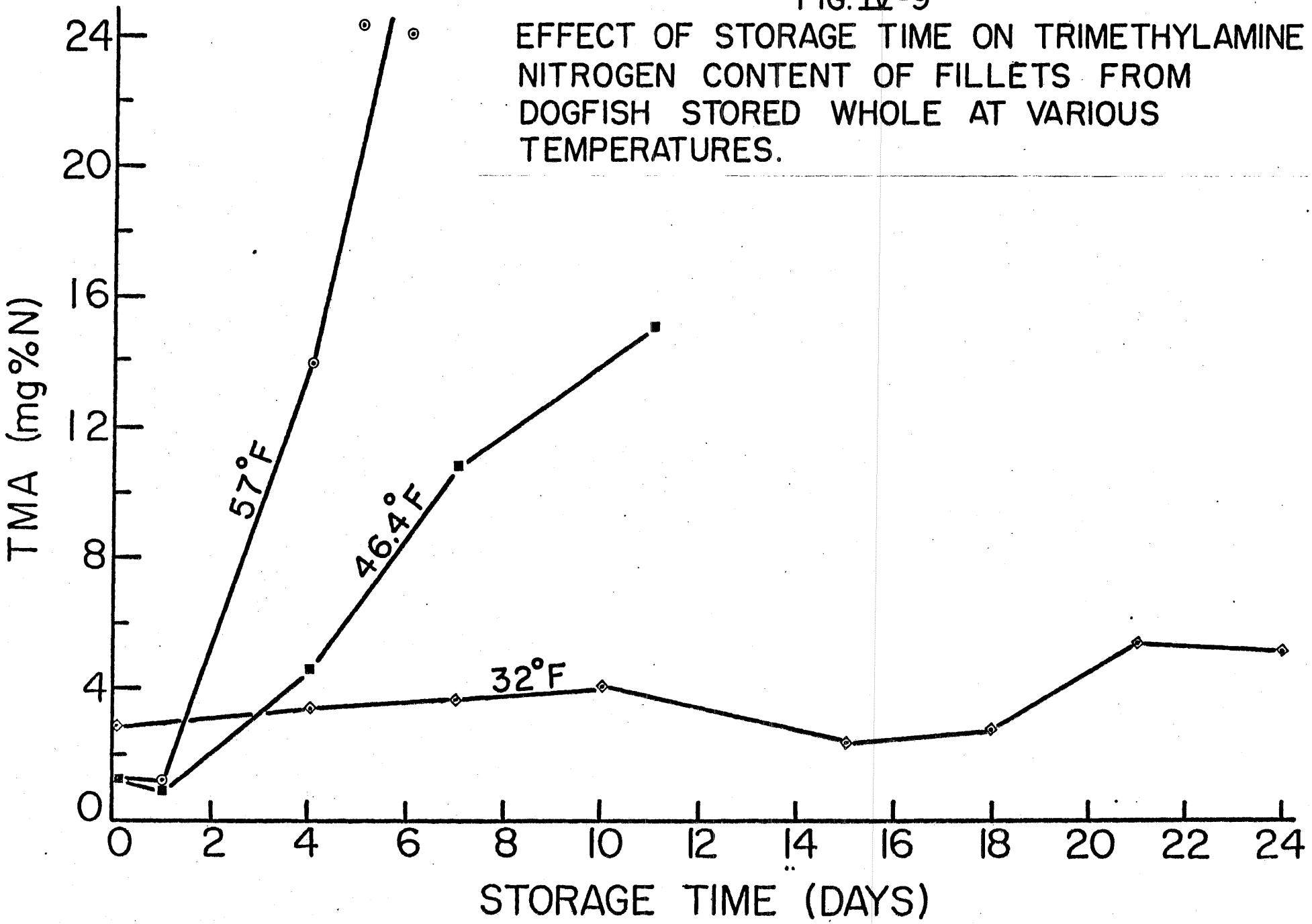


FIG. IV-10 EFFECT OF STORAGE TIME ON DIMETHYLAMINE NITROGEN CONTENT OF FILLETS FROM DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

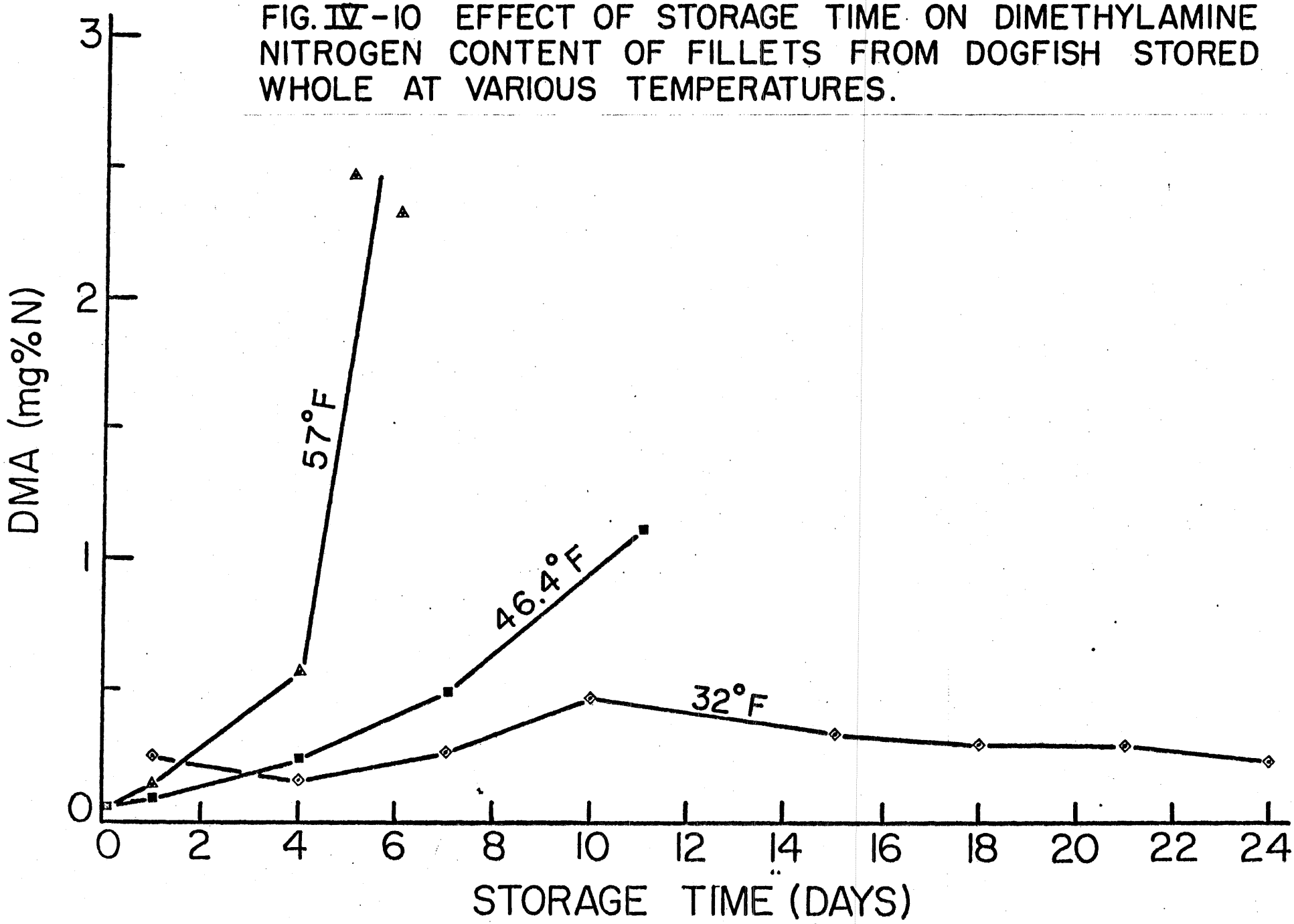


FIG. IV-11 BACTERIAL CONTENT OF DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

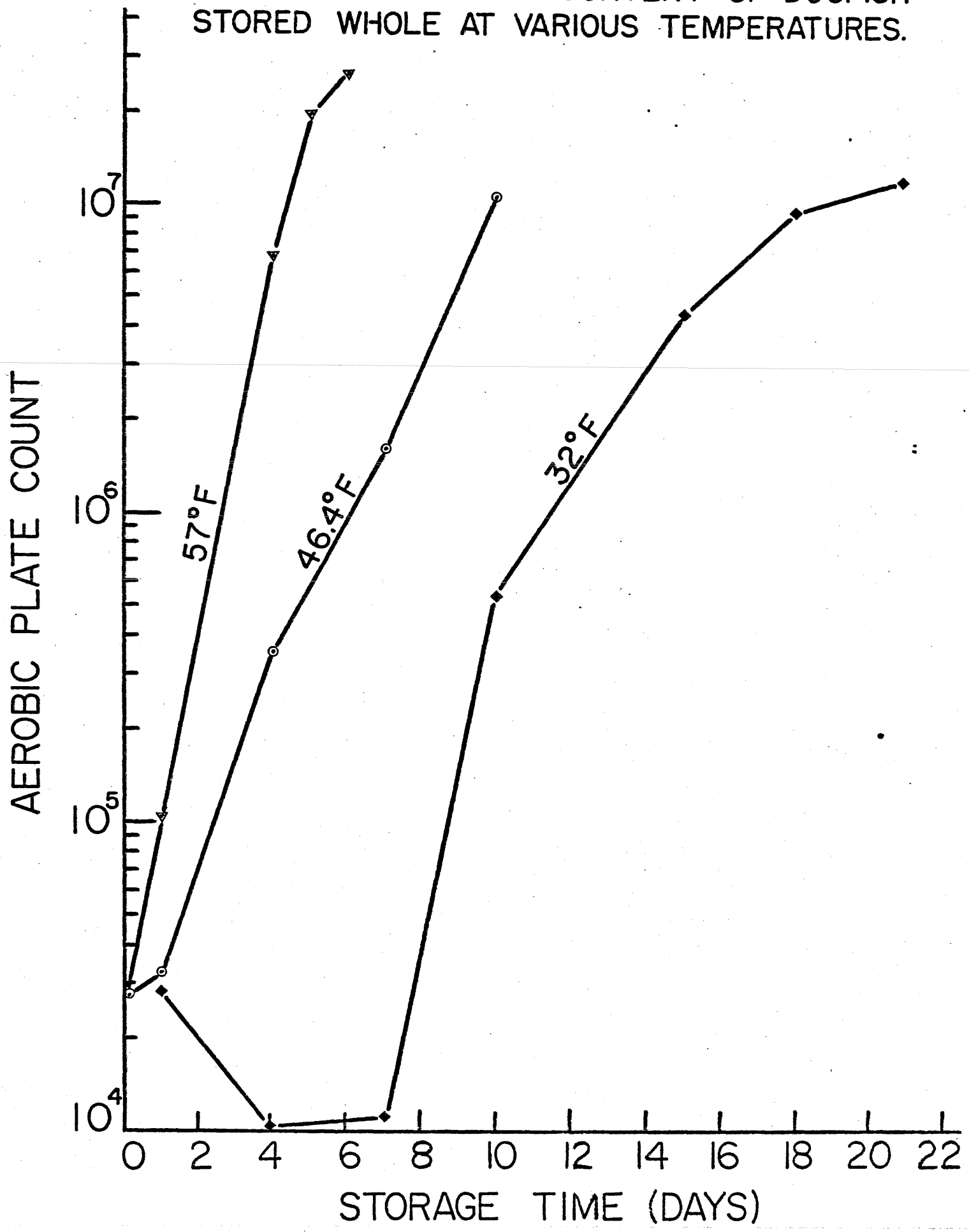


FIG. IV-12 EFFECT OF STORAGE TIME ON TORRYMETER READING ON DOGFISH STORED WHOLE AT VARIOUS TEMPERATURES.

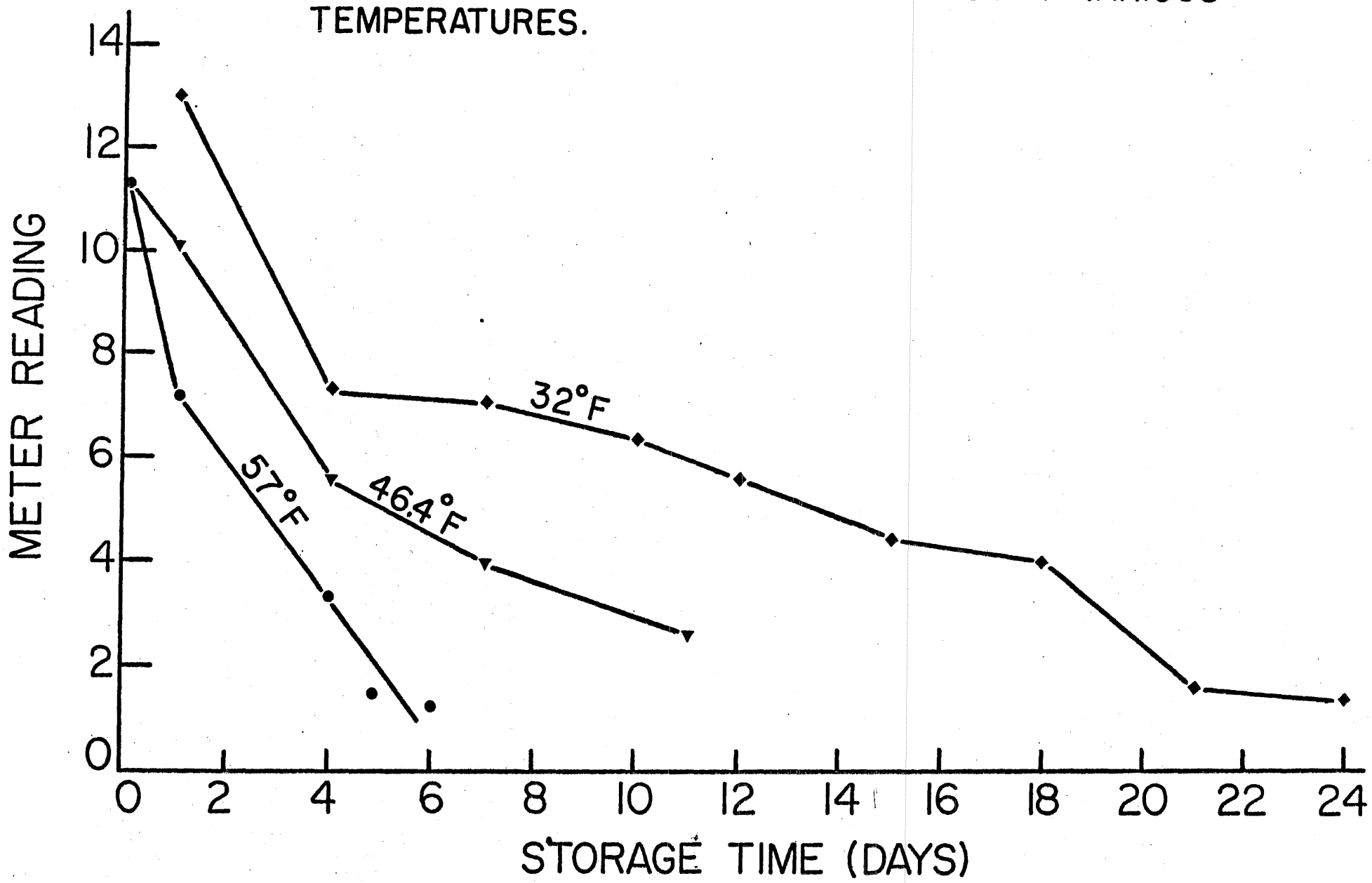
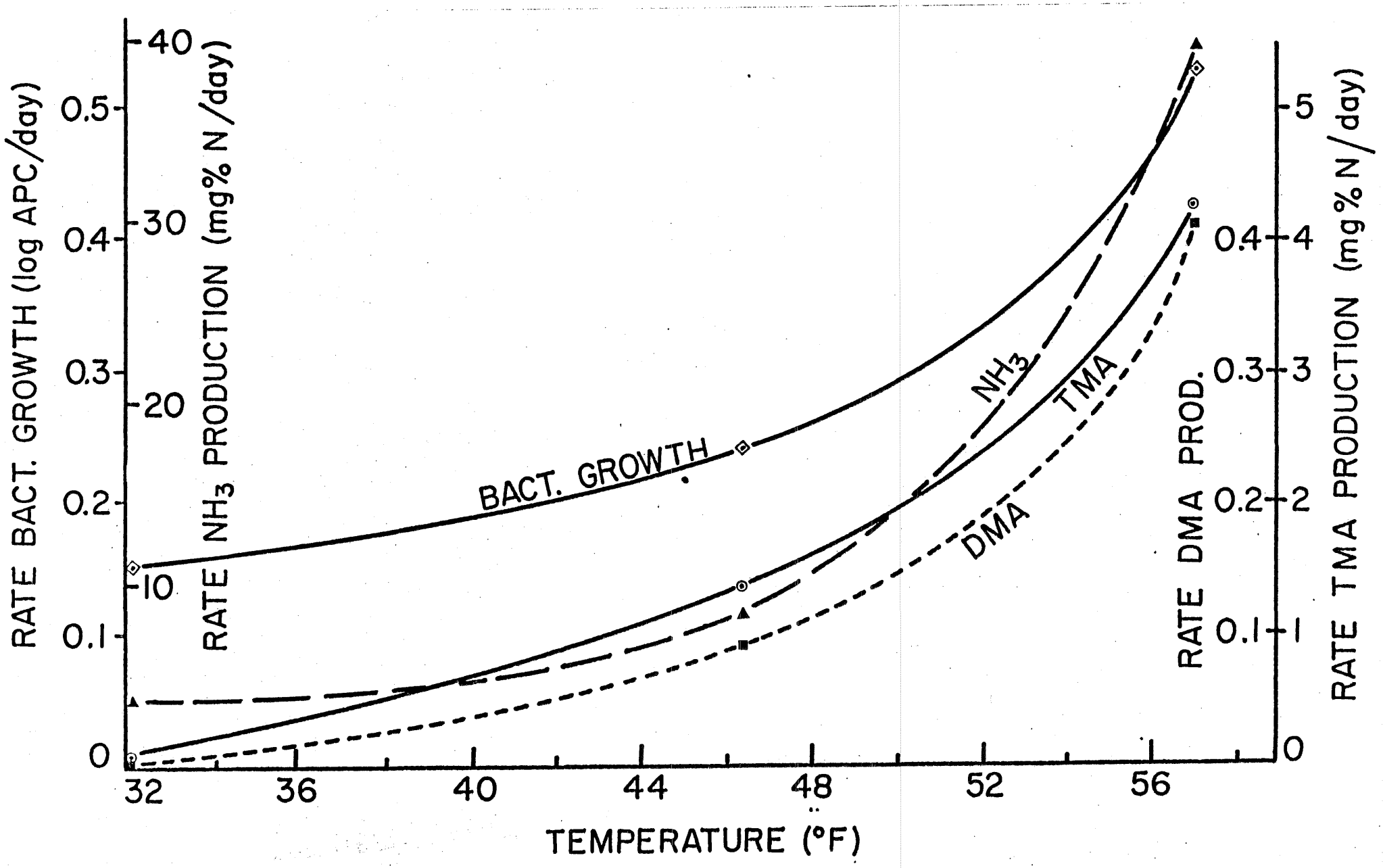


FIG. IV-13 RATE OF BACTERIAL GROWTH, TRIMETHYLAMINE, DIMETHYLAMINE AND AMMONIA PRODUCTION IN WHOLE-STORED DOGFISH AS A FUNCTION OF TEMPERATURE.



Fresh study V

The purpose of this study was to determine the role, if any, of autolytic (tissue) enzymes in the production of ammonia in dogfish post-mortem.

One day old iced dogfish obtained 10-21-82 were filleted and the fillets were individually packaged in nylon bags and marked for identification. One fillet from each fish was subjected to an irradiation dose of 200 Krads with gamma rays from a cobalt-60 source. The other fillet served as a control. Following the irradiation all samples were stored at 46.4^{o}F and at various intervals of time two matching fillets, an irradiated and a control from the same fish, were examined by chemical, physical and microbiological tests.

Although the primary interest was in comparing ammonia production, it was felt that monitoring other parameters would yield useful information regarding shelf life extension by the irradiation treatment. In this study the purpose of the irradiation was to significantly reduce the viable bacterial load on the fish without damage to enzymes. Under this condition any ammonia production in the irradiated samples would be attributed to autolytic enzymes, whereas in the control samples both microbial and autolytic enzymes would be responsible.

Results. The first obvious differences between the two treatments that were immediately apparent at the first testing period (7 days) were the color and amount of drip within the package. The package with the irradiated fillet contained more drip which was grayish-brown, whereas the drip from control fillet was blood-red colored. The grayish drip of the irradiated fillet was due to the enhanced oxidation of myoglobin by the irradiation resulting in the formation of the gray-brown pigment, metmyoglobin. This

reaction can be mitigated by irradiation in the absence of air. Controlling the excessive drip following irradiation might be more difficult, however, one should bear in mind that in this study the fillets were held at a relatively high storage temperature which would accelerate drip formation. Percent drip has been plotted as a function of storage time in Figure V-1. During a 22 day period the amount of drip from control fillets ranged from 1 to 2 percent, whereas in irradiated samples the drip increased progressively and had reached a value of about 9 percent at the end of that time.

Another discernable difference was that the pink flesh color had faded in the irradiated samples after 7 days and much yellow discoloration was present from the 15th day on. A rancid odor was detected in the irradiated samples after 7 days and this odor increased in intensity with continued storage. A comparison of the TBA numbers for the two treatments in Figure V-2 shows that the irradiation treatment caused an immediate increase and this relatively high TBA number persisted in the irradiated samples throughout storage whereas TBA number of the control samples remained unchanged. It is considered that the development of oxidative rancidity in irradiated dogfish could be mitigated by vacuum packaging. A slight-moderate ammonia odor was detected in the control samples at the first test period (7 days) and by the second testing (15 days) that had developed into a strong ammonia odor. In contrast no ammonia odor was detected in the irradiated fillets at any time during 39 days of storage. The curves displaying the ammonia content during storage (Figure V-3) corroborate these sensory findings. The lack of any significant ammonia production in the irradiated samples would indicate that ammonia production in dogfish was due principally to bacterial activity. Simidu and Oisi (1951 a, b) only found traces of urease in fresh

shark muscle and they concluded that urea decomposition in shark was caused by bacterial urease.

There was a sharp difference in the rate of pH increase during storage between the irradiated and unirradiated fillets (Figure V-4). This would most probably be associated with the difference in ammonia content between the two treatments.

Production of the volatile amines, TMA and DMA, was also depressed in the irradiated samples (Figures V-5, 6). It is a well established fact that ionizing radiation treatment will reduce the numbers of TMA producing bacteria on fish to an insignificant level (commensurate with the applied dose), and the spoilage flora that eventually emerges has weak TMA forming activity.

Formation of total volatile bases also occurred at a low level in irradiated fish (Figure V-7) and this was to be expected since production of ammonia, TMA and DMA, which collectively constitute TVB, was low.

Trimethylamine oxide content decreased rapidly during the first two weeks in both the irradiated and unirradiated samples, but to a slightly greater extent in the unirradiated samples (Fig. V-8). Nevertheless, only a very small quantity of TMA (5 mg % N) was detected in irradiated sample at the end of 39 days and yet the net loss in TMAO was about 30 mg % N. This leaves about 25 mg % TMAO unaccounted for.

A comparison of the aerobic plate counts for the two treatments during storage can be seen in Figure V-9. The irradiation treatment with 200 Krad reduced the bacterial count to 0.35 percent of the initial value. It was not just this reduction in bacterial count that was responsible for the extended shelf life (disregarding oxidative rancidity) of the irradiated samples, but rather elimination of the principal spoilage types of bacteria was probably the primary reason. That there was a major shift in the

microbial flora after irradiation is evidenced by the fact that a bacterial count in the millions for the control sample was associated with a high content of volatile basic amines whereas this association did not exist in the case of the irradiated fish. It is not believed that the irradiated samples were yet spoiled as a result of microbial activity at the end of 39 days because there was no off-odor other than the rancid odor present and that problem could be controlled with proper packaging. The aerobic plate count of low-dose irradiated seafoods at spoilage has usually been reported to be of the order of 10^8 per gram. In this study the APC had not yet reached 10^7 per gram after 39 days.

In summary it can be stated that ammonia formation in dogfish is due mainly to the action of certain bacteria and these particular spoilage bacteria can be eliminated or reduced in numbers by low dose irradiation ($\sim$ 200 Krads) so that an extension in storage life results. Certain biochemical tests, such as TMA, ammonia and TVB content, will not be reliable for assessing spoilage with irradiated dogfish.

Development of simple method for measuring ammonia

One of the prime objectives of this investigation was to develop a simple rapid method that could be used in the field to monitor quality of dogfish destined for export markets.

Several chemical methods for the measurement of ammonia are available, but no one is so rapid and simple that it would serve as a useful test for fish inspectors or processors lacking a well-equipped laboratory. Even the official method adopted by the Association of Official Analytical Chemists (AOAC) for determining ammonia in seafood (crabmeat) is too complex for routine laboratory analysis. This test is based on the blue color formed when ammonia reacts with certain phenolic compounds, and the intensity of the blue color, which is related to the ammonia concentration, is read in a spectrophotometer. Moreover, some of these chemical methods are unsuitable because of interfering substances present in certain fish and fishery products. There is the need, therefore, for an alternative procedure.

For the past 15 years a number of scientific instrument manufacturers have researched the development of electrodes to measure selectively a wide variety of ions and dissolved gases in solution. Electrodes are inexpensive and simple to use. In comparison with wet chemical methods, electrodes eliminate many time consuming and error-generating steps. With these advantages considered an examination of an ammonia-sensing electrode was made to determine its suitability. The Orion ammonia electrode coupled with a Corning pH/mV meter was selected for the investigation. A major disadvantage of all the ammonia electrodes available today is the lack of absolute specificity for the amine compound being monitored. Certain other similar compounds can cross the interface between the electrode membrane and the solution and create a measurable potential difference that interferes with the desired response. The volatile amines such as TMA, a spoilage product in many

species of fish, cause such interference. It was, therefore, necessary to determine the extent of interference from concentrations of TMA which might be expected in spoiling dogfish. If the interference from these concentrations of TMA was considerable, a rapid and reliable method of measuring the TMA content of dogfish would also be needed. If, however, a tool, which would indicate the general quality of the fish by measuring the combined ammonia and TMA content, is adequate, it would not be necessary to obtain absolute values for ammonia. Since the production of TMA experiences the same lag period as ammonia production, its interference may be fairly constant throughout the test period. For the duration of the storage period, the concentration of TMA averaged about 25 percent of the concentration of ammonia.

To examine the degree of interference contributed by TMA, a series of standard solutions of ammonium chloride with concentrations ranging from 0 to 80 ppm nitrogen were prepared with TMA added at various levels ranging from 0 to 5 ppm nitrogen. A steadily increasing amount of interference was observed with increasing TMA content. A concentration of 5 ppm TMA caused a significant apparent increase in the measured ammonia response.

In a recent study by Ward et al (1979) the ammonia-specific Orion electrode was used for quality measurement of fresh and frozen shrimp. The ammonia production as indicated by the electrode showed a very close relationship with total volatile bases (TVB). The investigators in this study were probably measuring a mixture of ammonia and volatile amines, rather than ammonia alone as they believed. Under the circumstances the electrode was serving as an adequate quality control tool, however, its limitations should be kept in mind.

In recent years Chang et al (1976) by modification of the ammonia-specific electrode developed an electrode for measuring TMA. If this electrode could reliably measure TMA without interference from ammonia, it could be used in combination with the ammonia electrode to obtain a more accurate measure of the ammonia content. Several problems were encountered by us in working with this electrode. Erratic and non-reproducible results were obtained. Brown et al (1980) recently modified the TMA electrode by increasing the formaldehyde concentration to enhance the selectivity of the electrode for TMA over ammonia. The modified procedure also produced poor results in our investigation. On successive days shifts in the ammonia electrode potential were common and the electrode routinely showed poor response and considerable drift.

