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FISH IRRADIATION

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OFFICE OF ATOMIC ENERGY FOR PEACE
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RADIATION PRESERVATION OF FISH

Abstract

Chub-mackerel was chosen for the study because they are the most common fish in Thailand. Preliminary investigations were conducted to determine the maximum radiation dose of gamma-rays by organoleptic tests. The samples were subjected to radiation at various doses up to 4 Mrad. Many experiments were conducted using other kinds of fish. The results showed that 1 Mrad would be the maximum acceptable dose for fish. Later, the influence of the radiation dose from 0.1-1 Mrad was studied in order to find the optimum acceptable dose for preservation of fish without off-flavour. For this purpose, the Hedonic scale was used. It was found that 0.2 and 0.5 Mrad gave the best result on Chub mackerel. The determinations of optimum dose, organoleptic, microbiological and trimethylamine content changes were done. The results showed that Chub mackerel irradiated at 0.2, 0.5 and 1 Mrad stored at 3°C for 71 days were still acceptable, on the contrary the untreated samples were found unacceptable at 14 days. The trimethylamine increment was significantly higher in the untreated samples. At 15 days storage, trimethylamine in the non-irradiated Chub-mackerel was about 10 times higher than the irradiated ones. At 51 and 79 days storage, about 13 times higher in the control samples than the irradiated samples except 0.1 Mrad. Only 2 times higher was found for the 0.1 Mrad.

The microbiological results showed that the irradiation above 0.2 Mrad gave favorable extension of shelf-life of fish.

Fish Irradiation in Thailand

Introduction.

In Thailand, fish is the largest source of animal protein, followed by carcase meats, poultry and eggs. Marine fish constitutes approximately 90% of the total annual fish harvest. The main fishing ports are located along the long double coast lines in the south of Thailand. (Fig. 1)

Fish is caught by bamboo stake traps, set bag nets, Uan Tangkeh (Chinese purse seines), Thai purse seines, trawl nets or other types of nets.

Transport of the marine fish harvest to the fish markets in Bangkok by railway, truck and boat, the fish is usually packed in ice-one part of ice to two parts of fish by volume.

The fish market in Bangkok is controlled by the Fish Marketing Organization (FMO), a government-owned organization of the Ministry of Agriculture. The objectives of the FMO are to improve fish marketing and transport, to control the operations of fish purchasing agents, to promote the welfare of fishermen and to promote fishing cooperative societies and associations. The market is located on the left bank of the Chao Phya River and 30 kilometers from the sea. There is pier for fishing boats, cold storage facilities, ice plant and an auction hall at the market.

Yearly production figures of marine fish for Thailand are shown in table 1. The amount caught per year is increasing. Mackerel the most popular marine fish represents about 15 percent of the total harvest.

Many reports showed that fresh fish can be economically and safely preserved by irradiation Rhodes (1966) reported that fresh fish pasteurized by radiation retained quality at 4°C for 10 days and at 0°C for alonger period. Slavin et al (1966) reported that gamma rays from Cobalt-60 at levels of 150,000 and 450,000 rads could at least double the refrigerated shelf-life of 16 species of fish and shell fish.

It was early recognized from laboratory experiments that many fish and fishery products were among the more successful of the foodstuffs that could be preserved by irradiation. However, despite the large amount of work that has been done on irradiation preservation, no fish or fishery product is yet available commercially to the general consumer, Shewan (1966).

These informations and previous results at the Thai AEC encourage us to pursue further investigation in this field. Future studies will cover other species namely Red snappers, Sea bass, Snappers and shrimp.

Materials and Methods.

Preparation of samples:

Chub mackerel (Rastrelliger sp.) the most popular fish in Thailand were studied. Fish samples were obtained directly from the FMO. Samples were mixed with crushed ice in a bamboo basket for transportation to the laboratory. The fish were filleted before irradiation. Two hundred grams of fish fillets or shrimp were vacuum-packed into a cellophane-saran laminated bag and irradiated within 48 hours. Bags were maintained at 0°C before irradiation.

Storage temperature: Storage tests were carried out in the walk-in cooler maintained at 4°C.