Another electrode was purchased from the Orion Company, but results with this electrode were similar. A special time-response graph paper was used in an attempt to obtain clearer data, however this step converted the method from a field test to a laboratory test. In this procedure periodic readings taken over an interval of time, usually about 10 minutes, are plotted on this special graph paper and the line drawn through the points is extrapolated to infinite time to obtain the desired reading. The Orion Company was contacted for assistance or suggestions and their recommendation was to soak the electrode in acid daily to remove the build up of scale which apparently was causing the problem. However, this step changes the base line reading of the electrode and requires a standard curve to be made each day the electrode is to be used. It was decided that this electrode technique would not provide processors or inspectors with a simple method for measuring ammonia content of dogfish. For the assessment of general quality

of fish it has possibilities. It appears to have potential as a laboratory tool, but further investigation is necessary. This study was limited to the Orion electrode. Others such as those developed by HNU Systems or Graphic Controls may perform differently with respect to limits of detection, response time, reliability and operating lifetime. It might be advantageous to examine these other electrodes.

Currently, there are many kits available for the examination of contaminants or dissolved gases in aqueous samples. These kits are inexpensive, simple and rapid to use, and completely self-contained. One such kit available from Chemetrics, Incorporated, Warrenton, VA, for the measurement of dissolved ammonia in water samples was examined. In this kit are glass ampoules containing a pre-measured quantity of color-forming reagent (Nessler reagent) sealed under vacuum. To test the water sample, the ampoule tip is broken below the surface of the water sample with a special snapper device supplied with the kit. Vacuum draws sample into the ampoule where it is mixed. A yellow-orange color develops immediately allowing an instant comparison of the test sample with liquid color standards supplied in the kit. After mixing, the ampoule is allowed to stand for one minute and then read immediately because the color intensifies with time. The presence of TMA offers no interference with the measurement of ammonia by this kit.

The Model AN-10 kit is supplied with two comparitors covering the range 0-1 ppm ammonia and 1-10 ppm ammonia. In dogfish flesh the concentration of ammonia will usually be within the range of 0-1000 ppm or 0-100 mg nitrogen per 100 g. Therefore, after extraction of the sample, a final 100 fold dilution will provide a sample with an ammonia content within the range suitable for analysis with this kit. The comparitor block contains 10 color

standards representing ammonia concentrations ranging from 1 to 10 mg %. In most cases the unknown sample being compared will have a color intensity that will lie between two adjacent standards and an interpolation is required. Thus, an absolute value for ammonia concentration will not be obtained with the kit, but rather a probable range of concentration. However, an estimate of the absolute ammonia concentration can be attained from the median value of the range. Thus, if the actual range observed was 0-8 mg %, the absolute concentration is estimated as 4 mg %.

Portions of the dogfish extracts which had been prepared for analysis by Vyncke's method were examined with the Chemetric kit. The comparative results are presented in Table V-2. There was excellent correlation ($r = 0.99$) between the Vyncke ammonia content and the Chemetric median values. The regression line and 95 percent confidence limits are shown in Figure V-10. The slope ($b = 1.04$) of the regression line was found by a t test to not be significantly different at the one percent level from the slope of the hypothetical line ($B = 1.00$) denoting perfect correlation between the two test results. The parametric equation for the t test was:

$$t = \frac{b - B}{S_b}$$

$$\text{where } S_b = \frac{S}{\sqrt{\sum n_i (X_i - \bar{X})^2}} \qquad \text{(Hald, 1957)}$$

Although the results with this kit lack the precision of a more involved chemical analysis such as Vyncke's method, it should provide a close estimate of the ammonia content of a fish extract. It certainly should enable a processor or inspector to judge whether or not a sample meets the specification for ammonia content. Chemetrics, Incorporated, had previously

produced a similar type kit which made more precise results possible. This "Scan Test Kit" as it was labeled consisted of larger ampoules which could be inserted in an inexpensive spectrophotometer to be read. This kit has been discontinued.

The Sigma Chemical Company also offers a chemical kit for measuring ammonia and this device has been applied for assaying ammonia in clinical samples and waste matter (Robbins and Weber, 1977) and also shrimp (Knight and Tooms, 1980). The test is relatively simple to perform and is based on the enzymatic (glutamate dehydrogenase) conversion of alpha-ketoglutarate to glutamate in the presence of ammonia. A special narrow-bandwidth spectrophotometer ($\sim$ \$1000) is required in this test and we believe that this requirement precludes this method from the field test category. Recently, Smith et al (1980) reported on the application of this test for measuring ammonia in dogfish.

In the previous studies ammonia and pH were found to correlate well in each independent study, but no attempt was made to determine the reliability of pH in predicting ammonia content from one lot of stored fish to another. All the ammonia/pH data from the five prior experiments, with the exception of those data in which the pH exceeded 8.2, have been plotted in Figure V-11. These latter data were excluded because they did not bear an apparent linear relation with the other data points. The regression line and 95 percent confidence limits ($\pm t_{0.95}$ x standard error) have been constructed on the figure. On the basis of these five combined experiments there was very good correlation ($r = 0.89$, $n = 112$) between ammonia-nitrogen content and pH; however, there also was considerable scatter among the data points. From the regression line a pH of about 7.1 is seen to predict an

ammonia-nitrogen content of 55 mg %. However, 95 percent of the time the ammonia nitrogen content at that pH will range from about 33 to 75 mg %. A processor could employ a pH value of 6.6 as the cut off the ensure that no fish with an ammonia nitrogen content in excess of 55 mg % would be exported, however, this plan would also reject some perfectly good fish. Although it does not appear that pH measurement can be used as a precise method for monitoring ammonia content in dogfish, there still is some potential utility in this parameter for rapidly screening fish quality on the basis of either acceptable, questionable or not-acceptable. By increasing the number of replicate samples taken for pH analysis, the average variability may be reduced and the reliability of the predicted ammonia concentration may be increased. Waller (1978) had suggested using pH measurement, either obtained with surface electrode or pH paper, to assess shark quality due to ammoniation and he designated pH ranges that would correspond to the post-mortem age of iced fish. However, he pointed out that at any given time there can be a variation in ammonia content either along the length of the fish or through a cross section of the body. In dressed fish ammonia content was higher at the head end because of the exposed cut surfaces, and was also high in the vent area because the folds of the belly flaps created insulated air pockets with slightly higher temperature than other areas of the fish thus promoting faster bacterial growth in that region. Another source of ammonia variation is related to sample thickness. Ammonia formation occurs mainly on the exposed surface of fish, therefore there will be a difference in ammonia content between two samples taken for analysis, both having the same ammoniated surface area, but of different thicknesses. This variability in ammonia concentration may account for some of the scatter of the data points in Figure V-11.

Regression analysis on flavor score as a function of ammonia nitrogen content was performed on the composite data (n = 87) of studies I, II and III. The correlation coefficient was found to be high (r = 0.89). The regression line and 95 percent confidence limits are presented in Figure V-12. Because of the considerable scatter about the regression line, ammonia nitrogen content cannot be reliably employed for accurately assessing quality. End of useful shelf life would be predicted by an ammonia nitrogen content of about 27 mg %, however, at this concentration the flavor rating could conceivably range from slightly poor to good, 95 percent of the time. It should be remembered that flavor deterioration can be caused by the presence of other compounds in addition to ammonia.

The regression of flavor score on pH is shown in Figure V-13 for the combined data (n = 102) of studies I, II, III and IV. There was good correlation (r =0.84) between flavor score and pH as would be expected since flavor score, ammonia content and pH are all interdependent. End of shelf life was predicted by a pH of 6.5, however, there will be a certain degree of unreliability with this predictor value as evidenced from the confidence limits for the regression line. Waller (1978) pointed out that physical mishandling of shark after capture can lower the quality of the fillets and this will not be reflected by the pH reading. However, he believed that a system of quality assessment could be established based on both surface pH measurement and physical appearance.

Table V - 1. Sensory evaluation of irradiated and non-irradiated packaged dogfish fillets during storage at 46.4°F.

Treatment	Storage Time (weeks)	Sensory evaluation of raw fillet
Control	0	clean fresh odor, no ammonia, sl. pink colored flesh.
Control	7	sl. ammonia odor, blood colored drip.
Irradiated	7	rancid odor, no ammonia odor, dark brown colored drip, pink color gone from flesh.
Control	15	putrid odor, strong ammonia odor.
Irradiated	15	strong rancid odor, no ammonia odor, much yellow discoloration, grayish colored drip.
Irradiated	22	)
Irradiated	29	) Same as for 15 days
Irradiated	39	)

Table V-2. Ammonia nitrogen content (mg/100 g) of dogfish samples as estimated by two different procedures (Chemetric kit and Vyncke method)

CHEMETRIC Range	CHEMETRIC Median	VYNCKE	CHEMETRIC Range	CHEMETRIC Median	VYNCKE	CHEMETRIC Range	CHEMETRIC Median	VYNCKE
0-8	4	6.06	0-8	4	6.5	9-16	12.5	11.3
0-8	4	6.63	0-8	4	5.6	9-16	12.5	8.8
0-8	4	3.8	0-8	4	7.4	9-16	12.5	6.6
0-8	4	5.9	0-8	4	6.6	9-16	12.5	7.3
0-8	4	7.8	0-8	4	5.4	17-24	20.5	22.8
0-8	4	6.5	0-8	4	5.9	17-24	20-5	15.2
0-8	4	5.1	0-8	4	4.8	17-24	20.5	29.5
0-8	4	5.1	0-8	4	3.2	17-24	20.5	23.5
0-8	4	6.1	0-8	4	7.4	25-32	28.5	23.0
0-8	4	6.4	0-8	4	4.6	25-32	28.5	46.1
0-8	4	6.8	0-8	4	5.6	25-32	28.5	43.9
0-8	4	3.9	9-16	12.5	10.0	33-40	36.5	21.4
0-8	4	7.3	9-16	12.5	7.9	33-40	36.5	47.1
0-8	4	4.9	9-16	12.5	9.7	41-48	44.5	51.1
0-8	4	5.7	9-16	12.5	10.6	41-48	44.5	41.2
0-8	4	5.5	9-16	12.5	12.9	41-48	44.5	67.4
0-8	4	8.5	9-16	12.5	9.7	41-48	44.5	47.3
0-8	4	6.8	9-16	12.5	8.4	49-58	53.5	51.1
0-8	4	7.0	9-16	12.5	8.9	49-58	53.5	64.1
0-8	4	5.5	9-16	12.5	11.5	72-81	76.5	84.0
0-8	4	5.7	9-16	12.5	7.7	82-99	90.5	82.6
0-8	4	5.3	9-16	12.5	7.9	82-99	90.5	91.7
0-8	4	6.5	9-16	12.5	9.8	82-99	90.5	90.1
0-8	4	3.4	9-16	12.5	7.9	198-230	214	256.2
0-8	4	8.8	9-16	12.5	7.6	198-230	214	213.3
0-8	4	3.5	9-16	12.5	11.3	198-230	214	220.6
0-8	4	6.9	9-16	12.5	11.0	231-264	247	237.3
0-8	4	4.8	9-16	12.5	4.7			

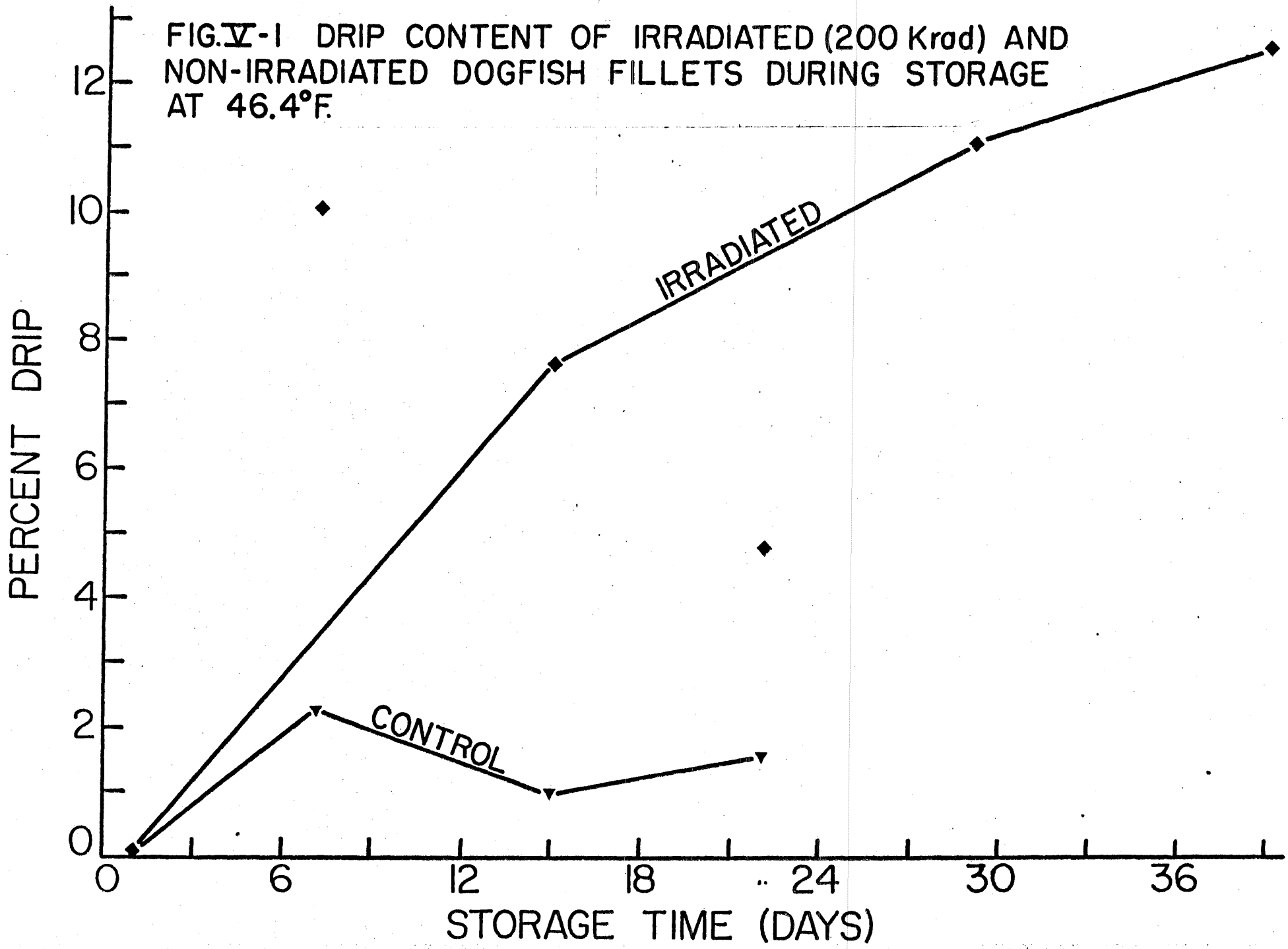


FIG. V-1 DRIP CONTENT OF IRRADIATED (200 Krad) AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.

FIG. V-2 TBA NUMBER OF IRRADIATED (200 Krad) AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.

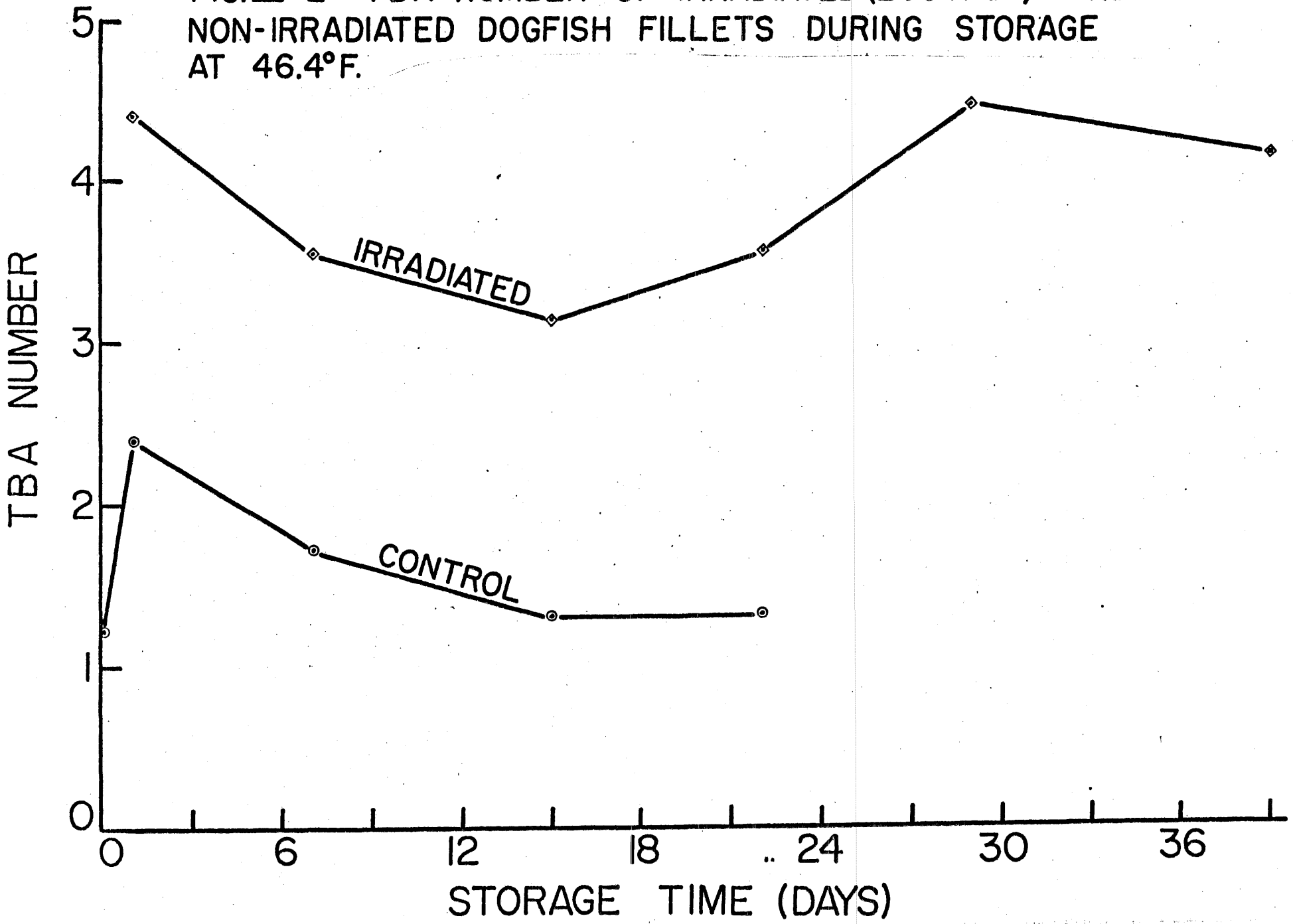


FIG. V-3 AMMONIA NITROGEN CONTENT OF IRRADIATED AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.

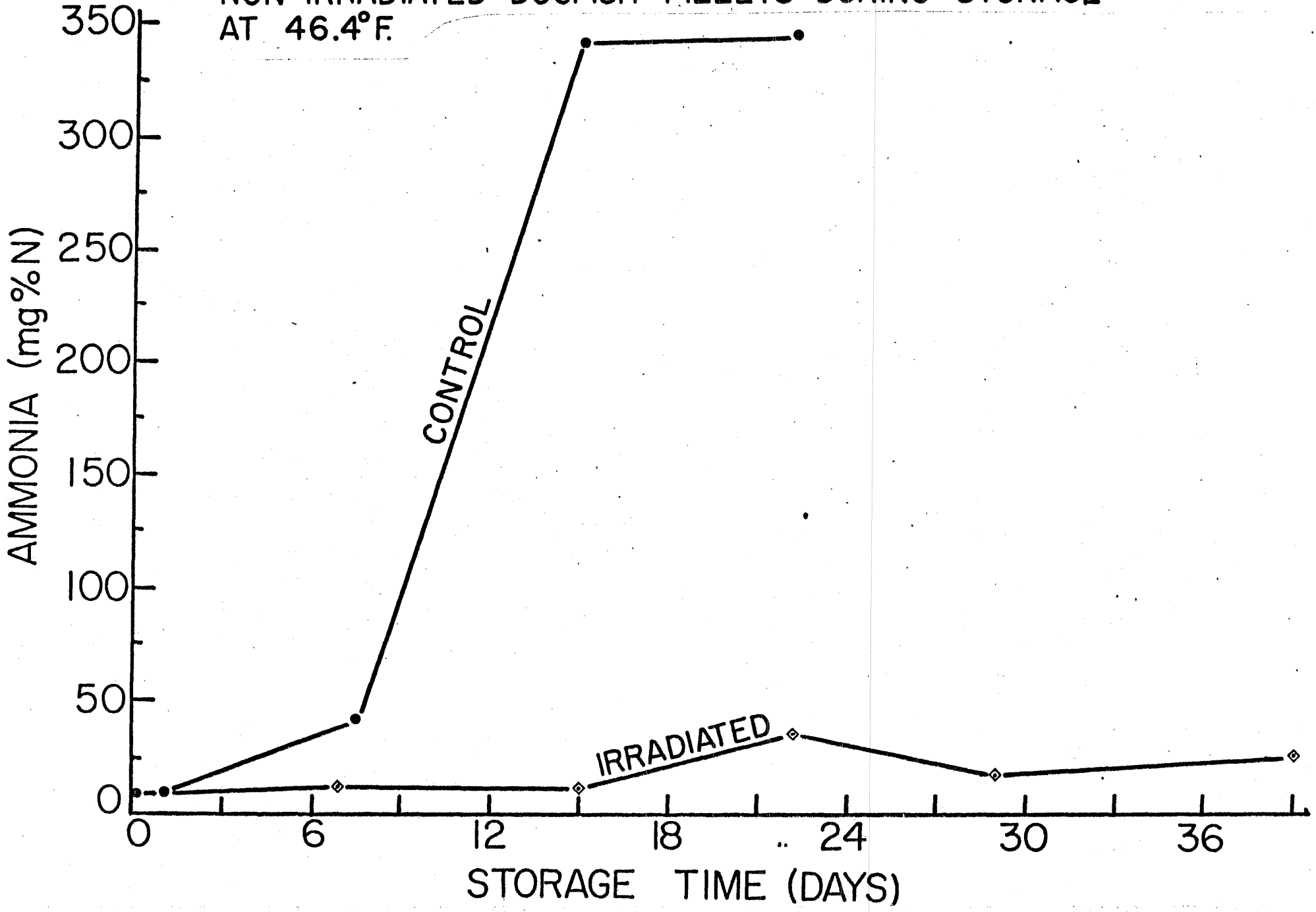


FIG. V-4 pH OF IRRADIATED (200 Krad) AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.

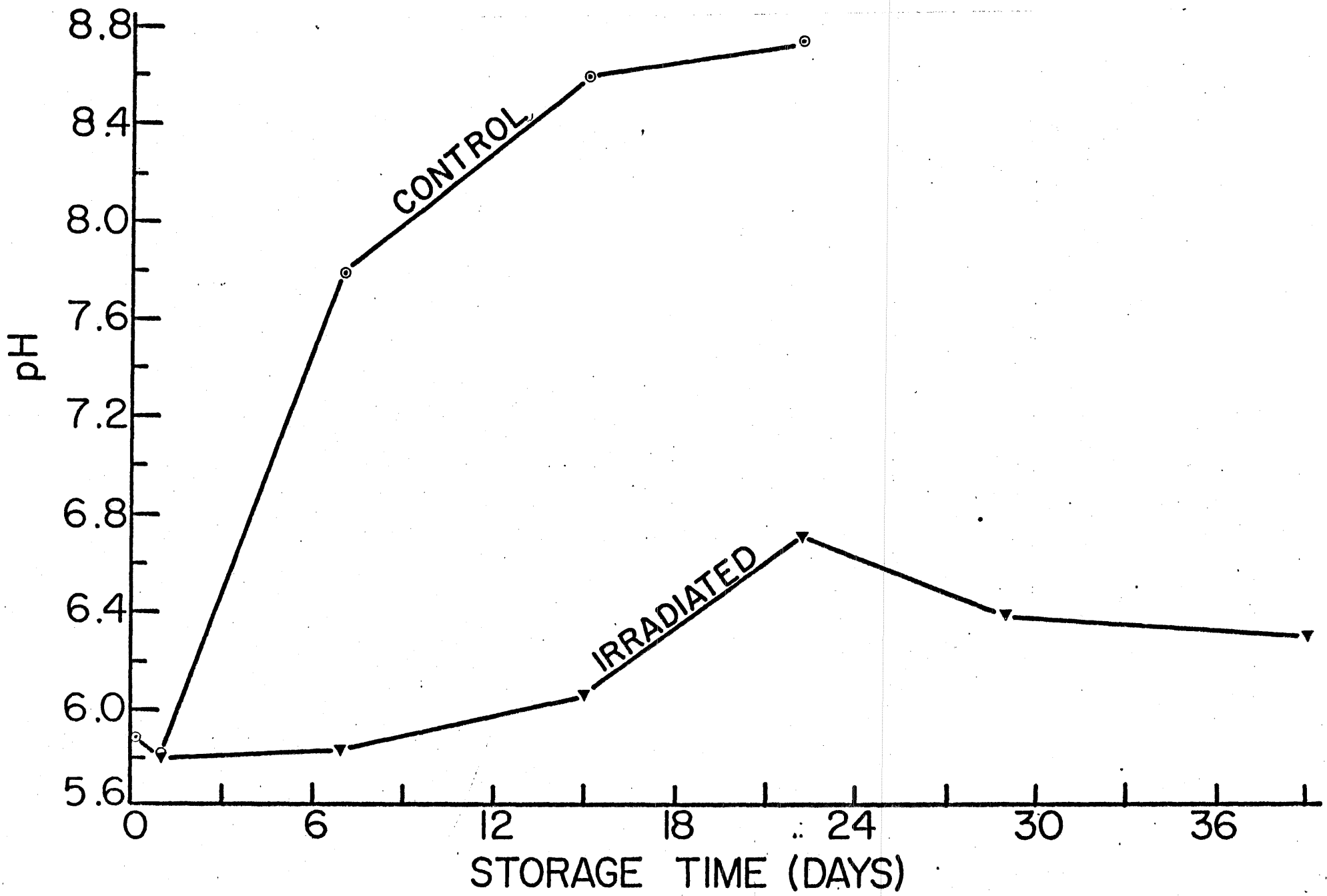


FIG. V-5 TRIMETHYLAMINE NITROGEN CONTENT OF IRRADIATED AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.

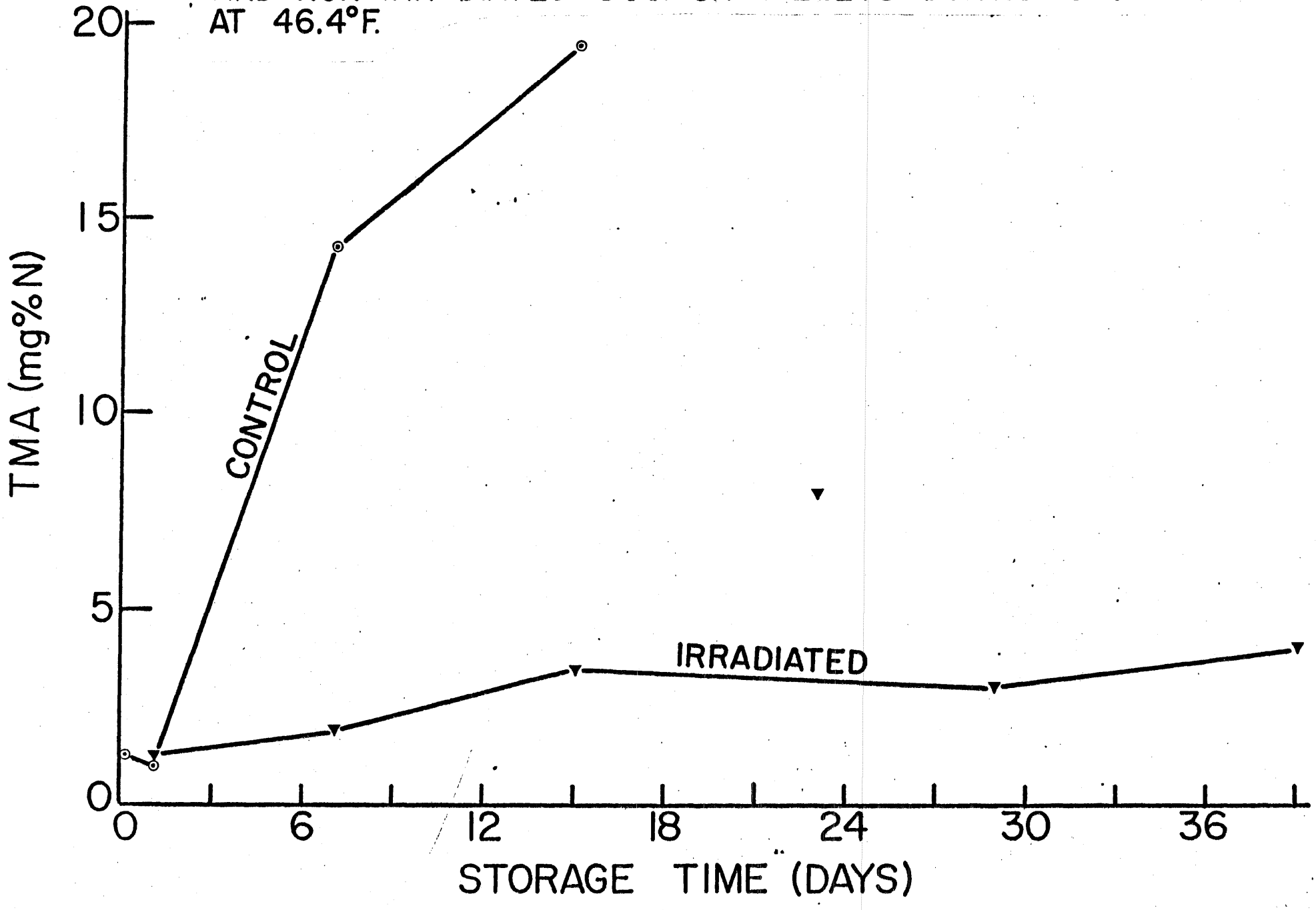


FIG. V-6 DIMETHYLAMINE NITROGEN CONTENT OF IRRADIATED AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4° F.

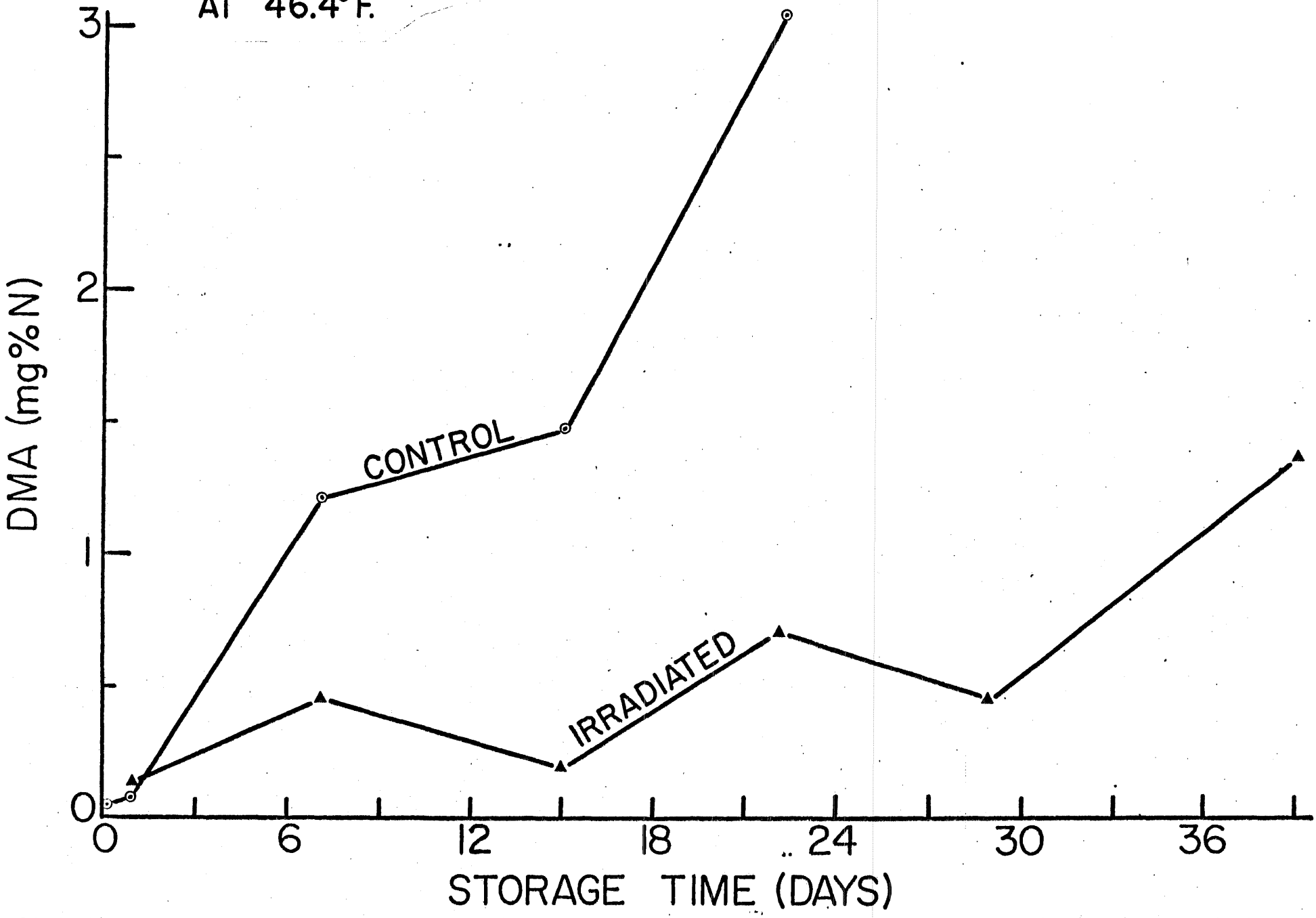


FIG. V-7 TOTAL VOLATILE BASE CONTENT OF IRRADIATED AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.

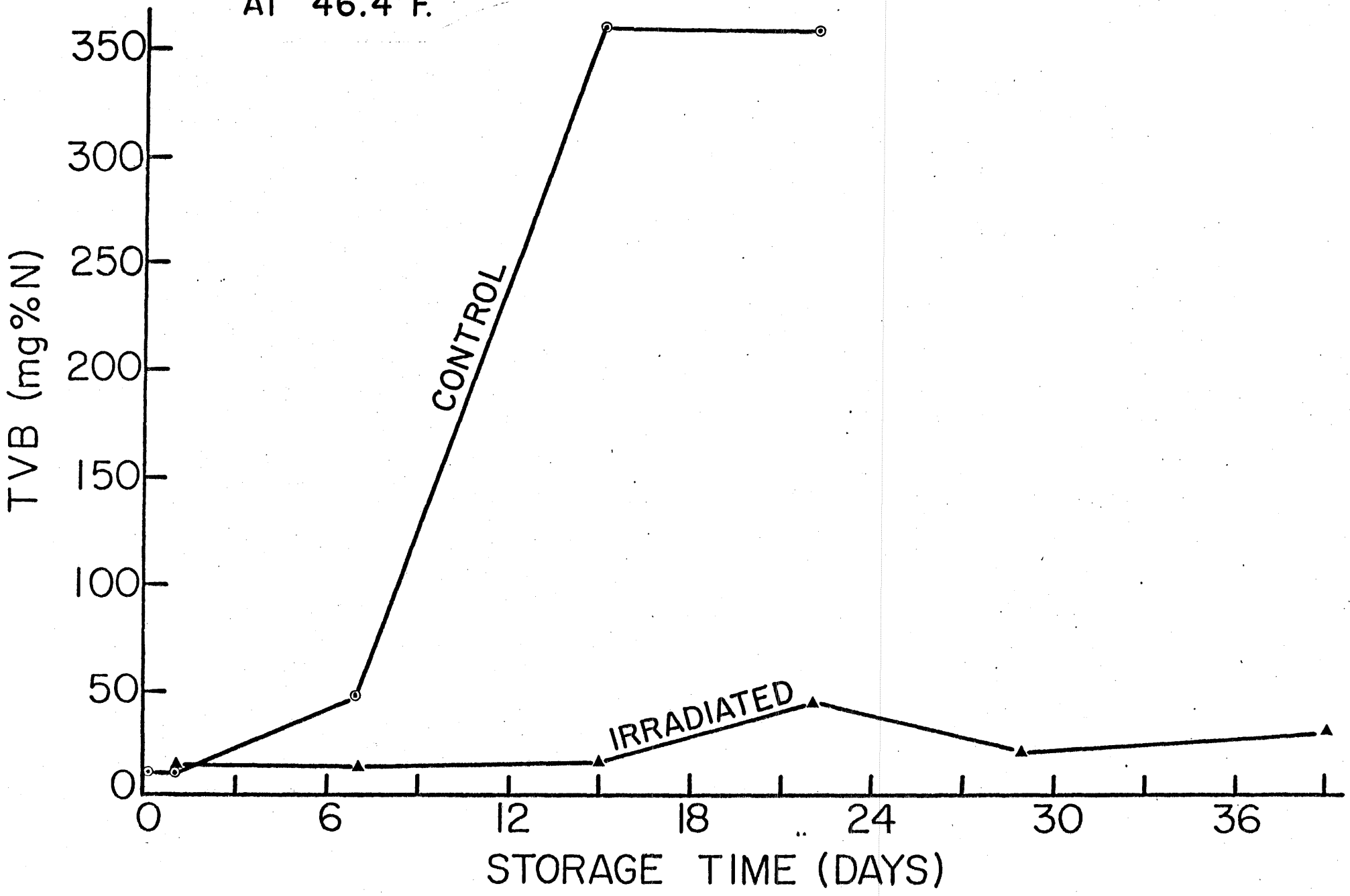
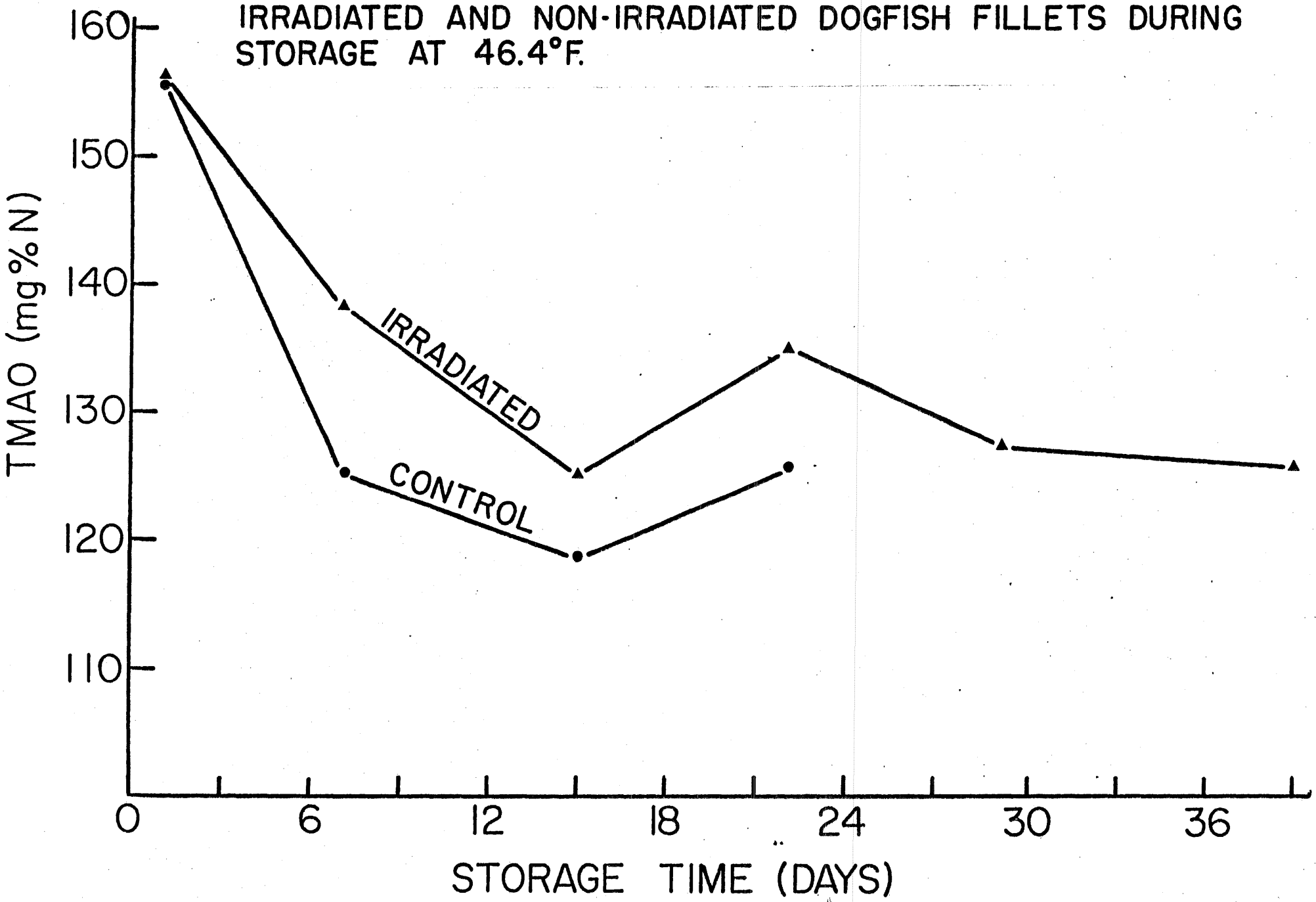


FIG. V-8 TRIMETHYLAMINE OXIDE NITROGEN CONTENT OF IRRADIATED AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.



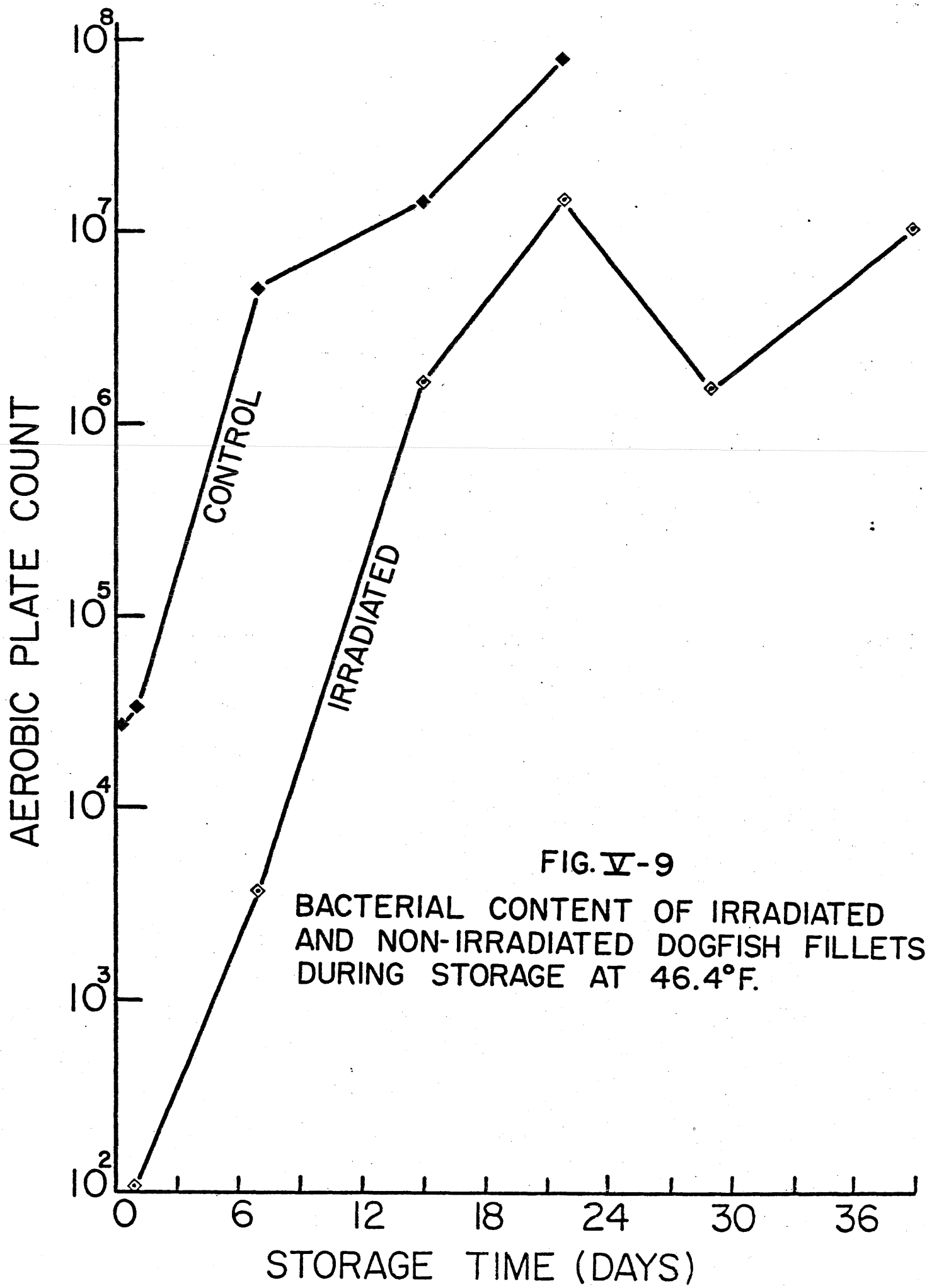


FIG. V-9

BACTERIAL CONTENT OF IRRADIATED AND NON-IRRADIATED DOGFISH FILLETS DURING STORAGE AT 46.4°F.

FIG. V-10 REGRESSION LINE AND 95% CONFIDENCE LIMITS FOR VYNCKE AMMONIA CONTENT AS A FUNCTION OF CHEMETRIC AMMONIA CONTENT.

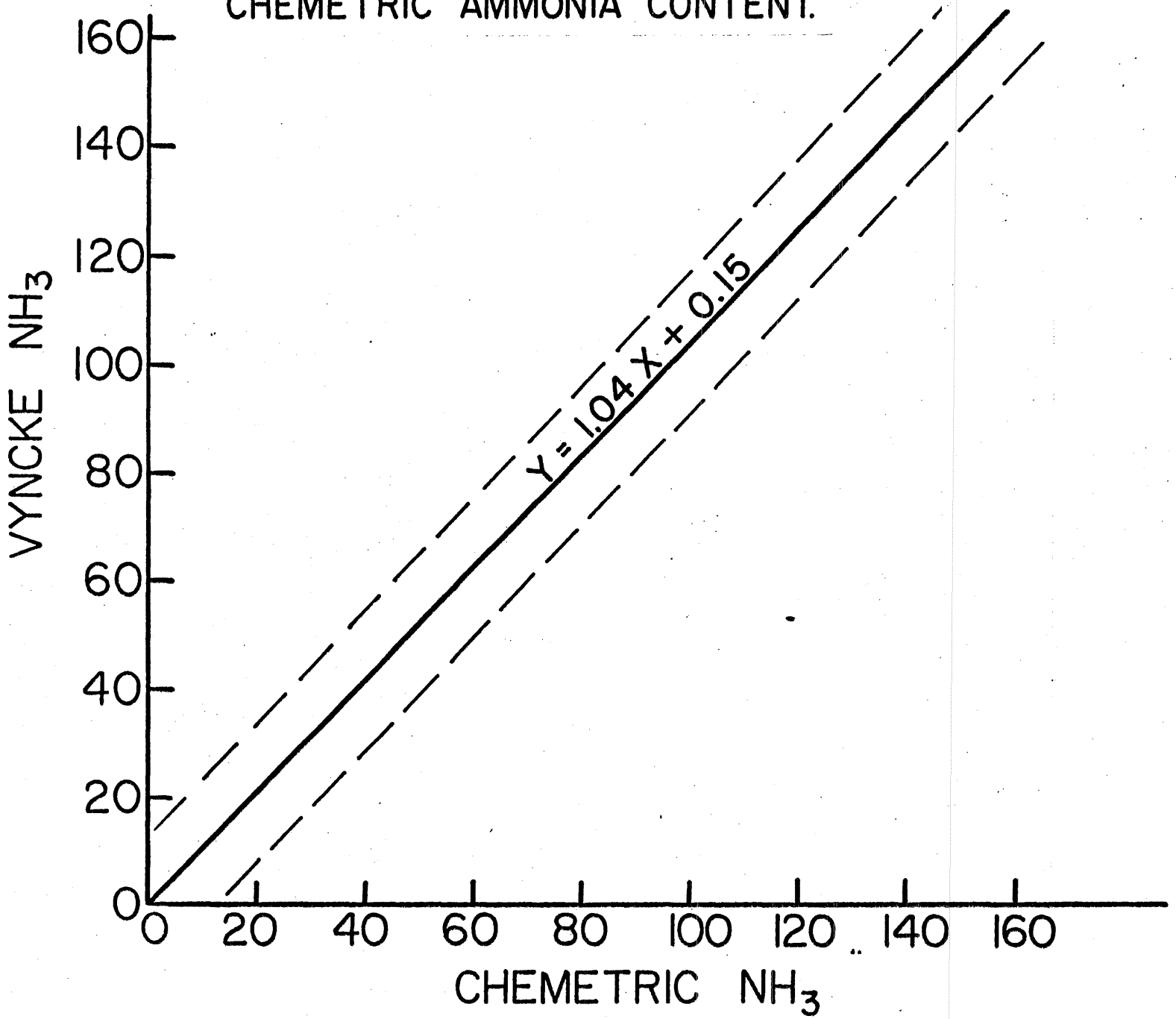


FIG. V-11

AMMONIA CONTENT OF DOGFISH AS A FUNCTION OF pH. (REGRESSION LINE AND 95% CONFIDENCE LIMITS).

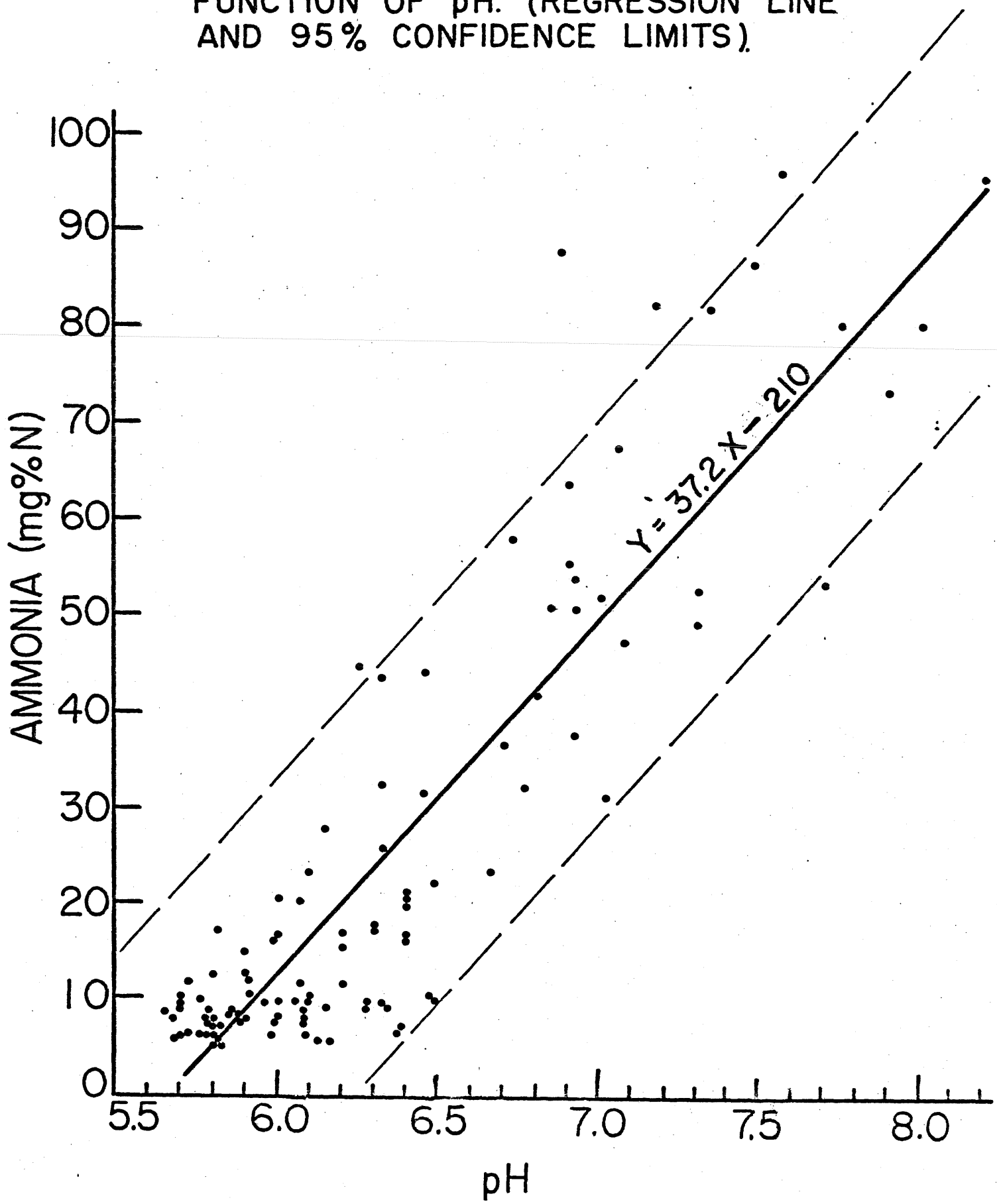


FIG. V-12 REGRESSION LINE AND 95% CONFIDENCE LIMITS FOR FLAVOR SCORE OF DOGFISH AS A FUNCTION OF AMMONIA CONTENT.