Irradiation and storage condition: Cobalt-60 of 8,000 curies is the gamma source for irradiation. The irradiation container is 12 x 15 x 16 cm. During irradiation, the temperature of the fish samples were kept about 0-5°C by ice. In the preliminary experiments the influence of the radiation was studied from 0.5 - 4 Mrad. It was found that 1 Mrad was the maximum acceptable dose. Later the influence of the radiation dose from 0.1 - 1 Mrad was studied in order to find the optimum acceptable dose for preservation without showing off-flavour.

The samples were stored at 0-4°C and 18°C during the post-irradiation period.

Three methods had been used in estimating the storage life of irradiated fish in the experiments. The first one was the measurement of the deterioration of the fish by a series of organoleptic tests. The second was the microbiological investigation of total count of bacteria. The third one was the determination of trimethylamine content by a spectrophotometric method (with picric acid color reaction).

Organoleptic tests: The organoleptic investigations are one of the most important part in determining changes following irradiation of fish. These changes may be followed only by means of organoleptic methods, i.e.

the results of effects caused by individual components can not be revealed unambiguously either by chemical or by means of organoleptic test, however depends on the following conditions, appropriate method of investigations and the proper evaluation of findings.

The current program consists of investigation of the changes on fish subjected to different treatments.

The purpose of determining taste changes caused by irradiation can be achieved by different methods:

a) difference tests (paired and triangular),
b) ranking tests,
c) scoring tests.

On the basis of theoretical requirements Jellinek (1964) & Pearson, Bennet (1942) to ensure changes by the number of judgements by difference tests(a) more tests results were required. The application of ranking tests(b) was rendered difficult by the necessity of comparing various treatment series for a single taster was able to classify 4-6 samples at the most and the ranking results could not be evaluated according to the rules of variance analysis, i.e. interaction could not be calculated. In view of the above mentioned the ranking and scoring(c) combined method based on the Hedonic-scale with 9 scores were adopted. The scores so obtained have been evaluated by means of variance analysis. The Hedonic-scale was not used continuously and no measured data were available at the evaluation. Both facts represent a certain problem. We have corrected this difficulty was minimized at the evaluation by working up the rather numerous data of the repetition tests and thus within the repetitions more scores were gained. This explained the non-application of the special transformation. The comparison in this arrangement for tasting was not entirely independent since each treatment was compared by pairs.

In the case of organoleptic tests the tasters involuntarily compared the test sample to the other samples instead of using the Hedonic-scale. With a view to saving the taster confusion caused by the cohesion between the individual data during the research work, few taste grades of the Hedonic-scale to each test were attached.

In our experiments two fold experiment series were applied and examined. In general 10 persons were used as taste panel members.

Complying with technological view-points, the average of treatments was examined. The differences between persons and the interaction (persons x treatments) were not dealt with detail since these two deviation components were connected mainly to physiological problems and not to problems of treatment and storage investigated.

Microbiological determination: Total bacterial count of the fish samples were determined by "Dilution Plate Methods."

Trimethylamine content determination: The trimethylamine content changes were determined according to Dyer (1959) method. The trimethylamine determination was based on color reaction in which the trimethylamine reacted with picric acid giving yellow complexes. For this purpose, trimethylamine was extracted by trichloracacetic acid.

Results and discussion.

Preliminary investigations were conducted to determine the maximum radiation dose of gamma rays by organoleptic tests. The samples were subjected to radiation at various doses up to 4 Mrad.

The results showed that 1 Mrad would be the maximum acceptable dose for fish (Chub mackerel). Later the influence of the radiation doses from 0.1 - 1.0 Mrad were studied in order to find the optimum acceptable dose for preservation of fish without off-flavour. By the combination of organoleptic method it was found that 0.2 and 0.5 Mrad gave the best result on Chub mackerel.

The results of the determination of total bacterial count of treated and stored fish are summarized in the next table.

Table 2.