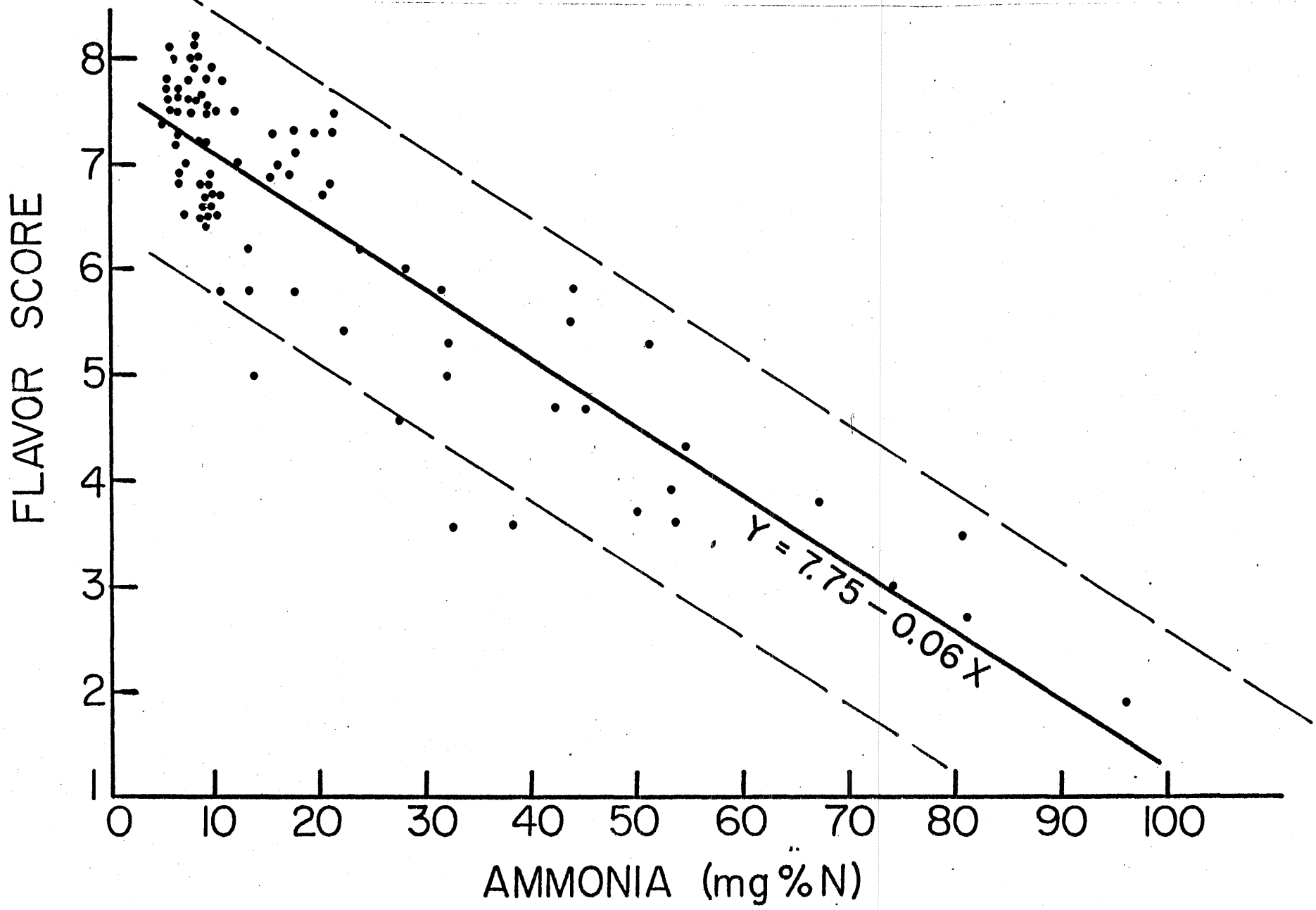
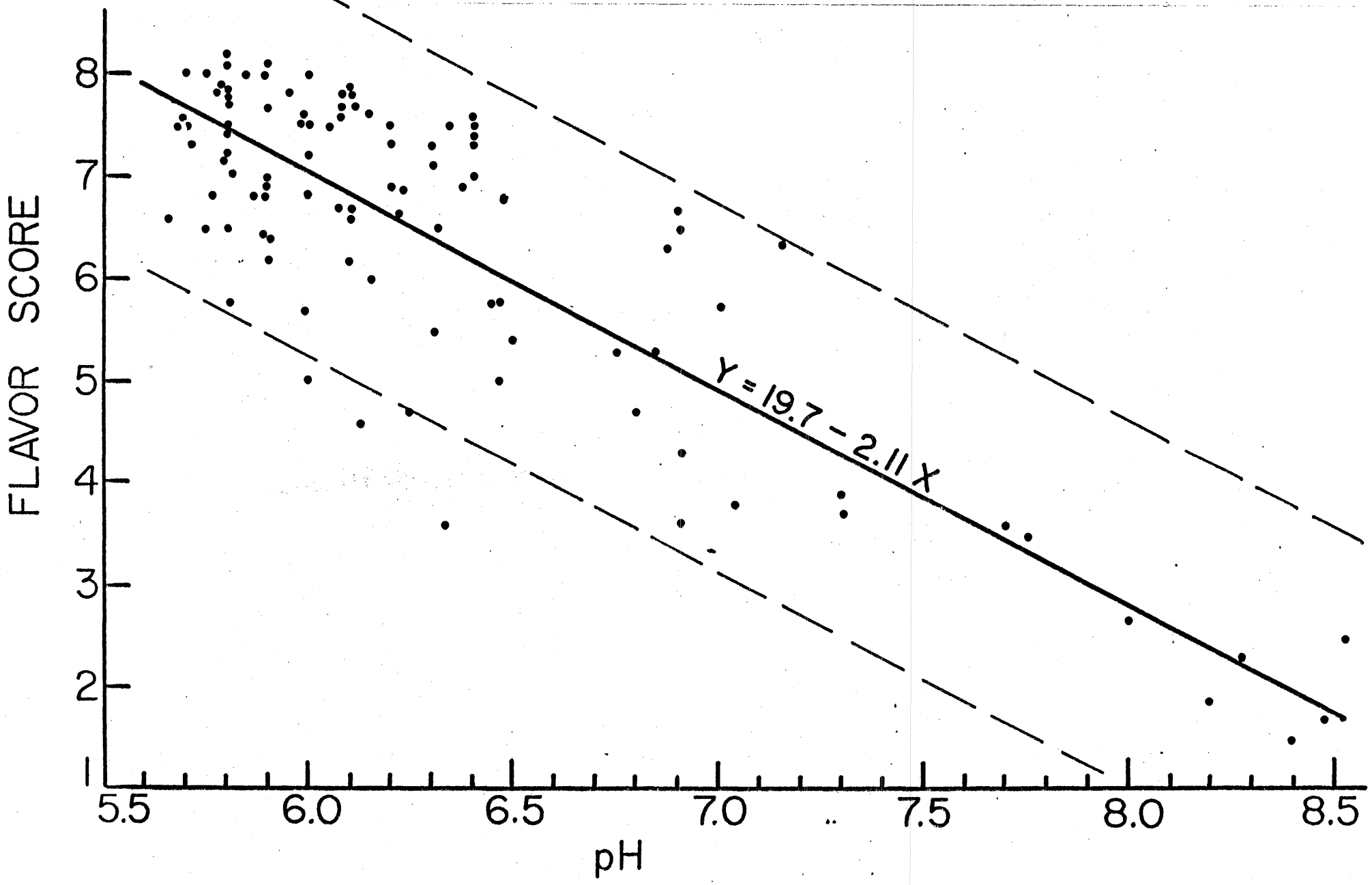


FIG. V-13 REGRESSION LINE AND 95% CONFIDENCE LIMITS FOR FLAVOR SCORE OF DOGFISH AS A FUNCTION OF pH.



Frozen Storage Study VI

The purpose of this study was to 1) determine the effect of initial quality on the shelf life of frozen dogfish fillets or belly flaps, and 2) determine the effect of vacuum packaging on stabilizing flavor of dogfish during frozen storage. Tarr (1958) had suggested that development of rancid flavors might be a problem with frozen dogfish and Boyd et al (1967) proved this to be a reality.

Three day post-mortem iced dogfish were obtained from the Pt. Judith, RI Fishermen's Cooperative on December 11, 1980. Forty eight fish, which was half the lot, were headed-gutted and re-iced for an additional 8 day storage period. The other 48 fish were filleted. Belly flaps were removed from the fillets for separate packaging. The fillets and belly flaps from 24 of the fish were paired and individually packaged in 2 mil polyethylene bags in an air atmosphere. An equal number was similarly packaged under vacuum in a Kenfield vacuum sealer model C-14 in bags made from Curlon S-660, a laminate of nylon-polyvinylidene dichloride-surlyn. All samples were stored at 0 ^{o}F. The 48 re-iced fish were treated similarly after 8 days.

At 5 week intervals during frozen storage a package of each treatment was withdrawn. From each package one fillet or belly flap was used for taste testing and the other paired fillet or flap was used for chemical analysis. The chemical tests included moisture content, pH, TBA number, TMA, DMA, TMAO and extractable protein nitrogen content.

Results

The effect of storage time at 0°F on the odor scores of either air or vacuum packed fillets or belly flaps cut from either 3 or 11 day post-mortem

iced dogfish is shown in Figures VI-1 and VI-2. The faint lines on the graph represent the regression or trend lines. Similar plots for flavor score and texture score are presented in Figures VI-3 through VI-6. Vacuum packaging afforded some protection against odor or flavor deterioration during frozen storage in either fillets or belly flaps from 3 day old fish, but there was no benefit in odor or flavor stability from vacuum packing these sections when cut from 11 day old fish. Textural degradation in either 3 or 11 day old fish was not affected by packaging method. In general, quality of the 11 day old fillets or flaps remained at a lower level at comparable storage times compared to 3 day old fish. The shelf lives of the fillets or belly flaps resulting from either odor, flavor or textural unacceptability were estimated from the regression lines to be as follows:

		shelf life (weeks) for fillets or belly flaps			
	packaging	fillet		belly flap	
attribute	mode	3 day fish	11 day fish	3 day fish	11 day fish
odor	air	35	16	32	6
	vac	65	16	42	13
flavor	air	19	13	10	5
	vac	28	15	17	5
texture	air	46	25	32	5
	vac	69	25	32	5

The above data demonstrate the efficacy of vacuum packaging in preserving frozen quality of fresh (3 day) dogfish, but not of older (11 day) fish. Flavor stability was the limiting quality attribute upon which frozen shelf life was dependent. The relatively short frozen shelf life of 11 day fish compared to 3 day fish is also evident. Thus, the commercial frozen ($0^{o}F$) shelf life

of good initial quality dogfish is estimated for fillets to be 19 weeks for air packed samples, 28 weeks for vacuum packed; and for belly flaps, 10 weeks for air packed samples and 17 weeks for vacuum samples. The higher fat content of the belly flaps enhances their susceptibility to quality deterioration during frozen storage. An Australian study with fillets of gummy shark also revealed a striking difference in frozen storage life at -4 ^{o}F between good and poor initial quality fish. Good quality fillets were still acceptable after 6 months whereas poor quality fillets were unacceptable after one month (Anon. 1978). The difference in post-mortem age of the fish at the time of filleting was not given.

Moisture content of both the fillets and flaps was monitored during frozen storage and results are given in Figures VI-7 and VI-8. There was considerable variability within each treatment and it is not known whether this was due to sampling error or a variation between individual fish. The trend (regression) lines generally showed that the vacuum packed samples increased in moisture content during storage whereas the air packed samples decreased. The decrease in moisture content in the air packed samples can be easily explained as evaporation (sublimation), but there is no rational explanation for an increase in moisture content in vacuum packed samples. The average moisture contents plus or minus one standard deviation over the entire storage term were as follows:

	fillets		belly flaps	
	3 day fish	11 day fish	3 day fish	11 day fish
air packed	69.9 ± 2.2	71.6 ± 1.1	63.5 ± 2.4	65.3 ± 3.7
vacuum packed	71.4 ± 1.5	70.8 ± 2.1	65.2 ± 4.1	63.7 ± 2.7

Because of the magnitude of the standard deviations, it is considered that there was no significant difference between comparable means due to packaging method. Thus, the average percent moisture contents were: 70.7 for fillets (3 day fish), 71.2 for fillets (11 day fish), 64.4 for belly flaps (3 day fish), and 64.5 for belly flaps (11 day fish). Boyd et al (1967) reported an average moisture content of 70 percent for dogfish muscle during frozen storage and this is in agreement with the results we obtained for fillets.

TBA numbers were generally erratic throughout storage, probably due to sampling error; however, the trend lines indicated that the air packed samples increased in TBA number with time, whereas in vacuum packed samples the TBA number remained relatively stationary (Fig. VI-9, 10). With air packed samples there was low correlation between flavor score and TBA number. Correlation coefficients ranged from 0.24 to 0.33 for fillets or belly flaps. This would infer that flavor deterioration was caused by some other factor in addition to rancidity. No attempt was made to correlate flavor score with TBA number of vacuum packed samples since the latter parameter remained relatively unchanged throughout storage.

pH values were also erratic during storage, most probably reflecting differences among individual fish (Fig. VI-11, 12). There was no apparent difference in pH due to packaging mode. The regression lines indicated a slight overall decrease in pH of about 0.1 to 0.2 pH units over the 60 week storage period. Boyd et al (1967) also observed a very slight decrease in pH of dogfish muscle during frozen storage at -4°F. The pH was 6.15 initially and 6.10 after 16 weeks. Olley et al (1962) reported no change in pH in dogfish muscle from an initial reading of 6.1 after 70 weeks at 7°F even though some free fatty acids had been produced. It was suggested

that perhaps the buffering capacity of the dogfish proteins and soluble salts, which might be different from other species, was responsible for the stability of the pH. Changes in pH during frozen storage of foods are known to occur and they result from changes in the composition of solutes in the cellular fluids due to differential solubility as the cellular water freezes out. Overall pH means plus or minus one standard deviation throughout storage are presented in the following table:

	fillets		belly flaps	
	3 day fish	11 day fish	3 day fish	11 day fish
air packed	6.07 ± 0.08	6.09 ± 0.10	6.21 ± 0.07	6.23 ± 0.10
vacuum packed	6.07 ± 0.10	6.10 ± 0.12	6.23 ± 0.11	6.21 ± 0.11

There was no difference in average pH value due to packaging method. The average pH of the belly flaps was higher than that of fillets and this must be due to compositional differences between the two. Correlation between flavor score and pH was low for the various treatments, ranging from r = 0.14 to 0.26. Thus, pH would not be a reliable parameter for assessing quality of frozen dogfish.

Values for trimethylamine nitrogen content fluctuated during storage, however, trend lines indicated that overall no change had occurred (Fig. VI-13, 14). This is to be expected since TMA is produced from bacterial enzymatic action on TMAO, and bacterial activity is arrested at 0°F. There was no effect on TMA content due to packaging method. Overall average TMA nitrogen content (mg %) plus or minus one standard deviation during storage is shown below:

	fillet		belly flaps	
	3 day fish	11 day fish	3 day fish	11 day fish
air packed	3.2 ± 0.65	3.3 ± 1.1	2.0 ± 0.59	2.4 ± 0.83
vacuum packed	3.0 ± 0.81	3.5 ± 1.6	1.9 ± 0.63	2.4 ± 0.90

The concentration of trimethylamine oxide, the precursor of TMA, did not change as a result of frozen storage although weekly readings did fluctuate (Fig. VI - 15, 16). This variability has been attributed to individual fish and also the particular section of fish muscle being sampled. Tokunaga (1970) examined 16 species of fish and found a difference between TMAO contents of dark and white muscle, and in some species there was variability within the white muscle. It is concluded that TMAO content would have no value as an index of quality of frozen dogfish. The average concentration of TMAO nitrogen (mg %) plus or minus one standard deviation was as follows:

	fillet		belly flaps	
	3 day fish	11 day fish	3 day fish	11 day fish
air packed	195.8 ± 39.6	158.0 ± 28.3	135.2 ± 30.7	109.3 ± 36.7
vacuum packed	192.6 ± 46.6	152.8 ± 26.0	132.9 ± 43.3	99.0 ± 24.8

From these data it can be seen that TMAO concentration was about 31 percent lower in belly flaps compared to fillets, and also about 20 percent less in older fish (11 day) compared to the fresher fish (3 day). This latter observation is of interest since there were only slight differences in TMA content between the 3 and 11 day fish which do not account for the disappearance of about 35 mg % TMAO.

A very small amount of dimethylamine was formed during frozen storage in both fillets or belly flaps from 3 day old fish, but none was formed in

11 day old fish (Fig. VI - 17, 18). Gadoid species are known to contain an enzyme, TMAO-ase, which decomposes TMAO to DMA and formaldehyde, but dogfish are not reputed to possess this enzyme. Nevertheless, the low rate of DMA production, and presumably formaldehyde production, during frozen storage would indicate that the TMAO degradation reaction, if indeed it was occurring, was of no significance in textural deterioration. It is interesting to note that the rate of DMA production in the 3 day old fish was greater with vacuum packed samples compared to air packed samples. This same observation has been made with frozen red hake (Licciardello et al, 1982). It was concluded that DMA content was of no practical use in evaluating textural quality of frozen dogfish.

Ammonia nitrogen content of the fillets or belly flaps from 3 day old fish increased slightly during frozen storage from an initial concentration of about 12 mg% (Fig. VI - 19, 20). The rate of increase was similar in air and vacuum packed samples. No change in concentration occurred during frozen storage in samples from 11 day fish. In the irradiation study discussed in a previous section it was demonstrated that ammonia formation in dogfish resulted from bacterial enzyme action and not from endogenous tissue enzymes. Therefore, it is difficult to explain the slight increase in ammonia that occurred in the 3 day fish since bacterial activity was stopped at $0^{o}F$. One might argue that bacterial enzymes could continue to operate (slowly) at $0^{o}F$, however, more of this enzyme activity would be expected in the 11 day old fish, yet there was no change in ammonia in these samples during frozen storage. In a study with skinned dogfish carcasses Boyd et al (1967) reported that no ammonia odor was detected during frozen storage; however, in some carcasses in which the kidney had not been removed

an ammonia-like odor was noticed. Nevertheless, in the present study since the increase was only slight, it is considered that ammonia content of frozen dogfish could serve as an index of the acceptability of the fish at the time of freezing.

The rate of decrease in extractable protein nitrogen was about the same for all 8 treatments (Fig. VI - 21, 22). At the end of 50 weeks frozen storage the EPN content had decreased to about 50-60 percent of its original value. In lean fish the EPN content at the end of textural shelf life has been found to be about 30-40 percent. Boyd et al (1967) reported a decrease in protein solubility in 5 percent Na Cl solution during frozen storage of glazed dogfish. After 120 days at 14°F or -22°F, the percent soluble protein was 67 and 89 respectively. Some lipid hydrolysis was also noted and it was suggested that the protein denaturation and lipid hydrolysis were both related. A decrease in protein solubility in 5 percent Na Cl solution to about 55 percent was also found by Olley et al (1962) in dogfish stored for 18 to 20 weeks at 7°F.

Correlation between texture score and percent EPN varied with the different treatments and in general would have to be considered overall as fair. Correlation coefficients ranged from 0.20 to 0.72. Textural deterioration (toughening) is more of a problem with lean fish compared to fatty fish, and EPN content has been generally found to correlate more favorably with texture of lean fish. From our results it would have to be concluded that EPN content would not be a reliable indicator of textural quality in frozen dogfish.

Summary

This study confirms the findings of Boyd et al (1967) that dogfish muscle and particularly the belly flaps are prone to the development of oxidative

rancidity during frozen storage. A water glaze was found by Boyd et al to act as a barrier against oxygen and thus retarded rancidity development. We showed that packaging the dogfish in an oxygen impermeable film at low oxygen tension was also effective to an extent in controlling rancidity. Although some protein denaturation was observed to have occurred during frozen storage, the limiting factor for shelf life was flavor change and not texture change. The frozen shelf life was also dependent on the initial quality of the fish.

Of the various tests conducted during the frozen storage study, only the sensory tests would be reliable in assessing quality. The TBA assay might be useful, but sampling can be a problem since oxidative rancidity is basically a surface phenomenon, and also there may be a non-uniform distribution between light and dark muscle in the fish with the dark muscle probably being more highly oxidized. Ammonia content, TMA and pH were relatively constant throughout frozen storage and might be considered of some value in predicting acceptability of the fish at the time of freezing. In iced dogfish there was practically no change in these variables (NH_3, pH, TMA) during the first 10 to 14 days. Thus, from these tests on frozen dogfish one could not predict the post-mortem age up to 10-14 days of the iced fish at the time of freezing. Yet, there was a significant difference in frozen shelf life of 3 day old compared to 11 day old iced fish. The EPN test might have some value in assessing the status of protein denaturation, however, the quality problem with frozen dogfish is related to flavor change.

FIG. VI-1 EFFECT OF STORAGE TIME AT 0°F ON ODOR SCORE OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

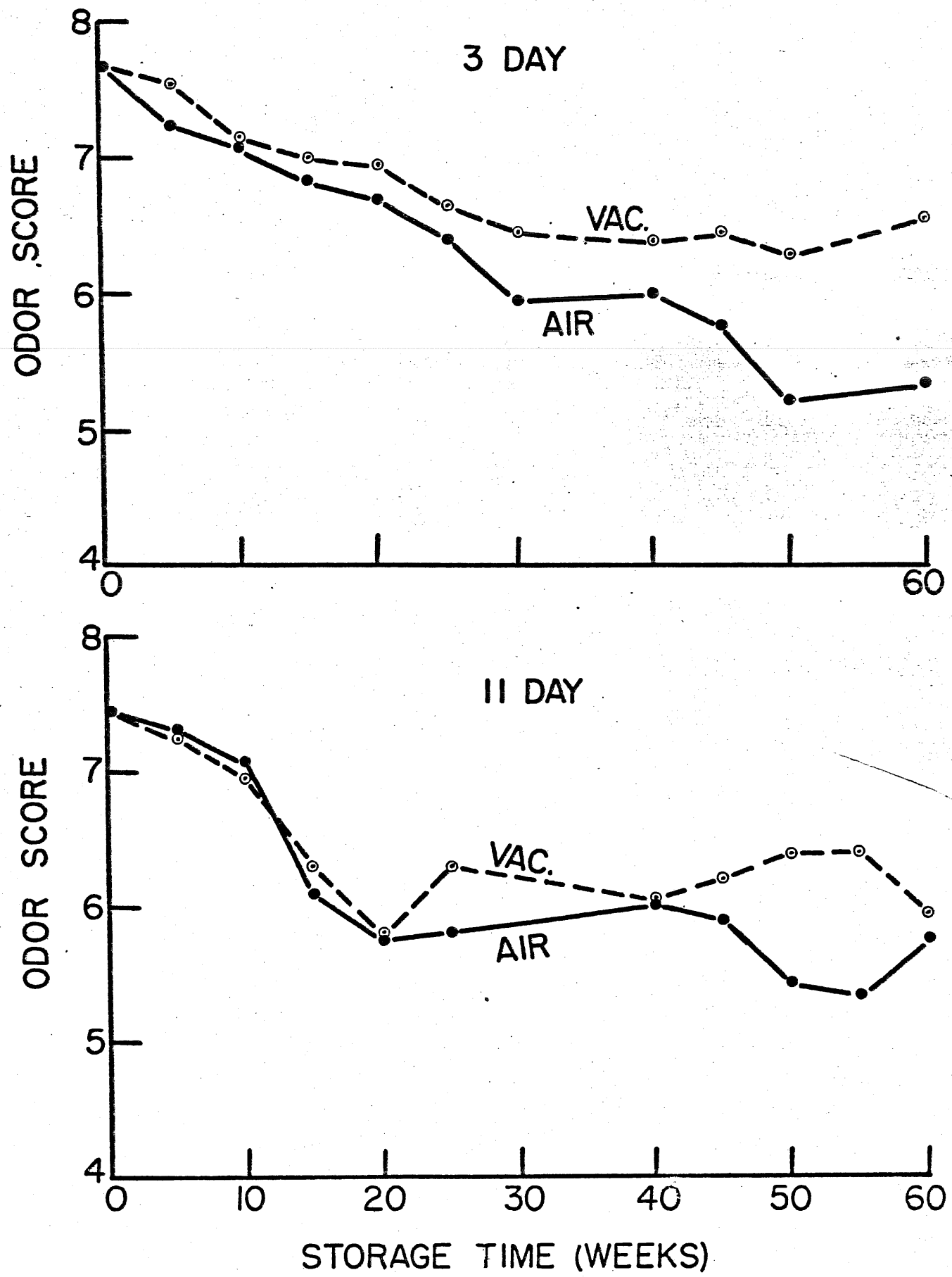


FIG. VI-2 EFFECT OF STORAGE TIME AT 0°F ON ODOR SCORE OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

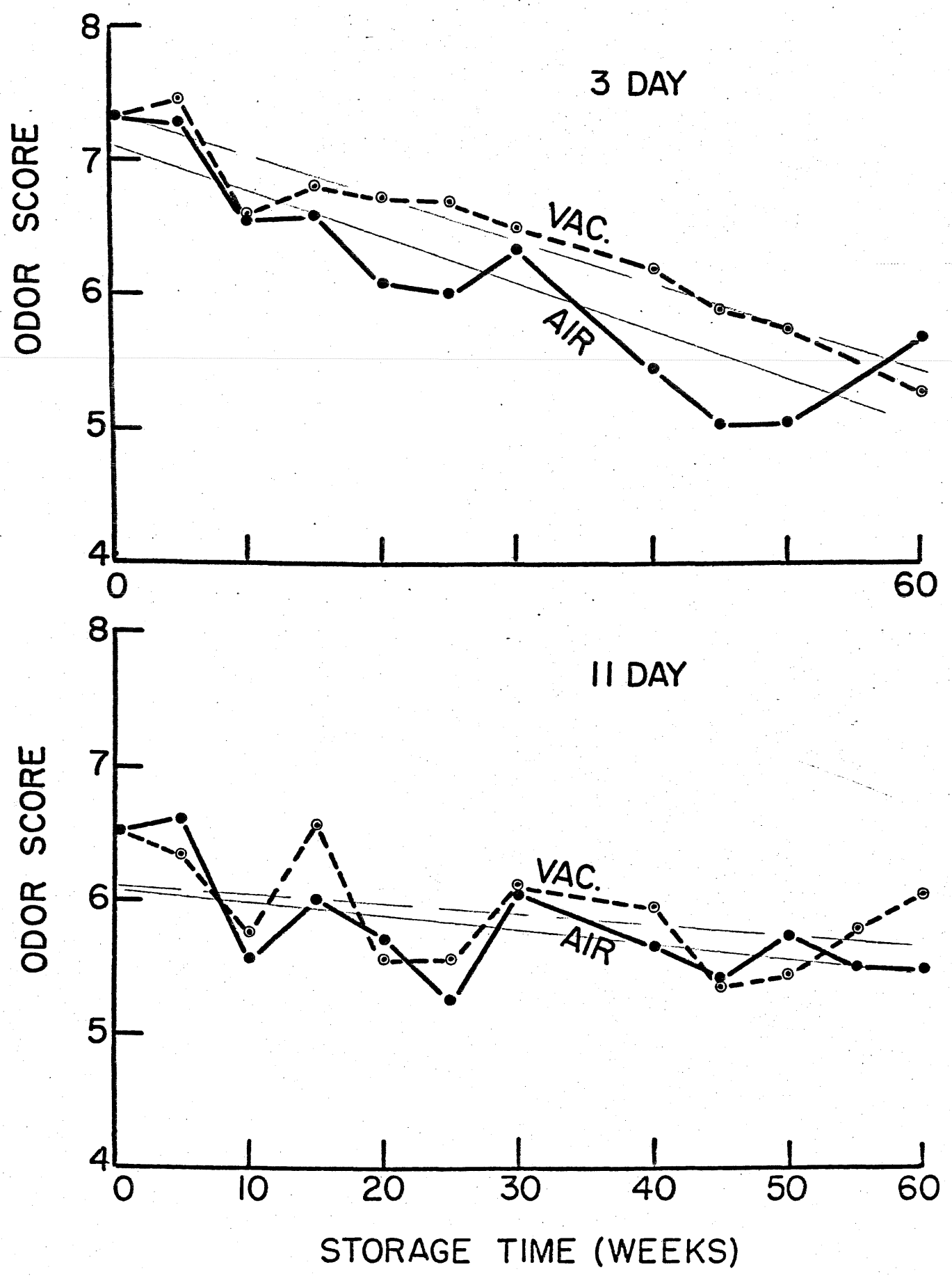


FIG. VI-3 EFFECT OF STORAGE TIME AT 0°F ON FLAVOR SCORE OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

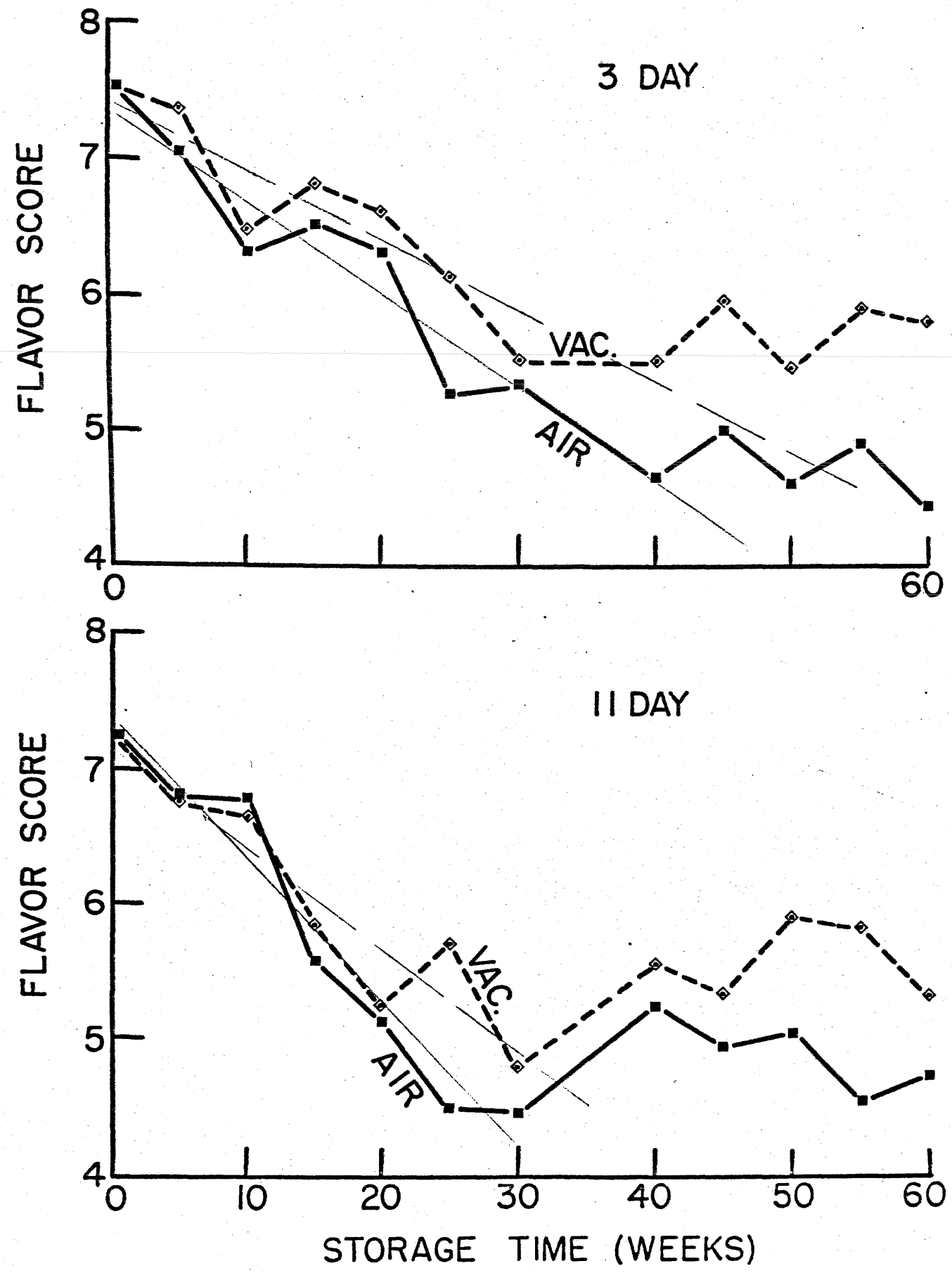


FIG. VI-4 EFFECT OF STORAGE TIME AT 0°F ON FLAVOR SCORE OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

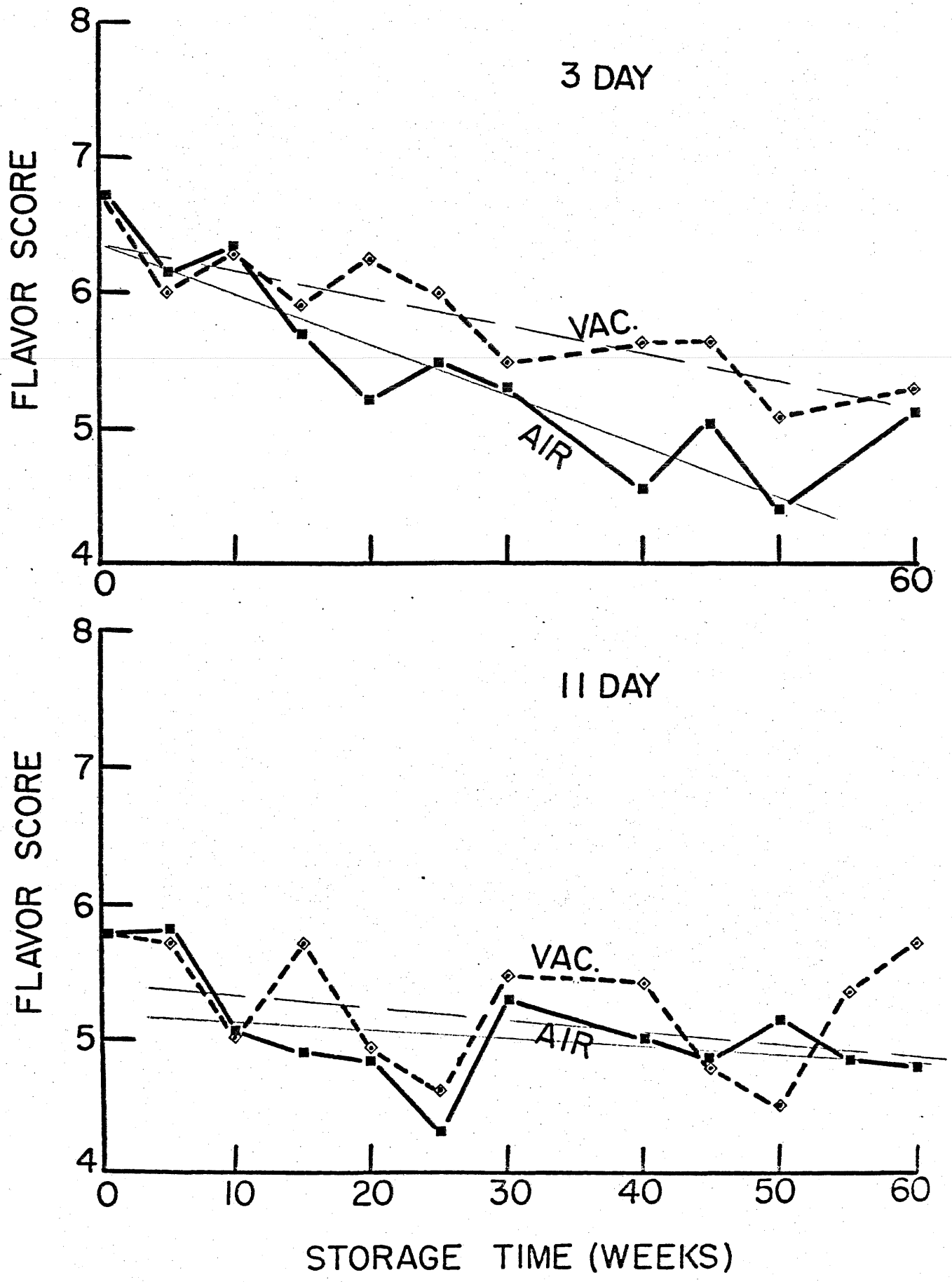


FIG. VI-5 EFFECT OF STORAGE TIME AT 0°F ON TEXTURE SCORE OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

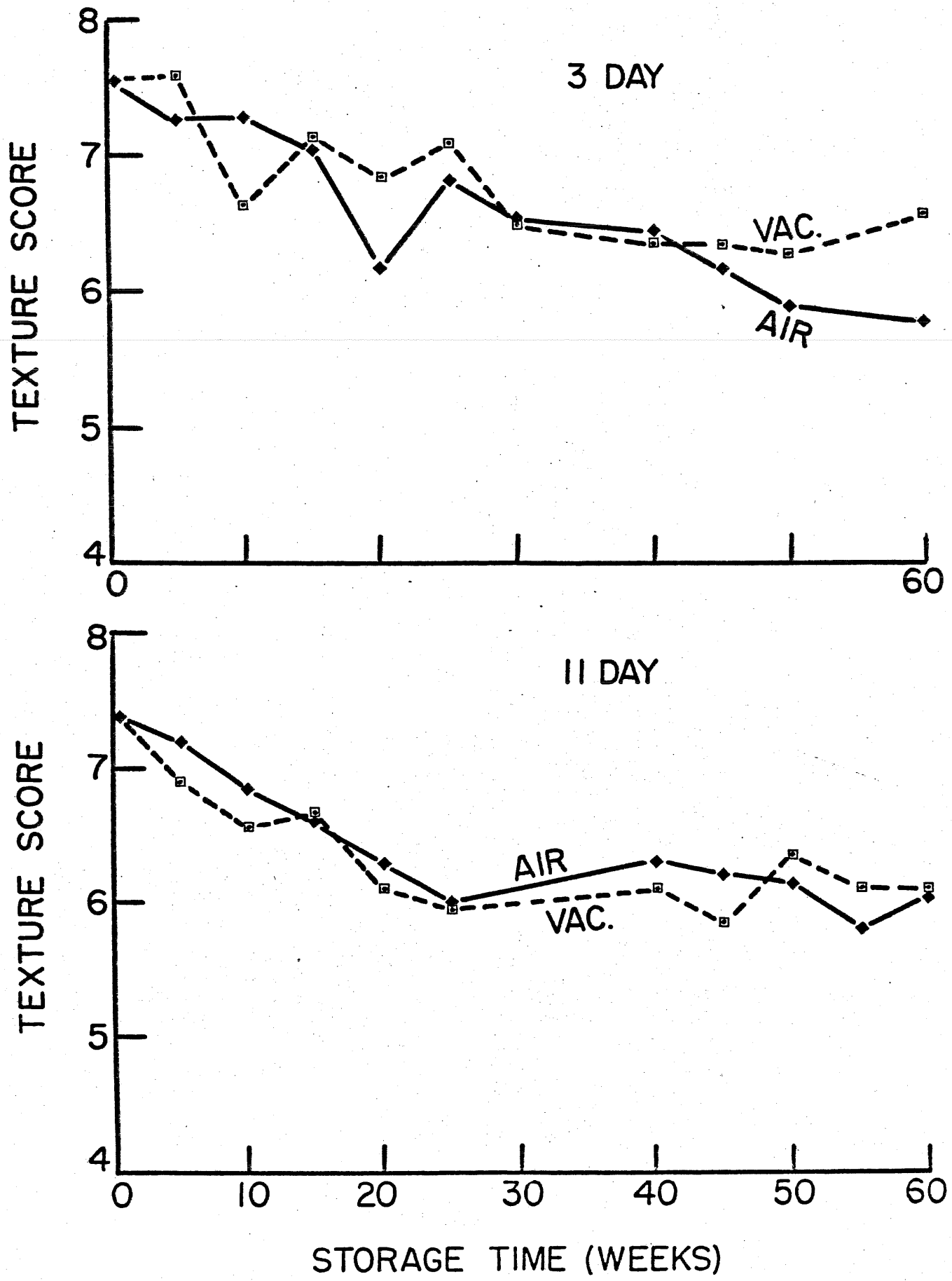


FIG. VI-6 EFFECT OF STORAGE TIME AT 0°F ON TEXTURE SCORE OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

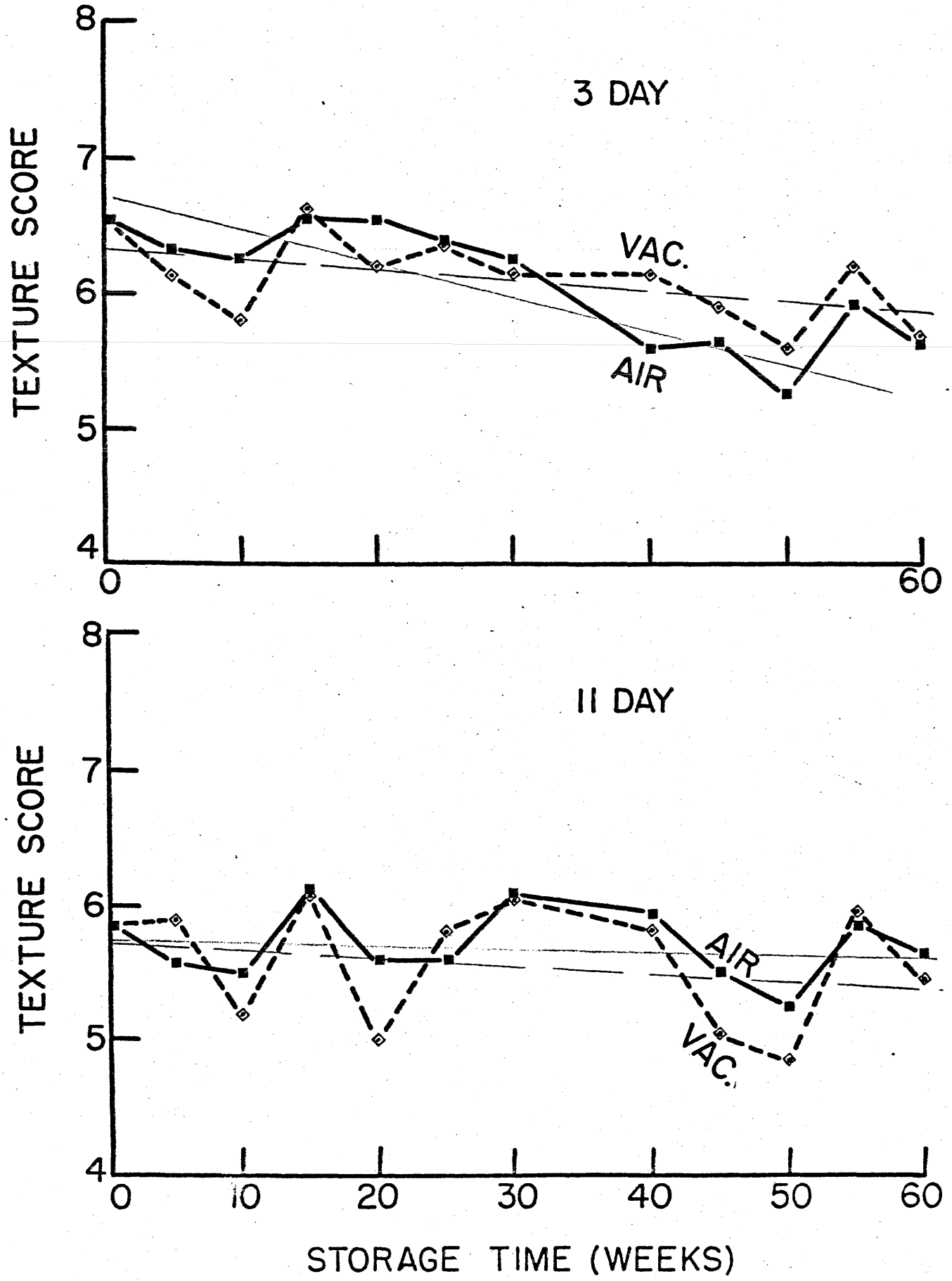


FIG. VI-7 EFFECT OF STORAGE TIME AT 0°F ON % MOISTURE OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

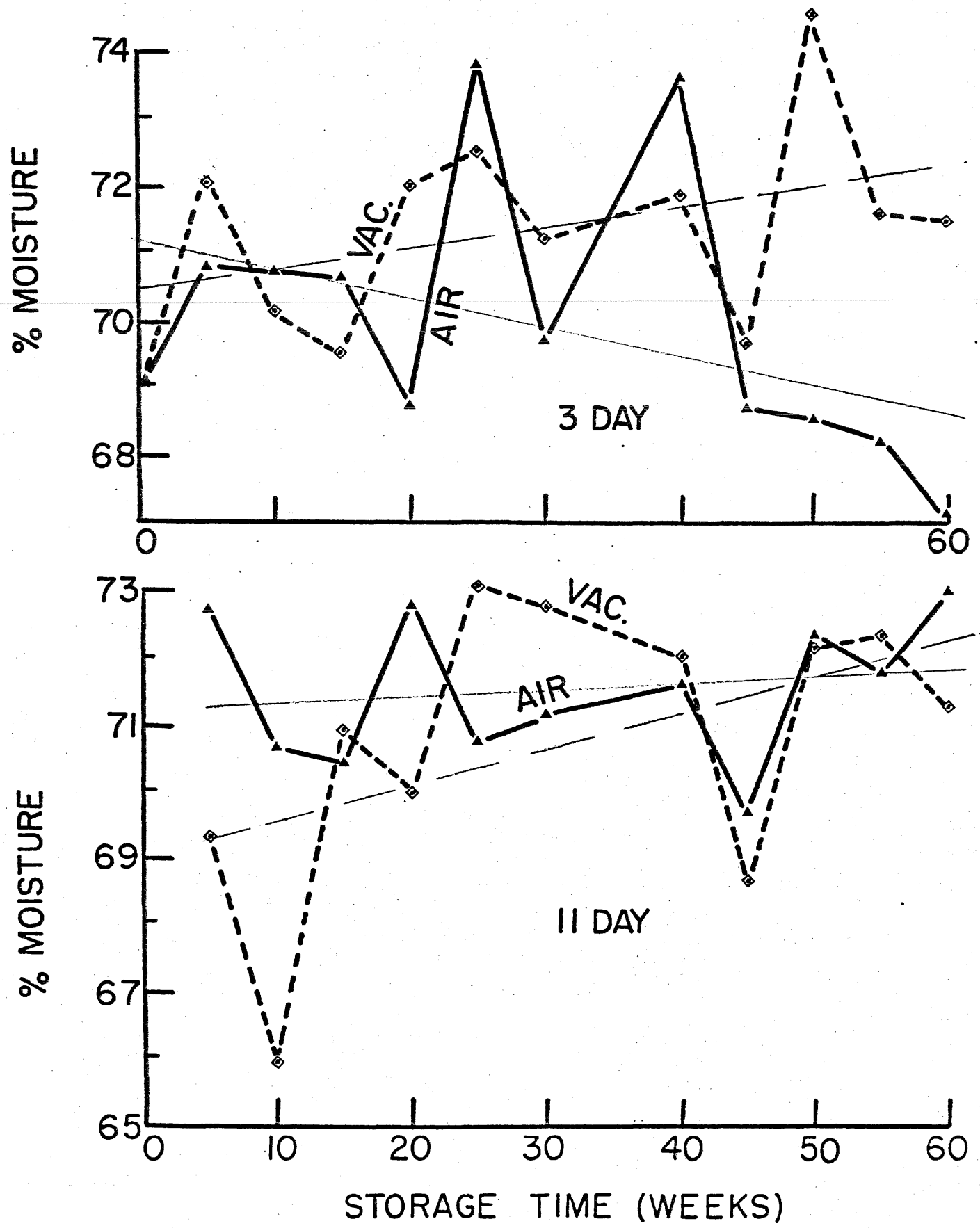


FIG. VI-8 EFFECT OF STORAGE TIME AT 0°F ON % MOISTURE OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

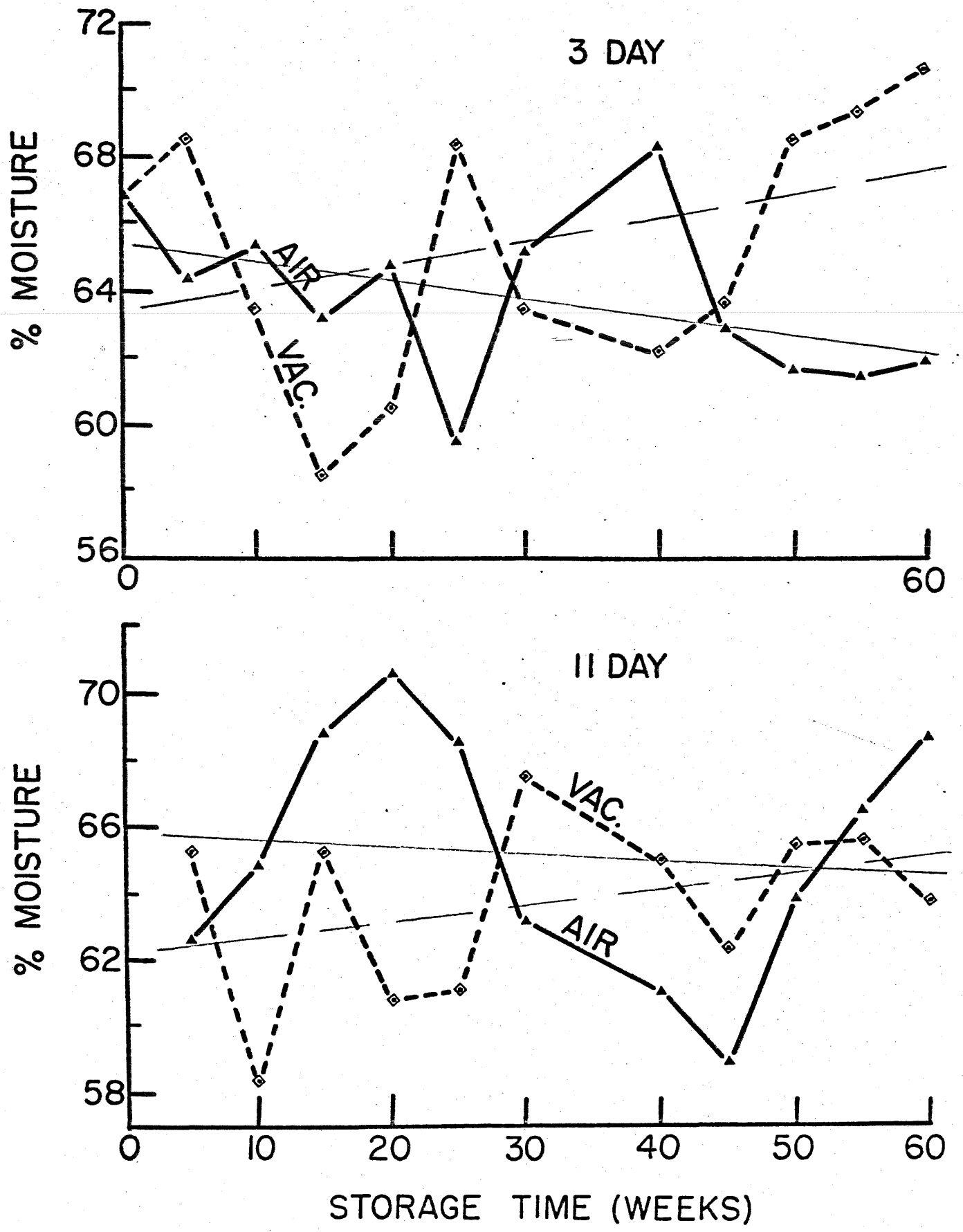


FIG. VI-9 EFFECT OF STORAGE TIME AT 0°F ON TBA NUMBER OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

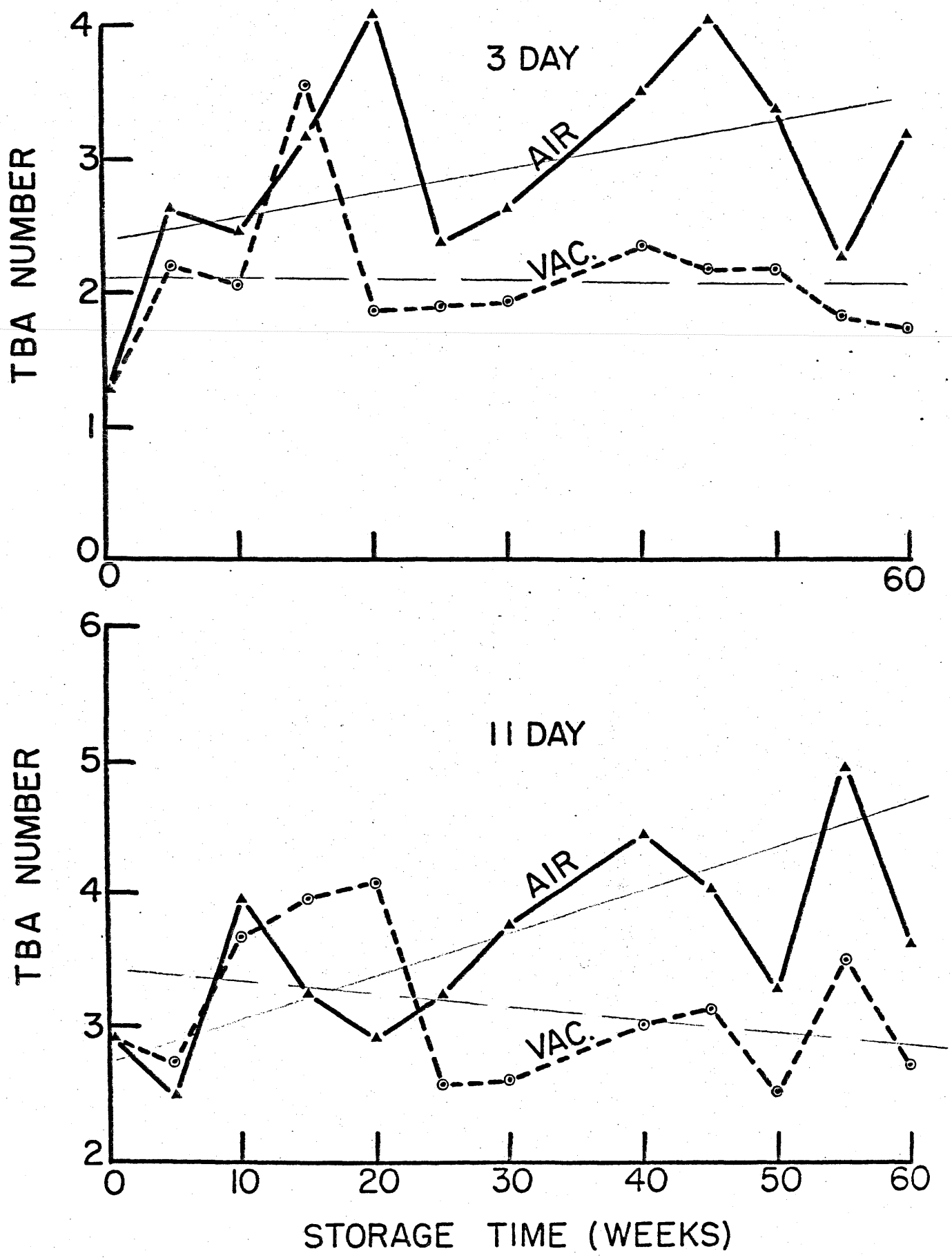


FIG. VI-10 EFFECT OF STORAGE TIME AT 0°F ON TBA NUMBER OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

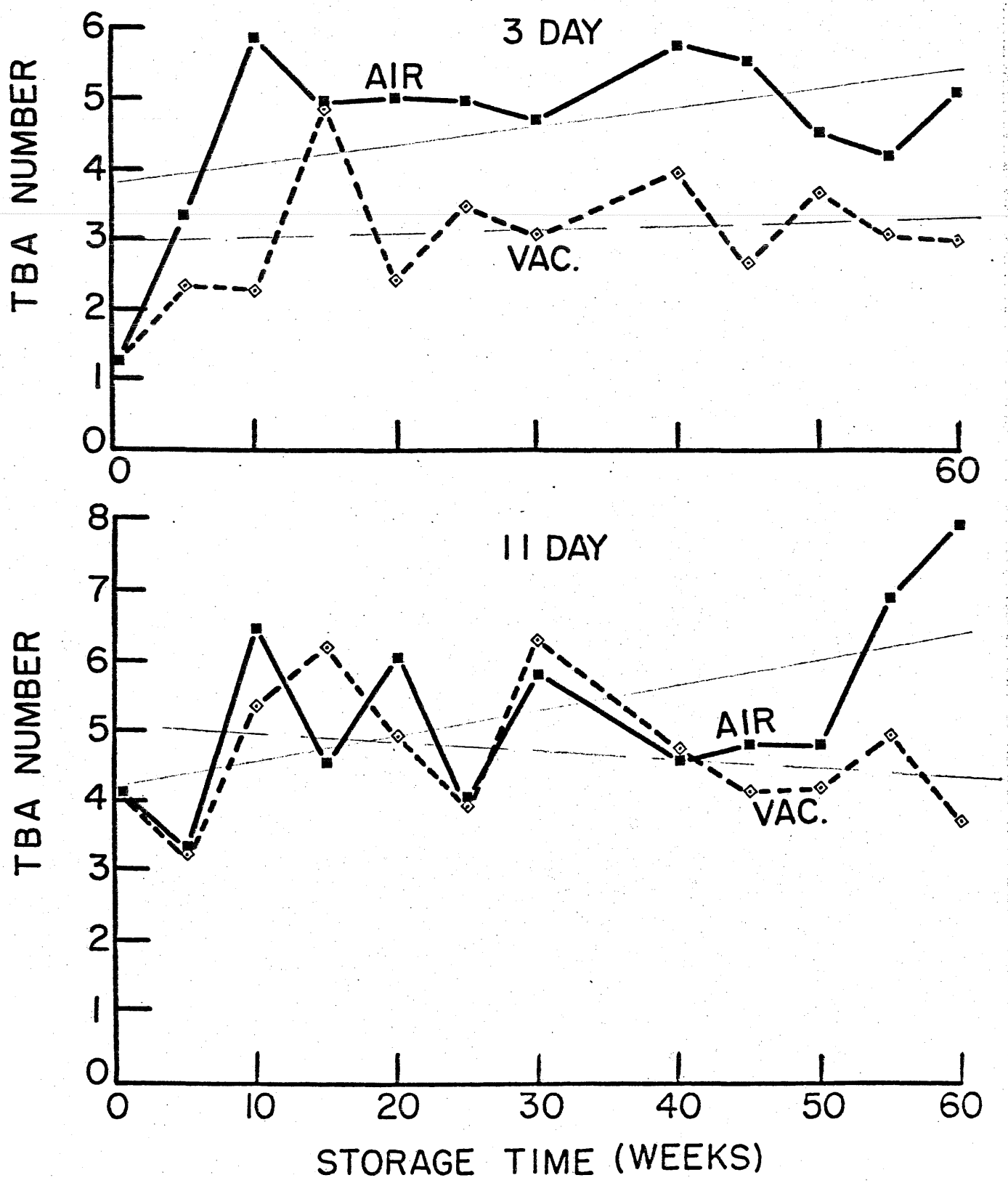


FIG. VI-11 EFFECT OF STORAGE TIME AT 0°F ON pH OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