Storage time (days)	Control		0.2 Mrad		0.5 Mrad		1.0 Mrad	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
1	$2.42x10^5$	$9.0x10^4$	$1.44x10^3$	$9.4x10^4$	$2.35x10^2$	$2.49x10^4$	9.5x10	$1.64x10^2$
7		$1.1x10^7$		$6.5x10^3$		1.3x10		0.5x10
14	$3.7x10^6$	$7.2x10^7$	$8.6x10^4$	$2.9x10^5$	$1.35x10^3$	$7.9x10^4$	$1.0x10^2$	$1.1x10^2$
21		$1.4x10^8$		$2.32x10^5$		2.5x10		0.26x10
28		$5.3x10^7$		$4.1x10^4$		$4.6x10^3$		1.0x10
35		$> 3.10^{10}$		$2.31x10^4$		$4.5x10^2$		1.2x10
50	$1.37x10^8$		$8.6x10^7$		$4.6x10^4$		8.5x10	

The total bacterial counts on fish were markedly reduced by doses of 0.2 ; 0.5 and 1.0 Mrad after 7 days storage time. The residual bacterial counts were reduced (from $1.1x 10^7$) to about the lowest level of measurement (to in order 10) at 0.5 and 1.0 Mrad ref. figure 1 - 3.

The initial non-irradiated values were exceeded by the 7th day of storage, increasing approximately 1000 fold (in lot 2) by the 14th day. By the 35th day of storage the count had reached 3.10^{10}. From the organoleptic test data, there was a significant difference between control and irradiated samples after 14 days storage time.

<u>Performing of organoleptic tests:</u> The samples chosen for comparison by organoleptic test had been dealt with by the tasters in a separate room serving this purpose.

The basis for judgement was the taste and smell. In each series of samples two samples were identical, the tasters had to declare which two samples were identical and which were different and what scores (1-9) the taster allocated to one or the other samples (from 1 = extraordinary weak, to 9 = extremely good).

Table 3.

Comparison of control and irradiated samples

Source of variation	Sum of squares (SS)	Degrees of freedom (DF)	Mean square (MS)	variance ratio (F)
Total	90	47		
Between Treatments	12	2	6.0	5.17^x
Between Persons	1	7	0.14	0.12
Interaction	49	14	3.5	3.0^x
Residual	28	24	1.16	

Sample: Fillet : Control : $\bar{x}_1$ = 5.87

0.2 Mrad : $\bar{x}_2$ = 5.75

0.5 Mrad : $\bar{x}_3$ = 5.31

Storage time: 14 days. Storage temperature 4 °C.

Within 14 days storage time the difference between treatment was significant at 0.05 probability level, but the judgements of tasters indicated significant interaction (persons x treatments). Some of the tasters preferred the control, while the others the irradiated ones. In the first period of storage tests there were no detectable differences between control and irradiated fish samples.

Table 4.

Comparison of control and irradiated samples

Source of variation	SS	DF	MS	F
Total	132	49		
Between Treatments	11	3	3.66	4.31^{x}
Between Persons	64	5	12.8	15.1^{xxx}
Interaction	35	15	2.33	2.74^{x}
Residual	22	26	0.85	

Sample: Control : $\bar{x}_1 = 3.77$
0.2 Mrad : $\bar{x}_2 = 6.77$
0.5 Mrad : $\bar{x}_3 = 5.25$
1.0 Mrad : $\bar{x}_4 = 6.08$

Storage time: 24 days ; Storage temperature 4°C.

In this experiment the data showed highly significant difference between persons and significant difference between treatments. The results as compared with the experiment 1 indicated the benefit of irradiation. Most of the tasters scored the control worst but the significant interaction showed that there were differences between the judgements of tasters in the case of irradiated samples.

Table 5.

Comparison of irradiated samples

Source of variation	SS	DF	MS	F
Total	375	95		
Between Treatments	110	5	22.0	26.8^{xxx}
Between Persons	144	7	20.57	25.0^{xxx}
Interaction	82	35	2.34	2.85^{xx}
Residual	39	48	0.82	

Sample :	Fillet	: 0.2	Mrad	: $\bar{x}_1$ = 5.62	} 5.43
Fish with	Bones	: 0.2	"	: $\bar{x}_2$ = 5.25	
	Fillet	: 0.5	"	: $\bar{x}_3$ = 3.12	} 4.27
Fish with	Bones	: 0.5	"	: $\bar{x}_4$ = 5.43	
	Fillet	: 1.0	"	: $\bar{x}_5$ = 6.0	} 5.53
Fish with	Bones	: 1.0	"	: $\bar{x}_6$ = 5.06	

Storage time 29 days ; Storage temperature 4°C.