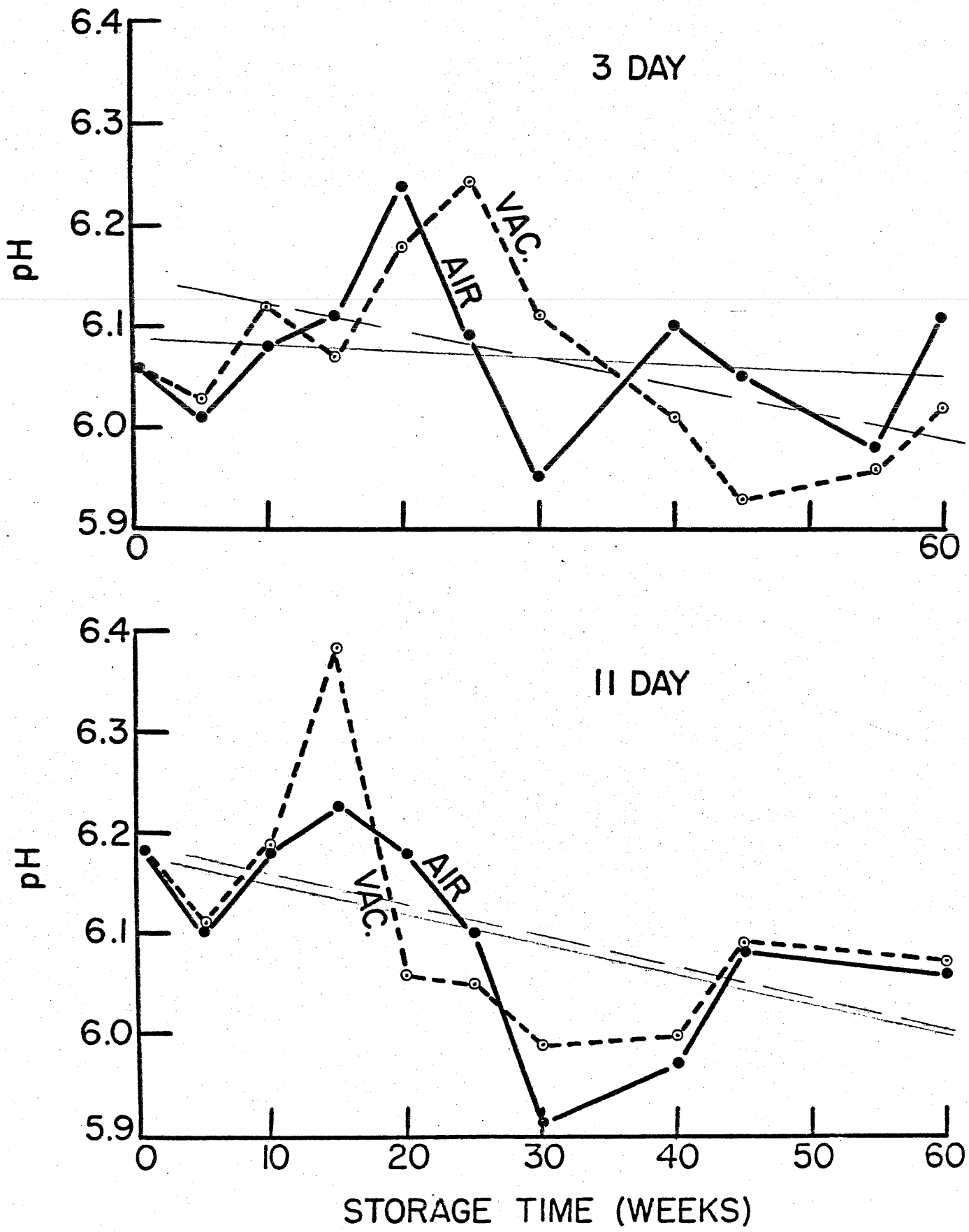


FIG. VI-12 EFFECT OF STORAGE TIME AT 0°F ON pH OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

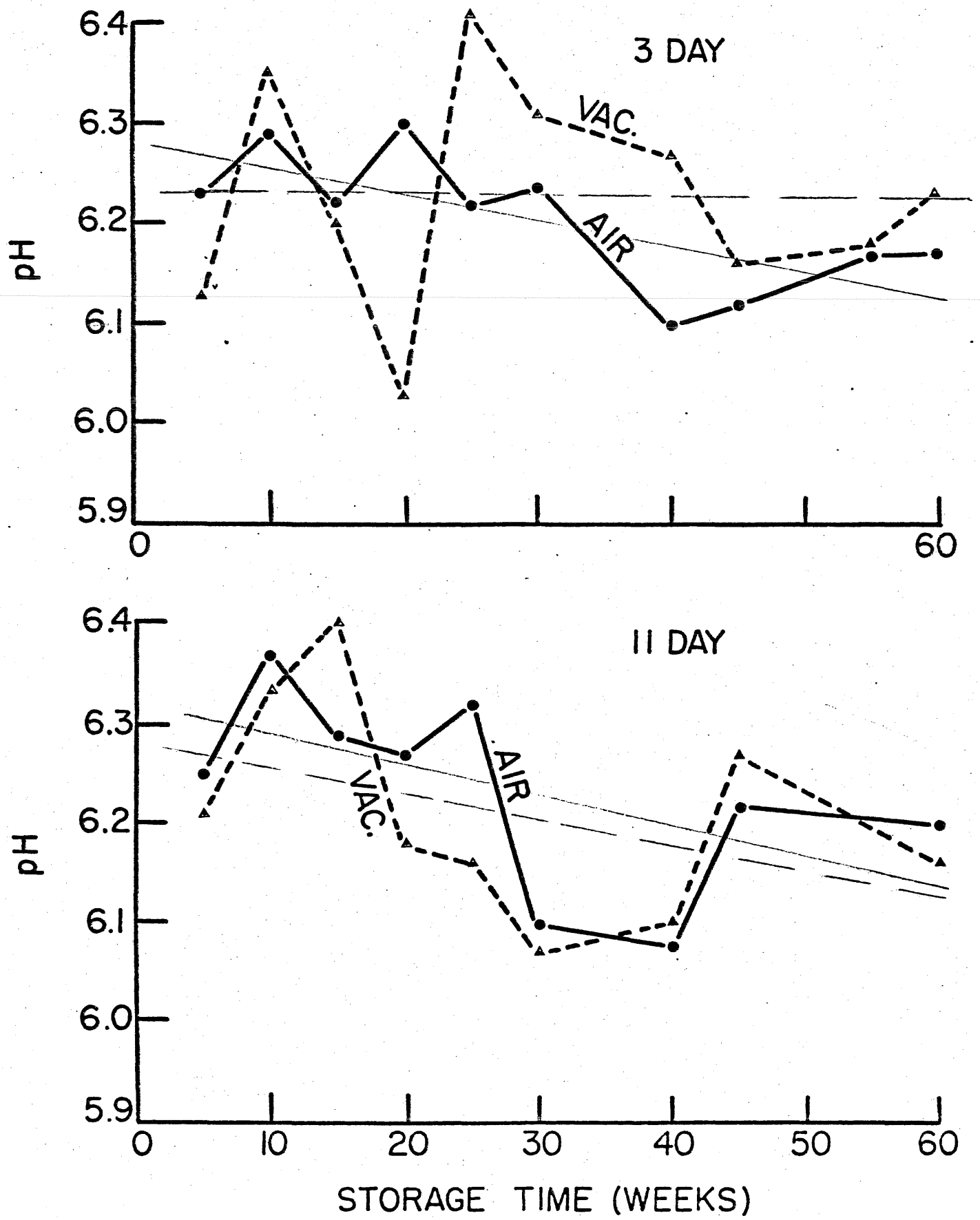


FIG. VI-13 EFFECT OF STORAGE TIME AT 0°F ON TMA CONTENT OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

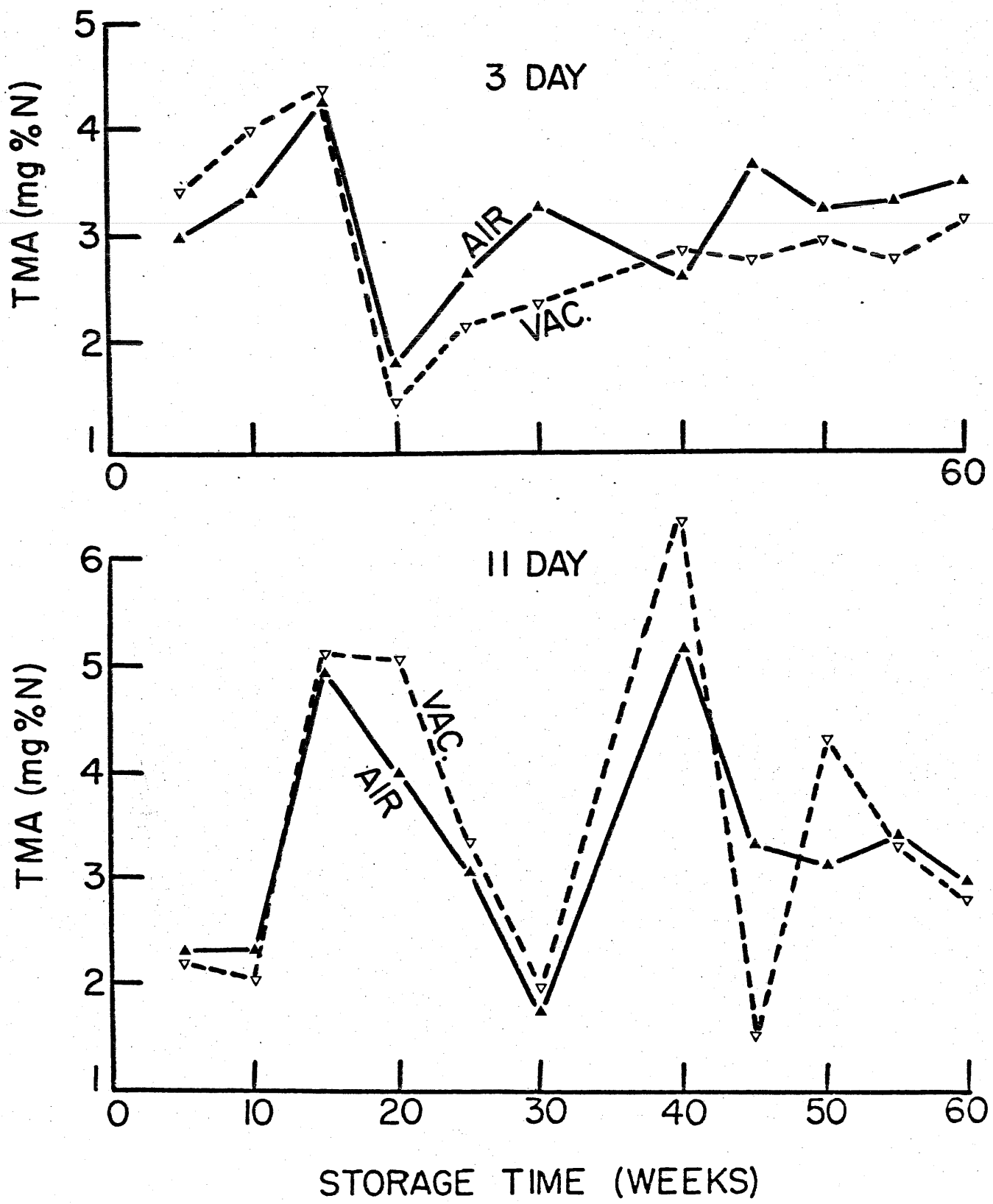


FIG. VI-14 EFFECT OF STORAGE TIME AT 0°F ON TMA CONTENT OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

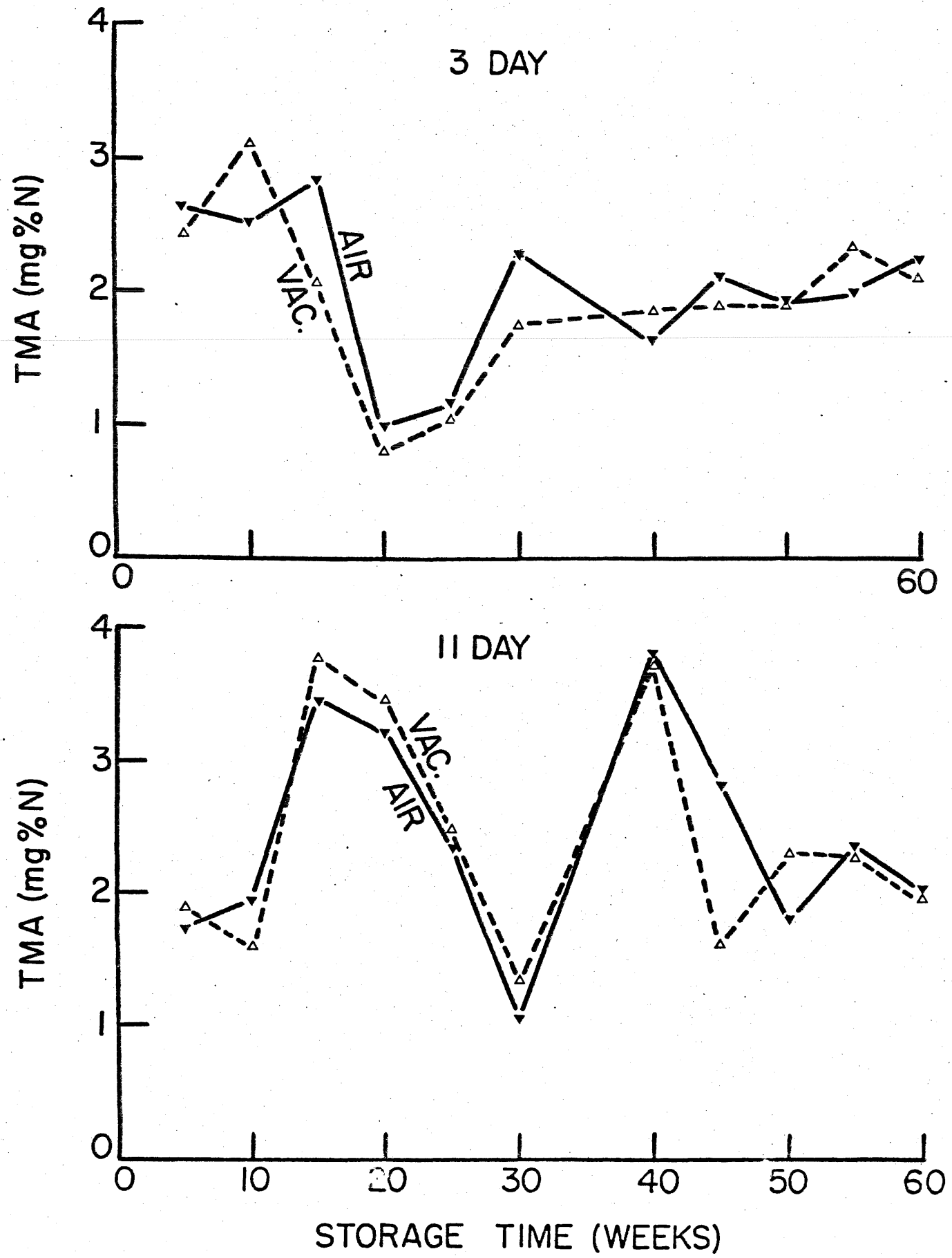


FIG. VI-15 EFFECT OF STORAGE TIME AT 0°F ON TMAO CONTENT OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

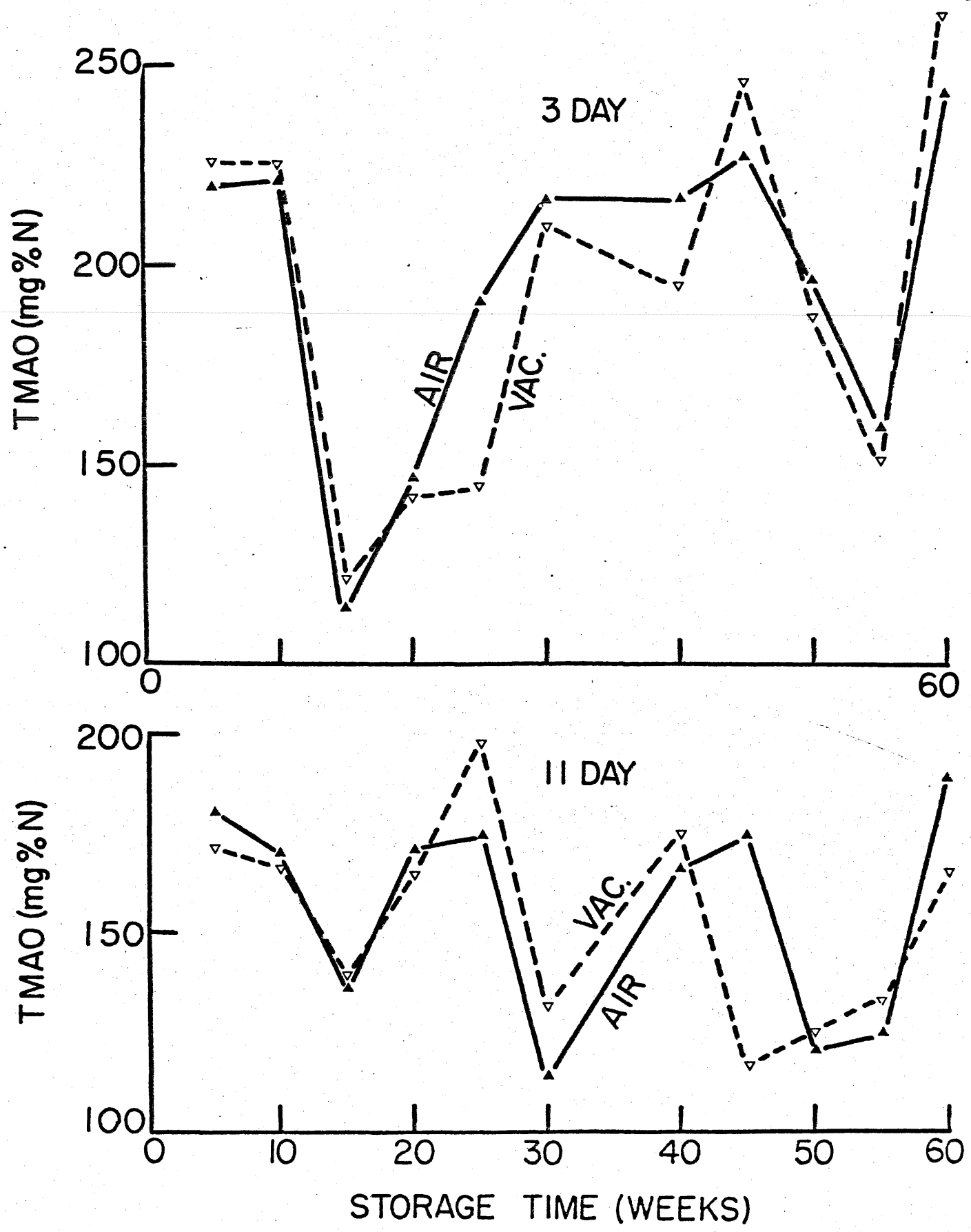


FIG. VI-16 EFFECT OF STORAGE TIME AT 0°F ON TMAO CONTENT OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

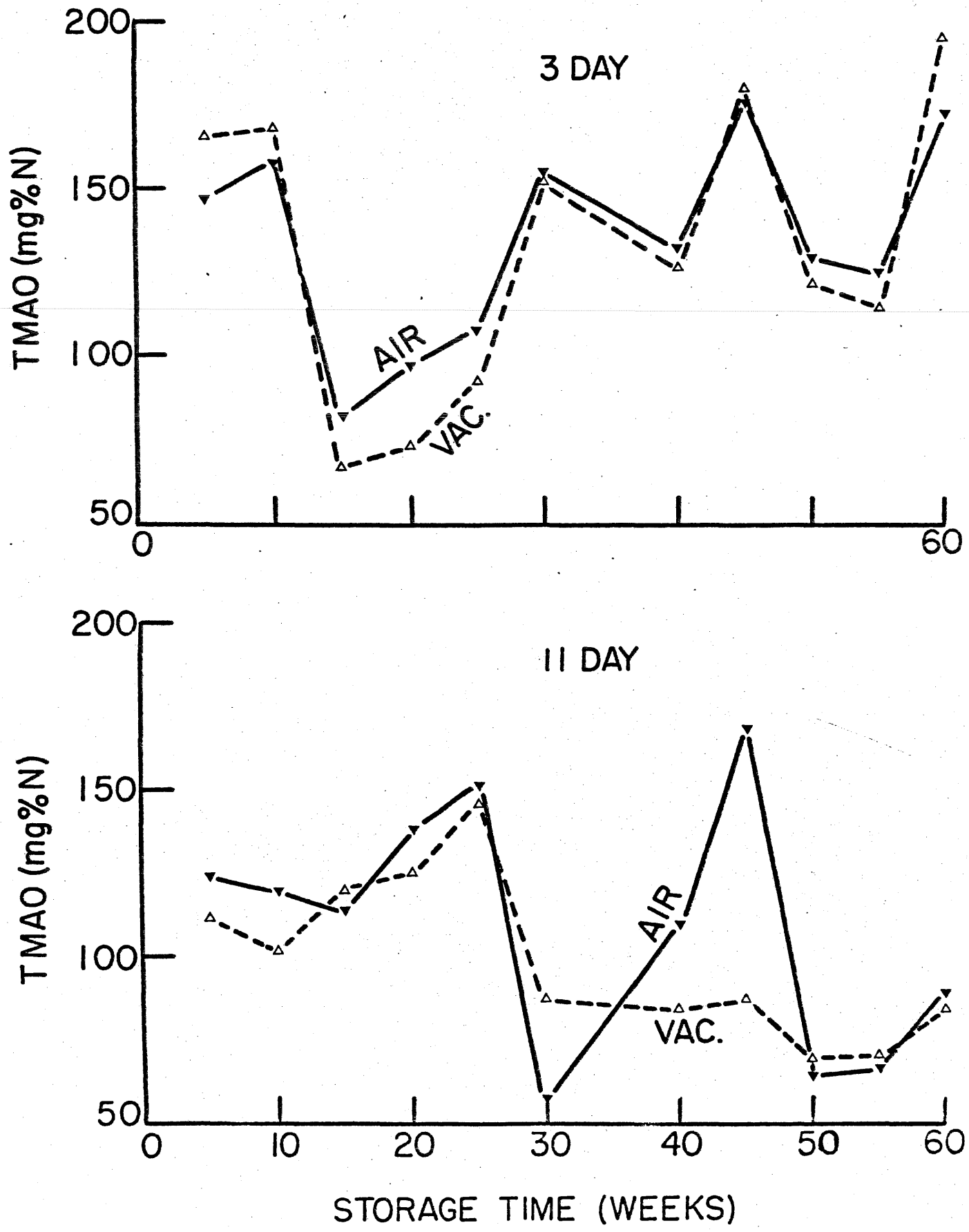


FIG. VI-17 EFFECT OF STORAGE TIME AT 0°F ON DMA CONTENT OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

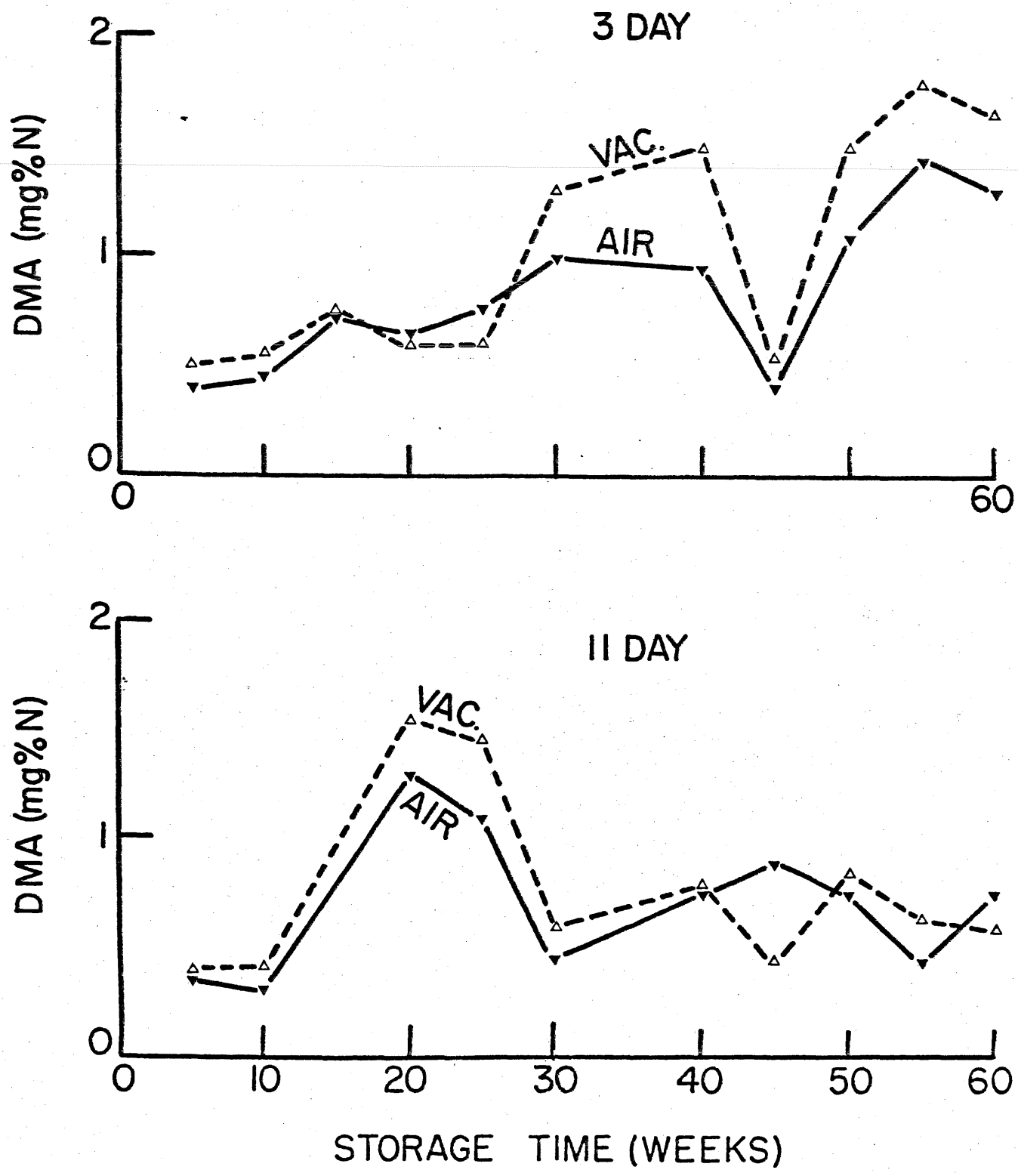


FIG. VI-18 EFFECT OF STORAGE TIME AT 0°F ON DMA CONTENT OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

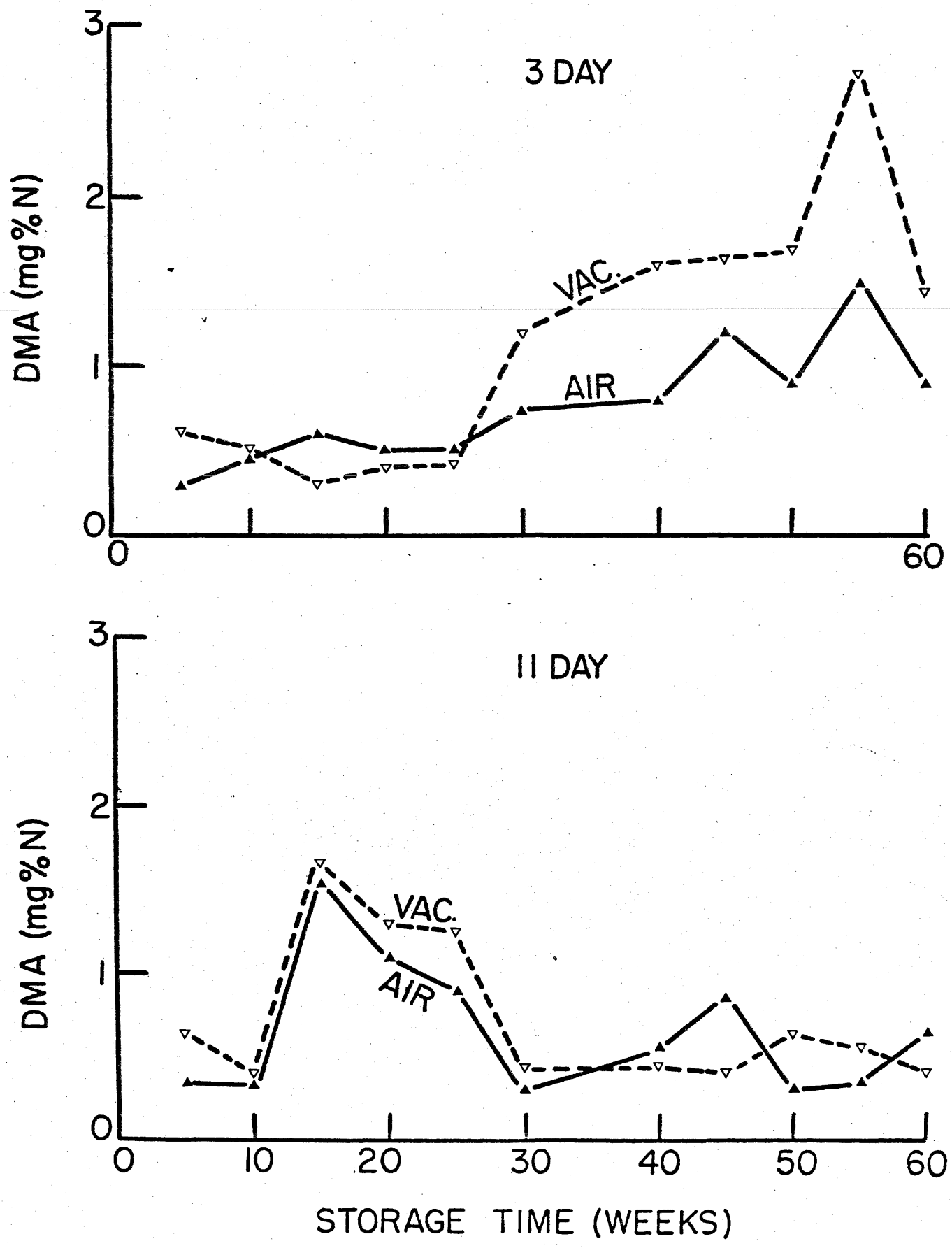


FIG. VI-20 EFFECT OF STORAGE TIME AT 0°F ON AMMONIA CONTENT OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

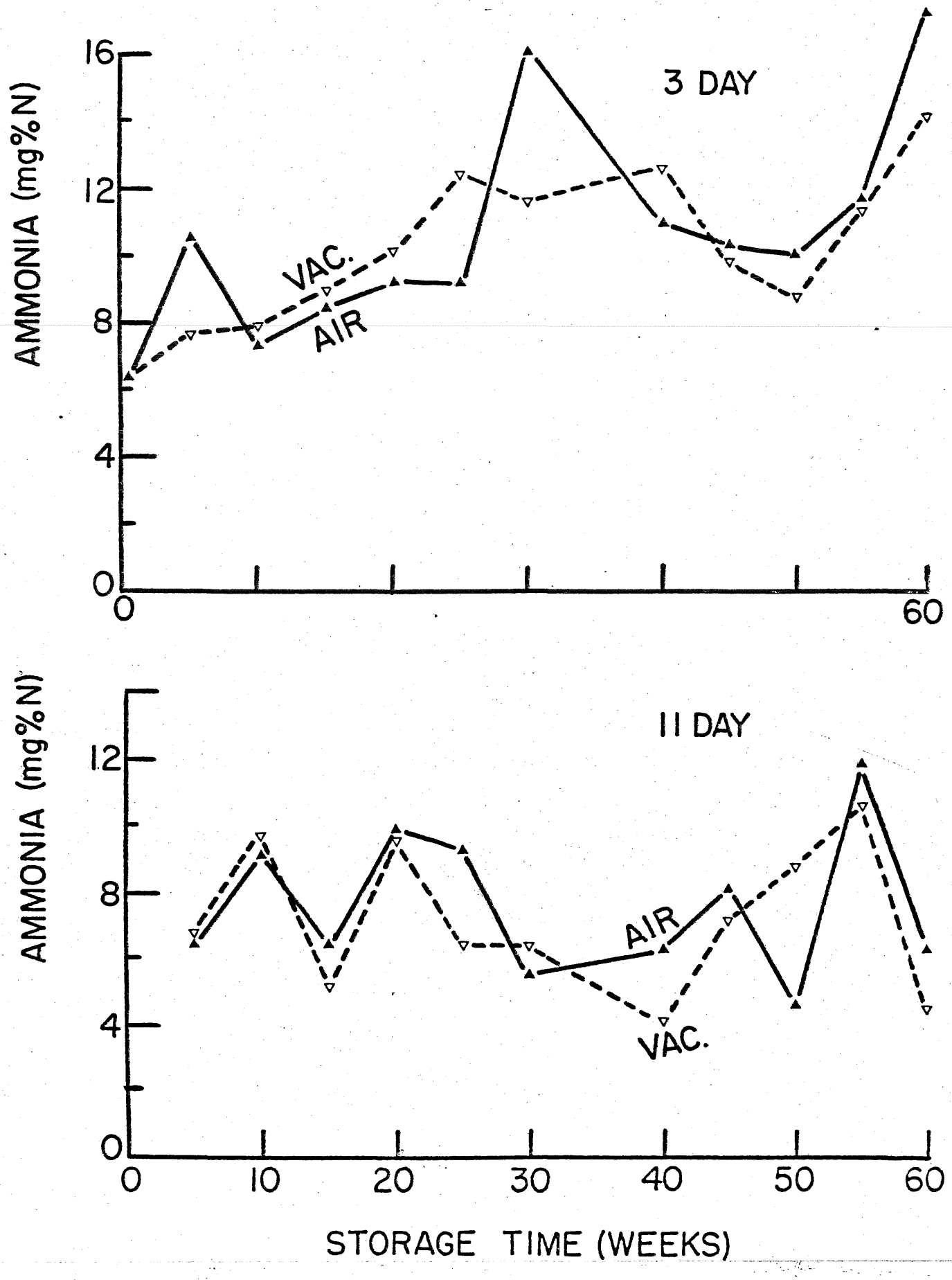


FIG. VI-19 EFFECT OF STORAGE TIME AT 0°F ON AMMONIA CONTENT OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

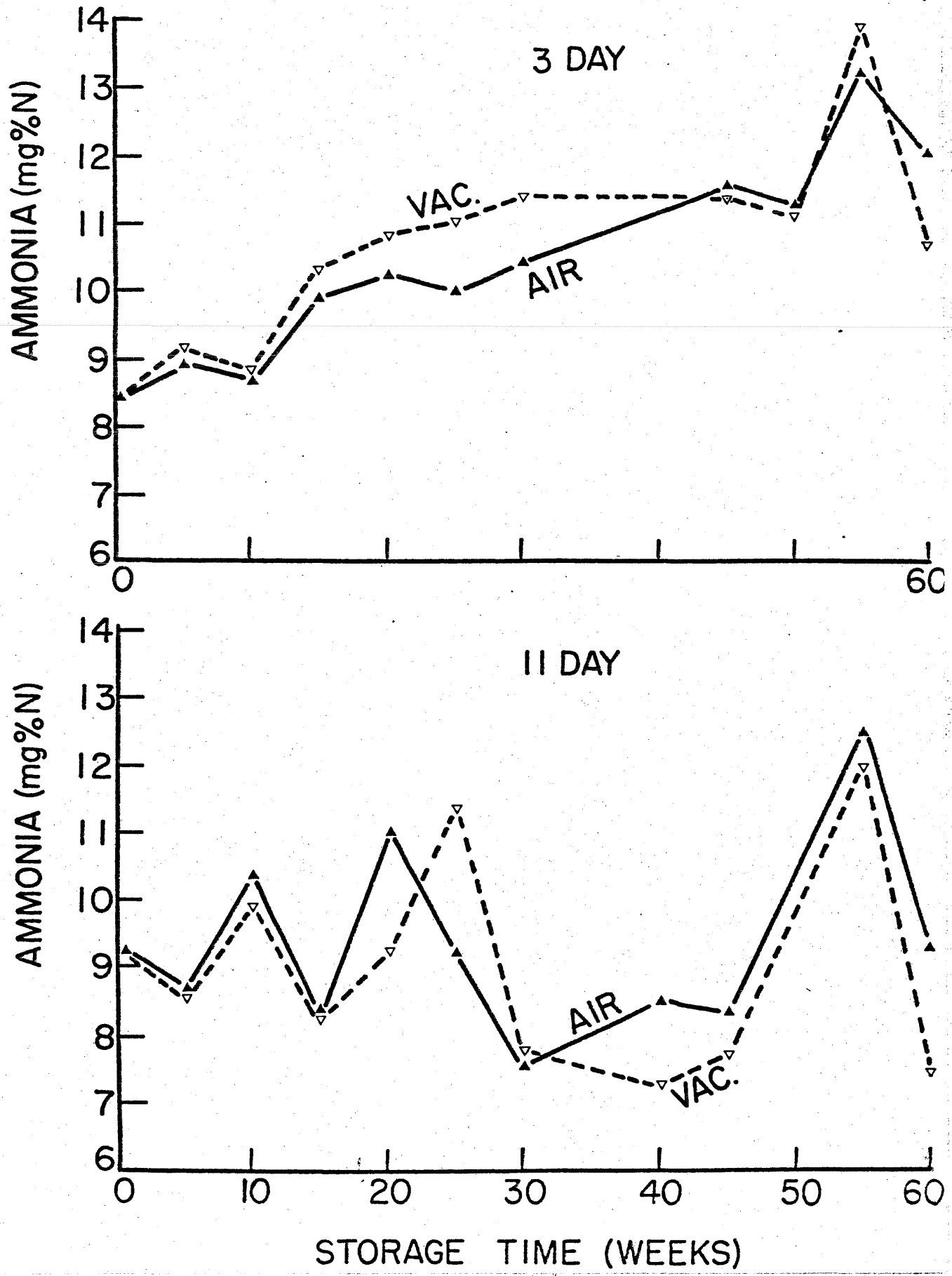


FIG. VI-21 EFFECT OF STORAGE TIME AT 0°F ON % EPN OF AIR OR VACUUM PACKED FILLETS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.

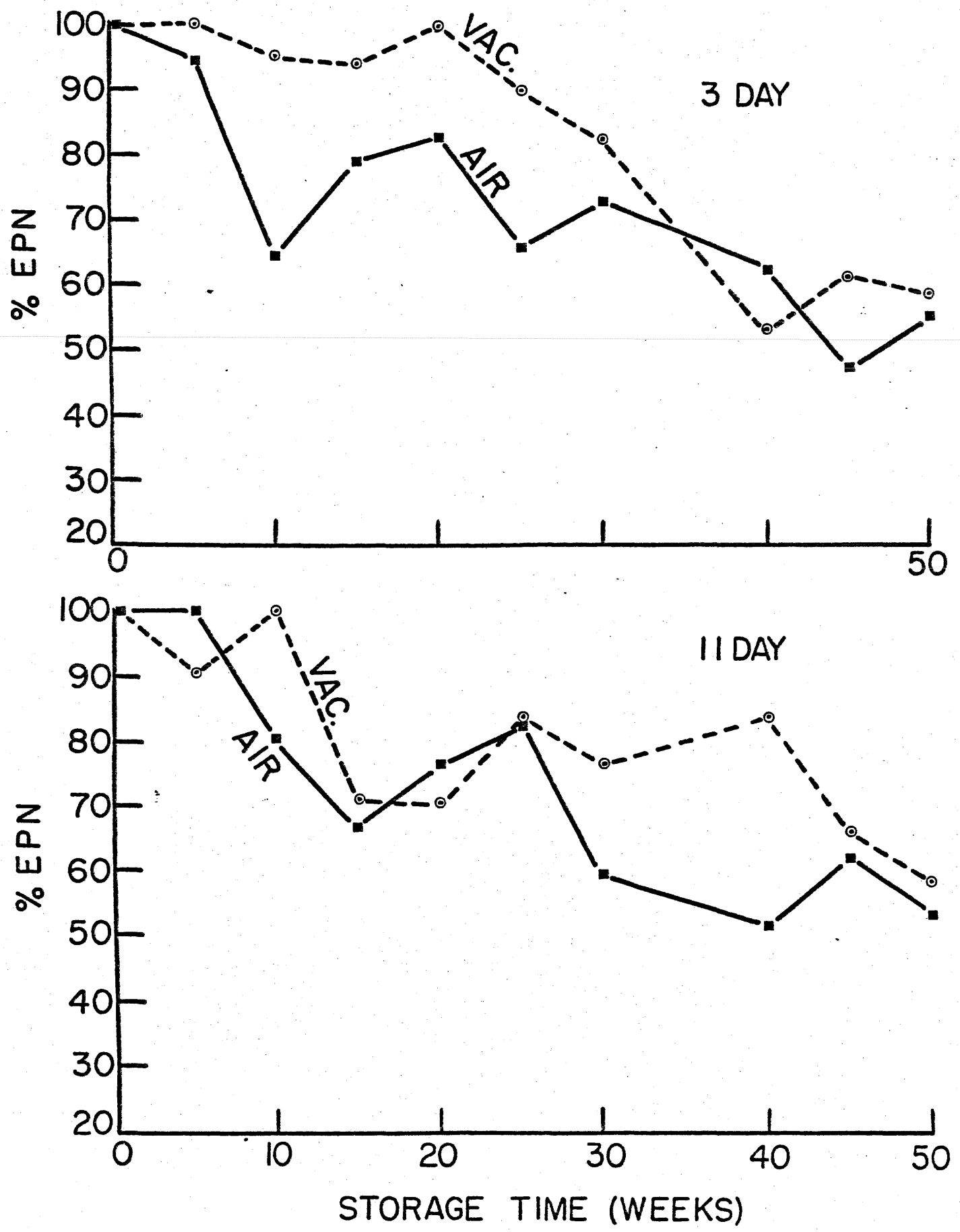
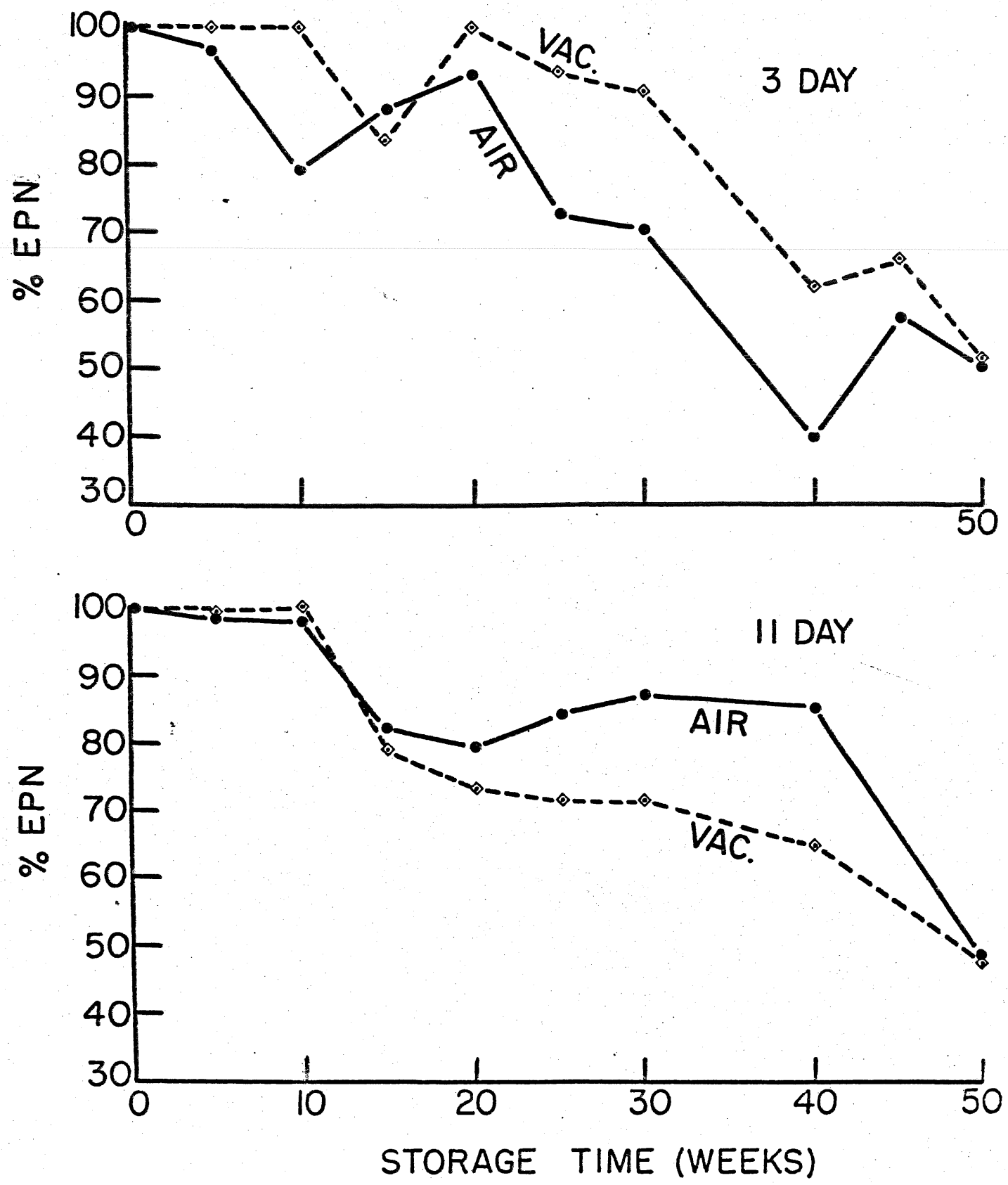


FIG. VI-22 EFFECT OF STORAGE TIME AT 0°F ON % EPN OF AIR OR VACUUM PACKED BELLY FLAPS FROM 3 OR 11 DAY POST-MORTEM ICED DOGFISH.



Frozen Storage Study VII

The purpose of this study was to determine the effect of treating dogfish fillets or belly flaps with an antioxidant (sodium erythorbate) on stabilizing the flavor during frozen storage, and also, to determine the frozen shelf life of raw breaded dogfish sticks.

One day post-mortem iced dogfish obtained from a local processor (10-14-81) were filleted with skin removed. Belly flaps were separated from the fillets. One fillet from each fish was reserved for control samples, the others for treatment with sodium erythorbate. This consisted of a 45 second dip in a 3 percent erythorbate solution followed by a one minute drain. Belly flaps were divided into two groups and similarly treated. Fillets were packaged in 5 pound cartons; the belly flaps in one pound cartons. All cartons were then sealed in 2 mil polyethylene bags and stored at 0 ^{o}F.

One 16.5 pound fillet block was prepared by freezing under pressure in a plate freezer. This block was cut into fish sticks which were batter-breaded with commercial product, packaged in one pound cartons and sealed within polyethylene bags.

At 5 week intervals samples were examined for sensory evaluation and chemical testing. For the taste tests the breaded sticks were cooked in oil. The stored blocks were cut into sticks, batter-breaded and cooked in oil.

Results

During the first 10 weeks of storage there was no flavor difference between control or erythorbate (ERY) treated fillets, however, beyond that time flavor score decreased more rapidly in the control samples (Fig. VII-1).

Erythorbate treated belly flaps were also generally scored slightly higher in flavor throughout storage in comparison with the untreated samples. Flavor deterioration due to the development of oxidative rancidity proceeded at a relatively rapid rate in the breaded sticks. Oxidative rancidity occurs mainly on the surface of fish flesh to a depth of a few millimeters and the high surface to volume ratio of a fish stick would favor its development. The batter-breading coating apparently is an ineffective oxygen barrier. Commercial shelf life at 0°F as determined from linear regression analysis was estimated as follows for the different treatments:

untreated fillet	24 weeks
erythorbate treated fillet	37 "
untreated belly flap	23 "
erythorbate treated belly flap	33 "
breaded sticks	6 "

Other investigators have reported on the efficacy of ascorbic acid, an isomer of erythorbic acid, as an antioxidant for stabilizing the flavor of dogfish during frozen storage. Boyd et al (1967) found that treatment of dogfish portions with one percent ascorbic acid retarded the development of oxidative rancidity. Jhaveri and Constantinides (1981) concluded that dogfish fillets which had been dipped for 90 seconds in a solution of 0.5 percent ascorbic acid and 8 percent tripolyphosphate were less rancid than untreated fillets during storage at 5°F. Although a water glaze has been shown to protect dogfish carcasses against oxidative rancidity during frozen storage, Bilinski (1981) observed that glazing with one percent sodium erythorbate or 2.5 percent Tenox was more effective. These results

and the results of the present study demonstrate that ascorbic acid or sodium erythorbate can retard development of oxidative rancidity in frozen dogfish to an extent, however, for maximum effectiveness one would have to experiment further to determine the optimum parameters of time and antioxidant concentration for the dipping treatment.

In comparison to flavor deterioration, texture remained relatively stable during frozen storage and did not appear to be affected by the erythorbate dip (Fig. VII - 2). The results of this frozen study confirm the findings of the previous study which indicated that flavor change was the major problem with frozen storage of dogfish.

Moisture content did not change during storage for any of the treated or control samples, however, as in previous studies considerable variation was noted within a given treatment. The moisture content of the erythorbate treated samples was not significantly changed as a result of the dip. The average moisture contents (and standard deviation) are presented in Table VII - 1.

For all practical purposes, pH of the different products remained unchanged during storage (Fig. VII - 3). The mean pH values and standard deviation are given in Table VII - 1.

There was no difference in pH between treated and untreated fillets, but the treated belly flaps were on the average one-tenth of a pH unit higher compared to untreated flaps. The belly flaps have a higher surface to volume ratio compared to the fillets which are thicker and therefore, would absorb a higher amount of the erythorbate solution per unit weight. Since the erythorbate solution is alkaline, the pH of the belly flaps

would tend to increase. The fillet on the other hand with a smaller surface to volume ratio might more effectively buffer the alkaline erythorbate solution so that a pH change would not occur in the treated fillet.

That erythorbate treatment did help to suppress development of oxidative rancidity during frozen storage can be seen in Fig. VII - 4. TBA numbers of untreated fillets or belly flaps were higher compared to treated samples during storage. The rapid increase in TBA number of the breaded sticks during early storage reflects the rapid flavor deterioration that occurred with this product. There was good correlation ($r = 0.74$) between flavor score and TBA number. The TBA number corresponding to end of shelf life was estimated from the regression line to be 4.

DMA content began to increase steadily after 10 to 15 weeks storage and the rate of increase was more rapid in erythorbate treated compared to control samples (Fig. VII - 5). It is believed that the erythorbate enhances the activity of the TMAO-ase enzyme either by lowering the oxygen tension or acting as an electron donor. From the very small quantities of DMA produced in any of the treatments, it is not considered that the activity of the TMAO degrading enzyme (if it was responsible for the DMA formation) was of any significance in affecting textural change. Correlation between texture score and DMA-N content was low ($r = 0.32$). Thus, DMA content would not be a reliable indicator for assessing textural quality in these products.

Ammonia content fluctuated during storage, but on the whole it was considered that no change occurred (Fig. VII - 6). The overall averages

and standard deviation are given in Table VII-1. For all practical purposes the ammonia content was the same for all treatments.

There was a progressive loss of extractable protein nitrogen during storage and the decrease occurred at a more rapid rate in either the erythorbate treated samples compared to controls, or in the breaded sticks compared to raw blocks (Fig. VII - 7). Except for the treated belly flaps, sensory textural unacceptability coincided with an EPN value in the range of 50 to 60 percent. In the previous frozen study this same range of percent EPN was also found to exist in samples on the threshold of textural unacceptability. In this study there was a low correlation ($r = 0.17$) between sensory texture score and EPN. Thus, although some protein denaturation may be taking place in dogfish during frozen storage, the high fat content apparently masks or overrides this protein damage thus exerting a protective effect.

In summary, treatment of dogfish fillets or belly flaps in 3 percent erythorbate solution for 45 seconds was found from sensory tests and TBA number to suppress the development of oxidative rancidity during storage at $0^{o}F$. It is possible that a greater protective effect than was observed in this study might be attained by increasing either the dip time or the concentration of erythorbate. Raw breaded dogfish sticks were highly susceptible to becoming rancid during frozen storage and would probably benefit from vacuum packaging in an oxygen impermeable film or prepared from erythorbate-treated fillet blocks. Although the erythorbate treatment seemed to enhance DMA formation and also caused some protein denaturation as evidenced by extractable protein nitrogen content, there was no apparent difference in sensory texture between treated and untreated samples.

Acknowledgment

The initial funding of this investigation by the New England Fisheries Development Program is greatly appreciated.

Table VII - 1. Average value and standard deviation for content of various parameters in frozen dogfish

Treatment	Parameter		
	Percent moisture	pH	Ammonia -N (mg %)
Untreated fillet	70.5 ± 1.40	5.95 ± 0.20	8.5 ± 1.4
Erythorbate treated fillet	70.7 ± 1.22	5.92 ± 0.18	8.2 ± 0.72
Untreated belly flap	64.8 ± 1.97	6.04 ± 0.06	8.9 ± 1.1
Erythorbate treated belly flap	65.1 ± 1.84	6.15 ± 0.13	8.2 ± 0.55
Raw breaded sticks	69.3 ± 2.17	5.85 ± 0.19	8.1 ± 1.1

FIG. VII-1 EFFECT OF STORAGE TIME AT 0°F ON FLAVOR SCORE OF DOGFISH FILLETS OR BELLY FLAPS TREATED IN VARIOUS WAYS.