In this experiment the quality of irradiated fish with bones and fillets were compared. The data in table (a) indicated highly significant differences between treatments and persons as well as significant interaction. The mean values of samples indicated the significant interaction, because of slight differences between mean values of samples and highly significant difference between persons. From these results it could be stated that no unfavourable organoleptic changes were caused by irradiation.

Table 6.

Comparison of irradiated samples

Source of variation	SS	DF	MS	F
Total	326	136		
Between Treatments	10	5	2.00	1.08
Between Persons	10	9	1.11	0.6
Interaction	163	45	3.62	1.96^{xx}
Residual	143	77	1.85	

1. Sample	: 0.2 Mrad	(DW)	: $\bar{x}_1$ = 5.4	} 5.7
2. "	: 0.2 "	(TW)	: $\bar{x}_2$ = 6.0	
3. "	: 0.5 "	(DW)	: $\bar{x}_3$ = 5.89	} 5.68
4. "	: 0.5 "	(TW)	: $\bar{x}_4$ = 5.48	
5. "	: 1.0 "	(DW)	: $\bar{x}_5$ = 6.10	} 6.02
6. "	: 1.0 "	(TW)	: $\bar{x}_6$ = 5.95	

Storage time 38 days ; Storage temperature 4°C.

From this experiment two statistical evaluations were made. In the first variance analysis all of the pretreatment and doses correlation were investigated. The samples were washed in (a) group by distilled water (b) group by tap-water.

In the second variance analysis the mean values of different doses were evaluated.

There was no reason to doubt that this difference between pretreatment had not significantly influenced the quality of irradiated products. (Table 7).

Table 7.

Source of variation	SS	DF	MS	F
Total	326	136		
Between Treatments	10	2	5.0	1.99
Between Persons	10	9	1.1	0.40
Interaction	38	18	2.1	0.84
Residual	268	107	2.51	

0.2 Mrad $\bar{x}_1 = 5.7$ 0.5 Mrad $\bar{x}_2 = 5.68$ 1.0 Mrad $\bar{x}_3 = 6.02$

Table 8.

Source of variation	SS	DF	MS	F
Total	250	55		
Between treatments	36	3	12.0	27.8^{xxx}
Between Persons	140	6	23.3	54.1^{xxx}
Interaction	62	18	3.45	8.1^{xx}
Residual	12	28	0.43	

Sample 1 : Control : $\bar{x}_1$ = 2.14
" 2 : 0.2 Mrad : $\bar{x}_2$ = 5.57
" 3 : 0.5 Mrad : $\bar{x}_3$ = 6.0
" 4 : 1.0 Mrad : $\bar{x}_4$ = 5.78

Storage time 47 days ; Storage temperature 4°C.

In this experiment the difference between treatments and persons was highly significant. The mean values of irradiated samples showed a slight difference. From the total variance analysis table there was evidence of interaction (persons x treatment). All of the irradiated samples were scored better than the control.

Table 9.

Comparison of control and irradiated samples

Source of variation	SS	DF	MS	F
Total	514	91		
Between Treatments	193	4	48.4	52.3^{xxx}
Between Persons	153	7	21.9	14.6^{xxx}
Interaction	90	28	3.2	2.13^{x}
Residual	78	52	1.5	

Sample 1 : Control : $\bar{x}_1$ = 1.68
" 2 : 0.1 Mrad : $\bar{x}_2$ = 3.32
" 3 : 0.2 Mrad : $\bar{x}_3$ = 4.43
" 4 : 0.5 Mrad : $\bar{x}_4$ = 4.63
" 5 : 1.0 Mrad : $\bar{x}_5$ = 5.68

Storage time 52 days ; Storage temperature 4°C.

From this experiment the statistical analysis of these data indicated that highly significant differences between treatments and persons but no significant difference from the earlier irradiated samples (at 1.0 Mrad) were detected by tasters.