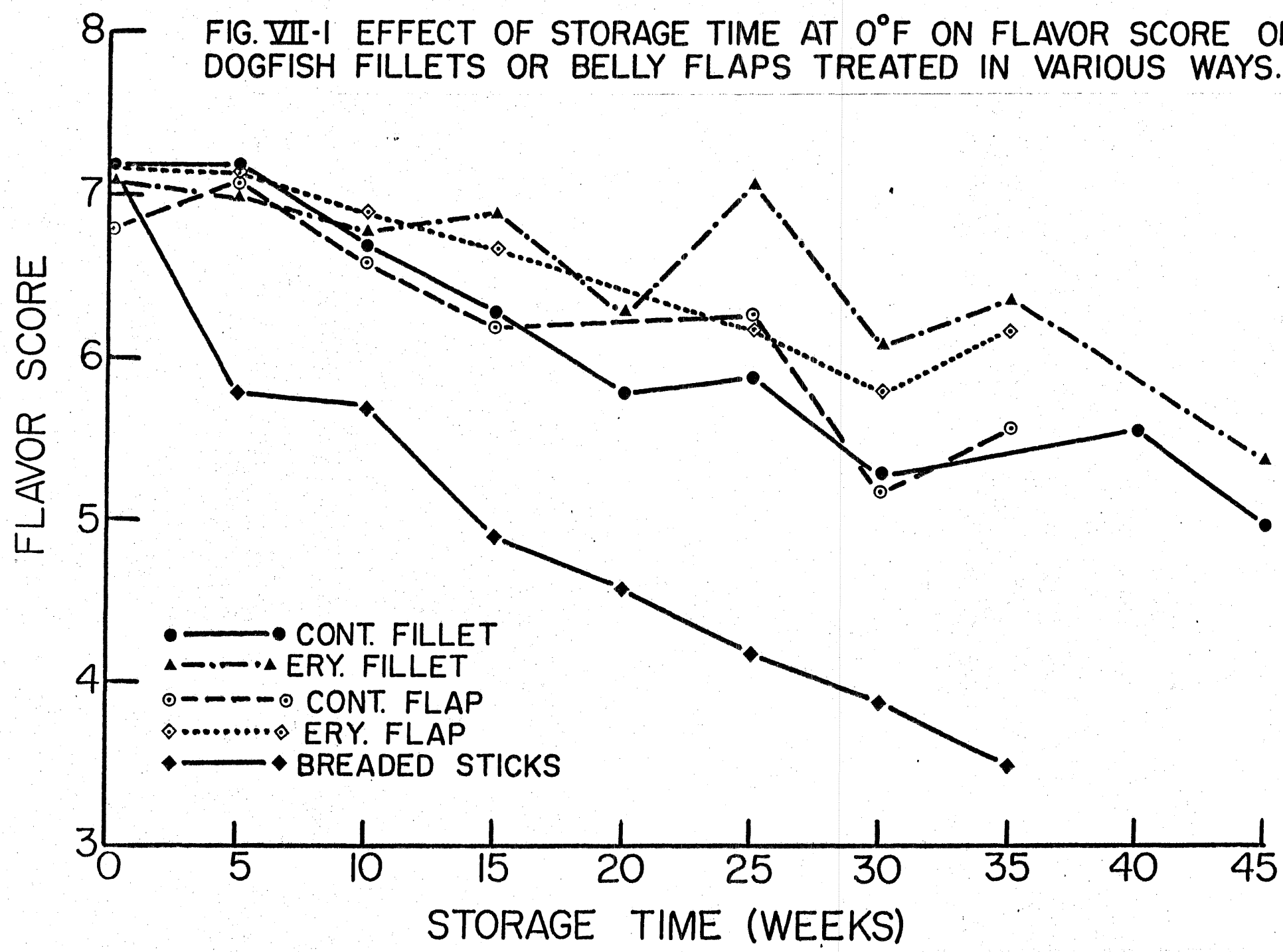


FIG. VII-2 EFFECT OF STORAGE TIME AT 0°F ON TEXTURE SCORE OF DOGFISH FILLETS OR BELLY FLAPS TREATED IN VARIOUS WAYS.

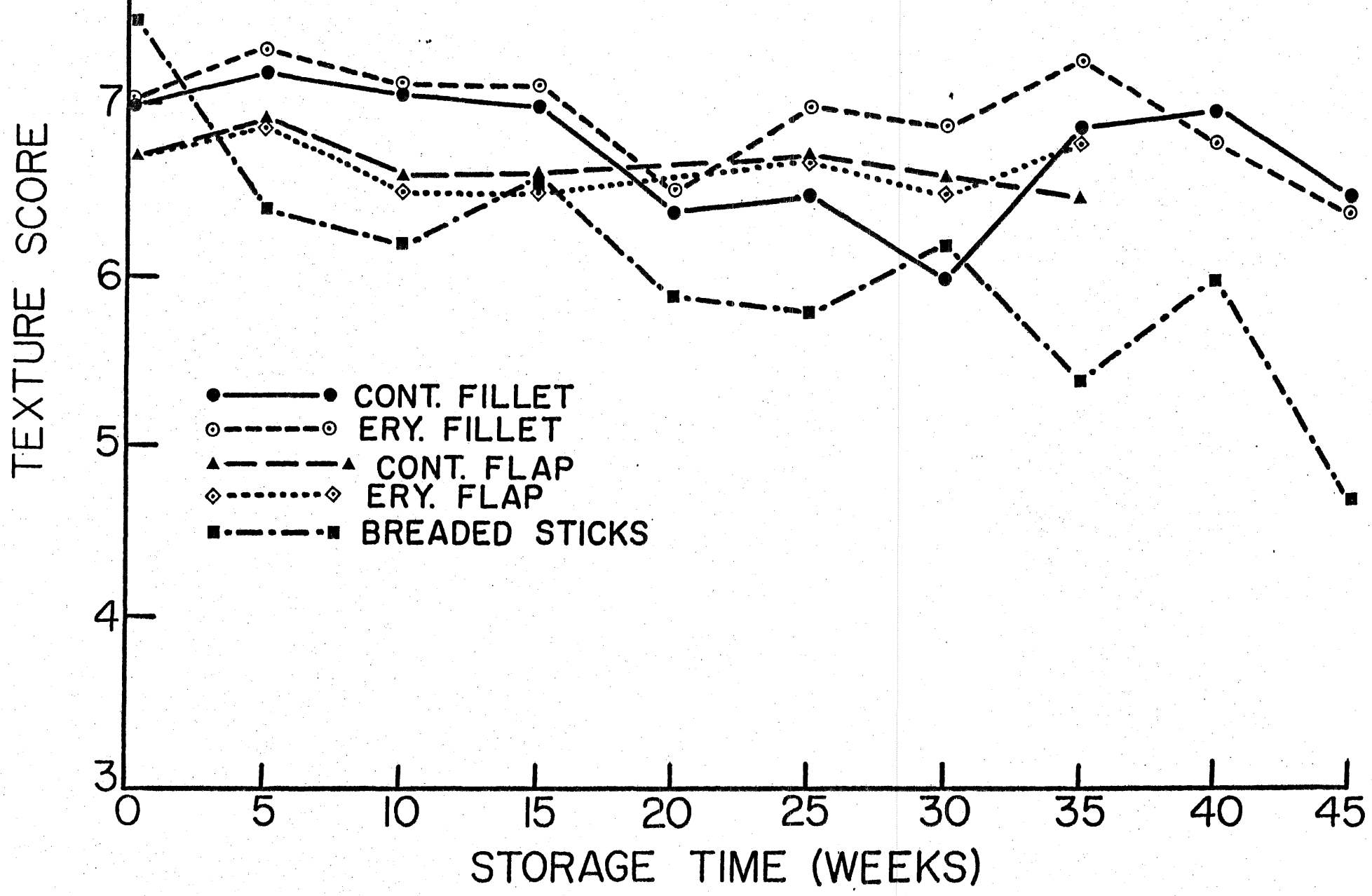


FIG. VII-3 EFFECT OF STORAGE TIME AT 0°F ON pH OF DOGFISH FILLETS OR BELLY FLAPS TREATED IN VARIOUS WAYS.

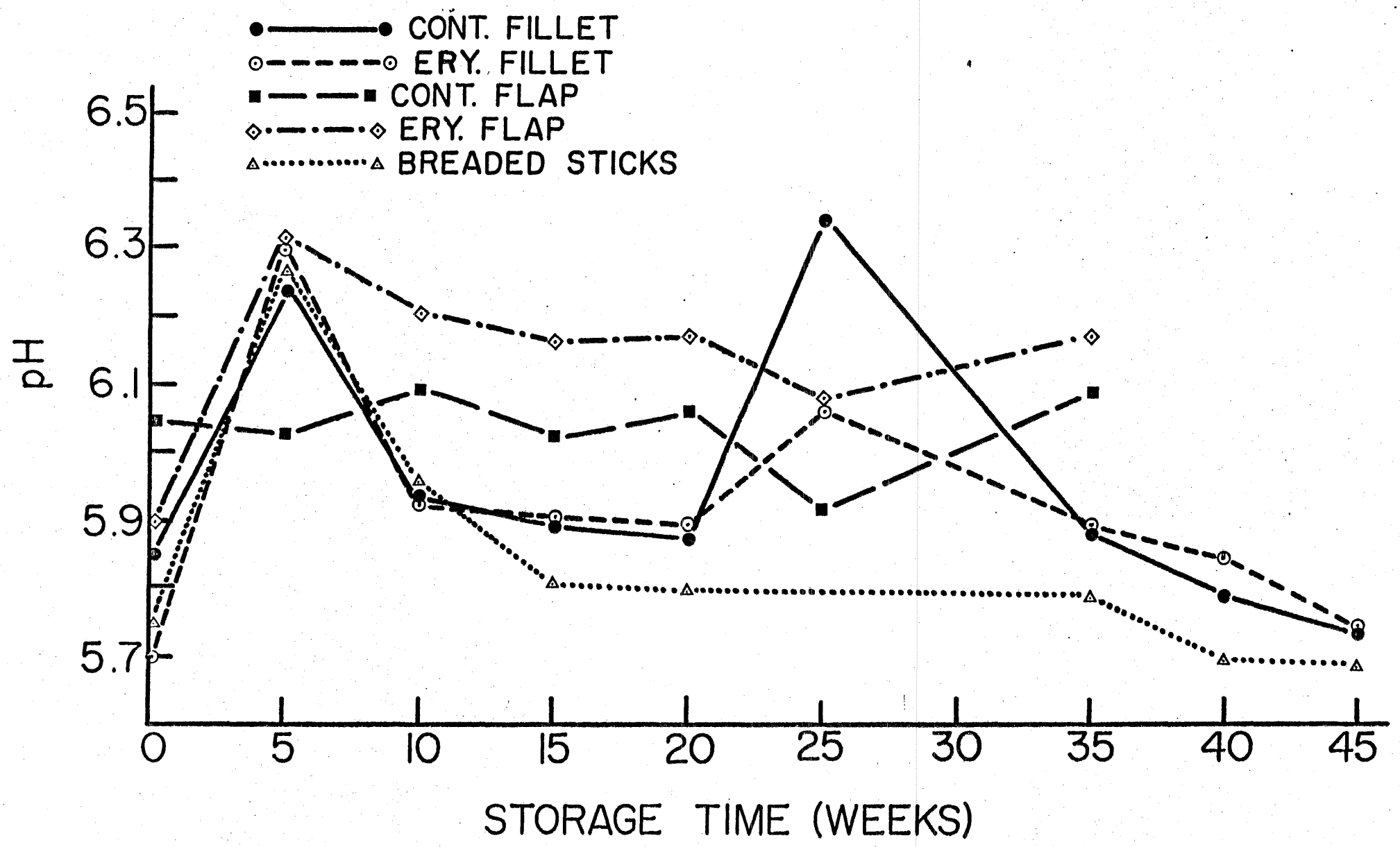


FIG. VII-4 EFFECT OF STORAGE TIME AT 0°F ON TBA NUMBER OF DOGFISH FILLETS OR BELLY FLAPS TREATED IN VARIOUS WAYS.

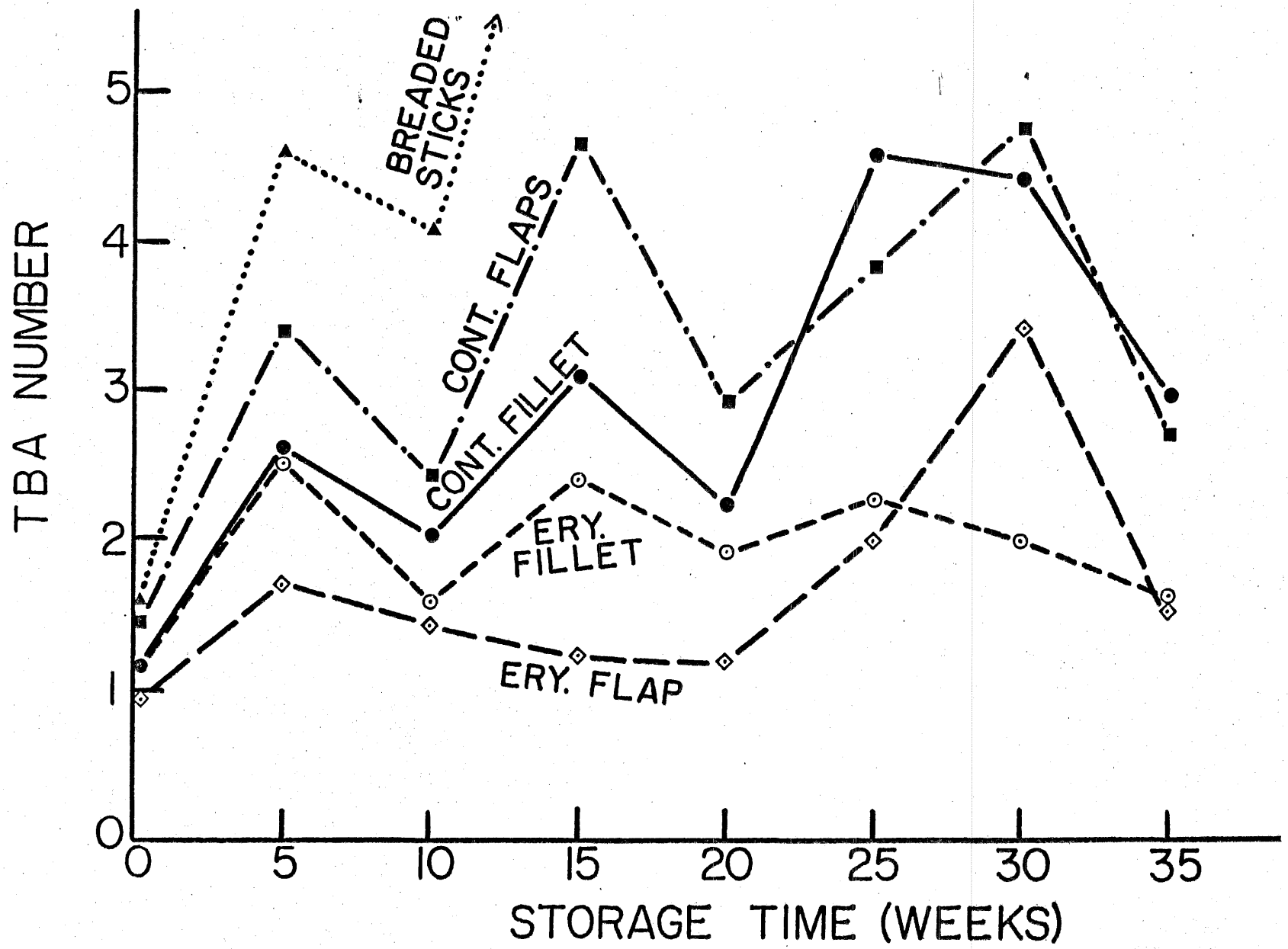


FIG. VII-5 EFFECT OF STORAGE TIME AT 0°F ON DMA CONTENT OF DOGFISH FILLETS OR BELLY FLAPS TREATED IN VARIOUS WAYS.

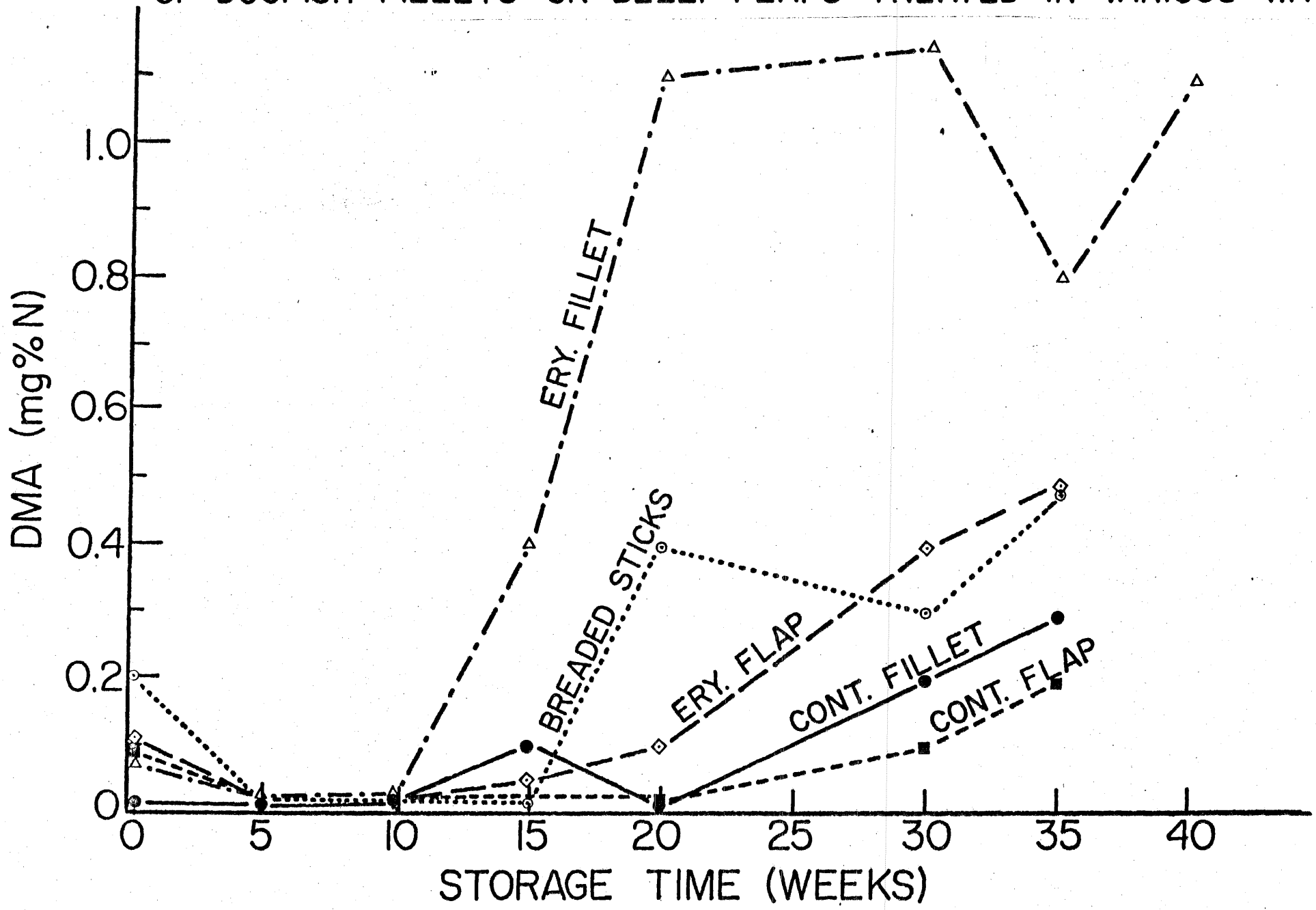


FIG. VII-6 EFFECT OF STORAGE TIME AT 0°F ON AMMONIA CONTENT OF DOGFISH FILLETS OR BELLY FLAPS TREATED IN VARIOUS WAYS.

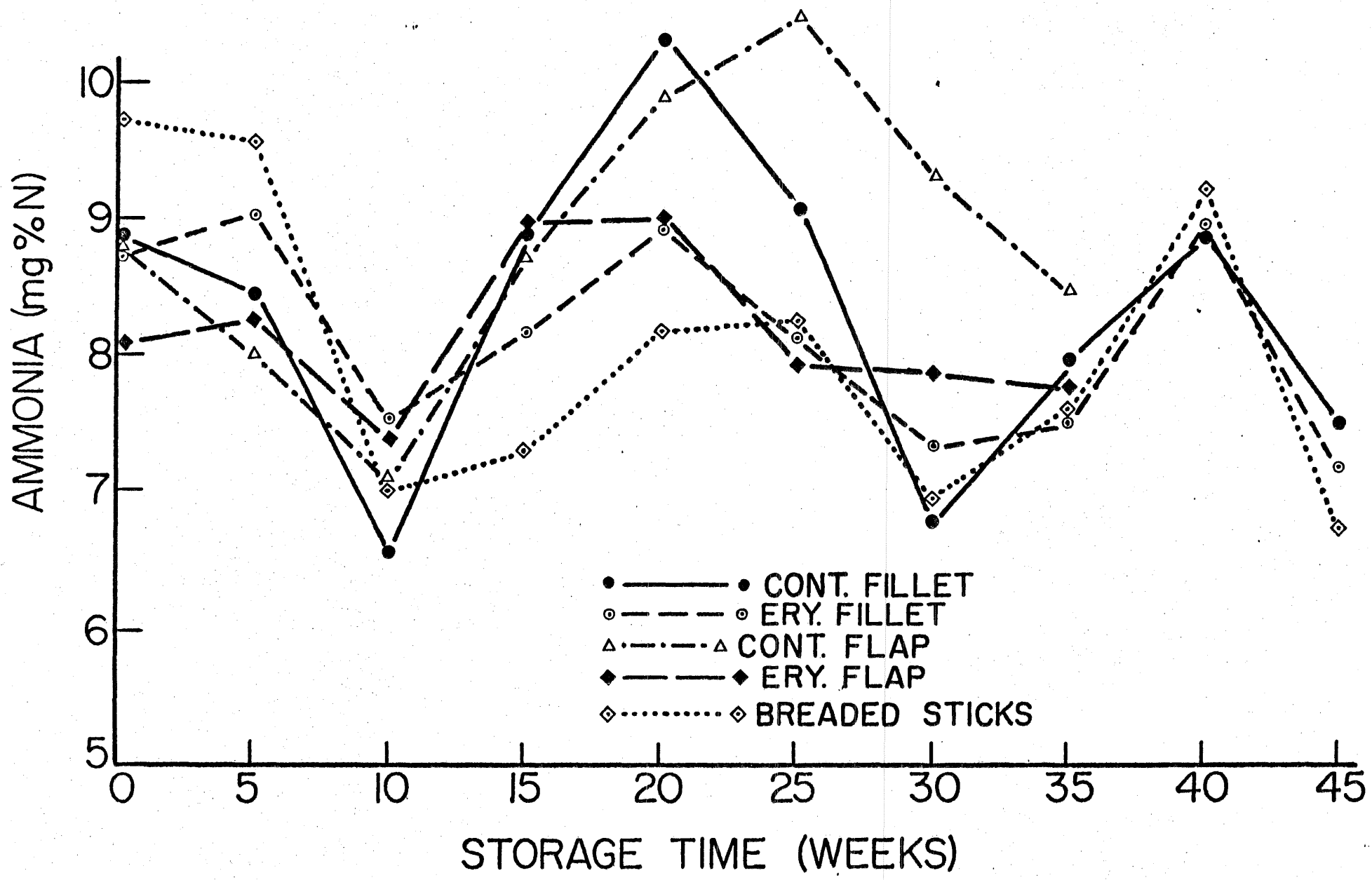
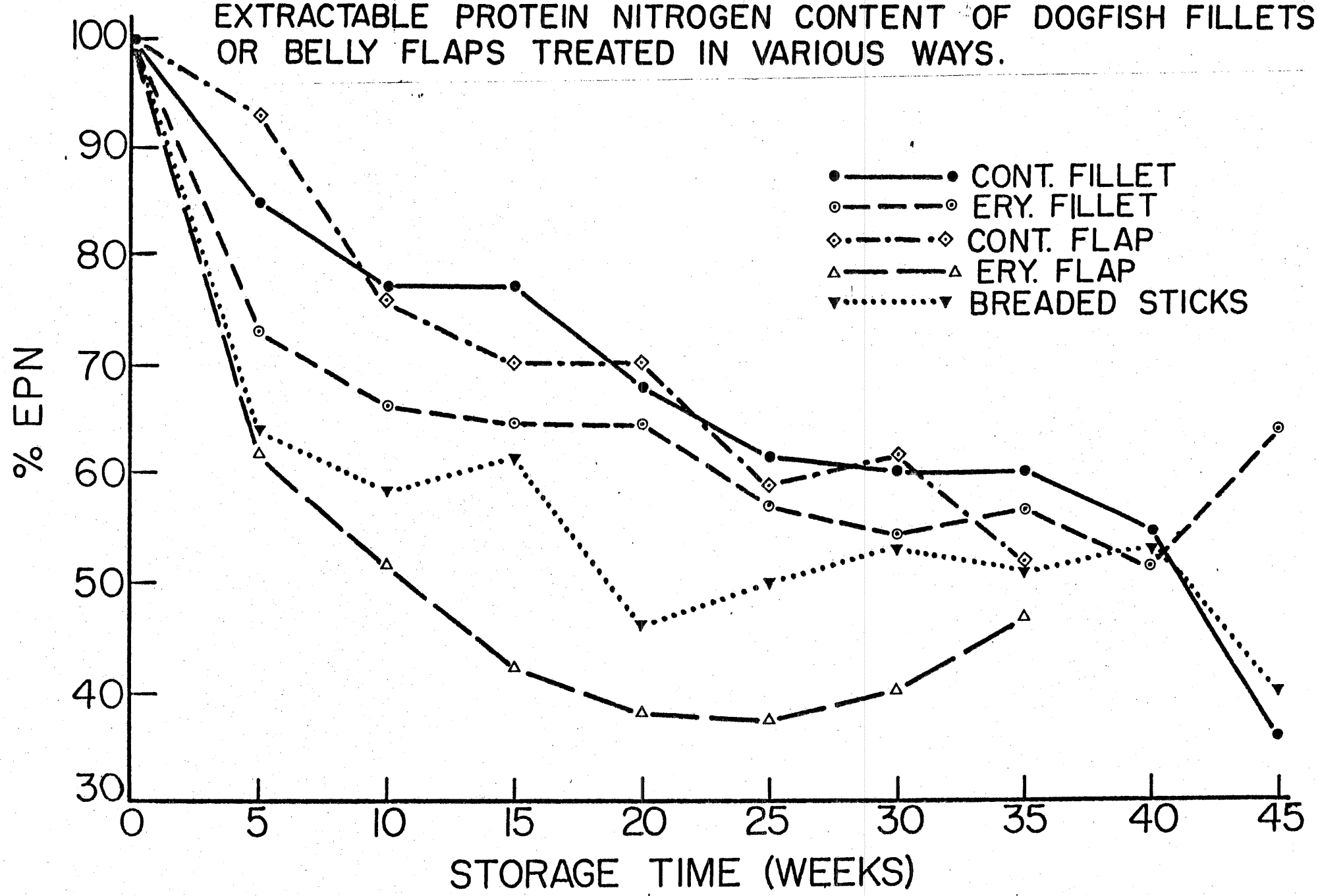


FIG. VII-7 EFFECT OF STORAGE TIME AT 0°F ON PERCENT EXTRACTABLE PROTEIN NITROGEN CONTENT OF DOGFISH FILLETS OR BELLY FLAPS TREATED IN VARIOUS WAYS.



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