Because of significant interaction in the total variance analysis of this experiment, in the second table, the judgements of the best (well-trained) tasters were analysed.

Table 10.

Comparison of control and irradiated samples

Source of variation	SS	DF	MS	F
Total	274.4	39		
Between Treatments	114.7	4	28.7	6.5^{xxx}
Between Persons	46.9	3	15.6	3.5^{x}
Interaction	24.8	12	2.06	
Residual	88.0	20	4.4	

Sample 1 :	Control	:	$\bar{x}_1$	=	2.5
" 2 :	0.1 Mrad	:	$\bar{x}_2$	=	3.5
" 3 :	0.2 Mrad	:	$\bar{x}_3$	=	4.5
" 4 :	0.5 Mrad	:	$\bar{x}_4$	=	4.5
" 5 :	1.0 Mrad	:	$\bar{x}_5$	=	5.5

The results showned that there was a highly significant difference between treatments and significant difference (at 0.05 probability level) and there was no interaction.

Table 11.

Comparison of irradiated samples

Source of variation	SS	DF	MS	F
Total	438	91		
Between Treatments	137	3	45.6	23.2^{xxx}
Between Persons	20	9	2.22	1.13
Interaction	179	27	6.62	3.36^{xx}
Residual	102	52	1.97	

Sample 1 :	0.1	Mrad	:	$\bar{x}_1$	=	4.71
" 2 :	0.2	Mrad	:	$\bar{x}_2$	=	5.05
" 3 :	0.5	Mrad	:	$\bar{x}_3$	=	5.19
" 4 :	1.0	Mrad	:	$\bar{x}_4$	=	5.15

The results after 21 days storage time showned an interesting distribution. The difference between treatments was highly significant but there was a significant interaction (treatments x persons). The quality of irradiated samples at 0.5 Mrad and 1.0 Mrad were acceptable though of poor texture.

<u>Trimethylamine determination.</u>

In the first investigation the sensitivity of method was checked. In table 12. The data of treated and stored fish samples could be summarized. The storage temperatures were 3°C and about 25°C.

Table 12.

Trimethylamine content in stored Chub mackerel

Storage days	Storage temperature	Trimethylamine nitrogen mg/g wet fish Control	0.1	0.2	0.5	1.0 Mrad
2	3°C	0.15	0.20	0.10	0.10	0.40
8	"	0.60	0.40	0.20	0.30	0.20
11	2 days at about 25°C	20.4	-	-	1.50	1.15

Initially, the trimethylamine nitrogen content in each sample was the same. The difference between the highest and the lowest at the first determination was due to the heterogeneous samples. After 8 days storage time, there was a trend of increment in trimethylamine content in the samples (at 3°C). The increment of trimethylamine content was significantly higher in the control samples.

After 10 days storage time, for study purpose, 10 bags of each treatment were stored at room temperature (about 25°C). Two days later the results were evaluated by sensory analysis and trimethylamine determination.

All of the control samples (100 percent) were spoiled and the bags were full of released. The smell produced as the result of proteolysie by bacterial growth. In control samples the smell was very strong. The trimethylamine content of control samples was approximately 15 times higher than the treated samples. Among the irradiated samples, the degree of deterioration was small and no bag was gas-filled.

To follow the quality changes of stored fish, some other trimethylamine determinations were made parallel with microbiological investigations. In the table 13, the data of trimethylamine determination could be summarized.

Table 13.

Storage days	Storage temperature	Trimethylamine nitrogen mg/g wet fish			
		Control	0.2 Mrad	0.5 Mrad	1.0 Mrad
1	3°C	0.030	-	-	-
17	"	0.58	0.10	0.09	0.08
29	"	1.04	0.08	0.05	0.06
51	"	1.38	0.10	0.10^{x}	0.09
80	"	0.87	0.13	0.08	0.09
92	"	0.70	0.10	0.06	-

The trimethylamine content during 51 days storage time was slightly increased on irradiated samples. There was a significant tendency towards an increase in trimethylamine content of control samples during the same period of storage. The correlation between control and irradiated samples could be demonstrated in figure 4.

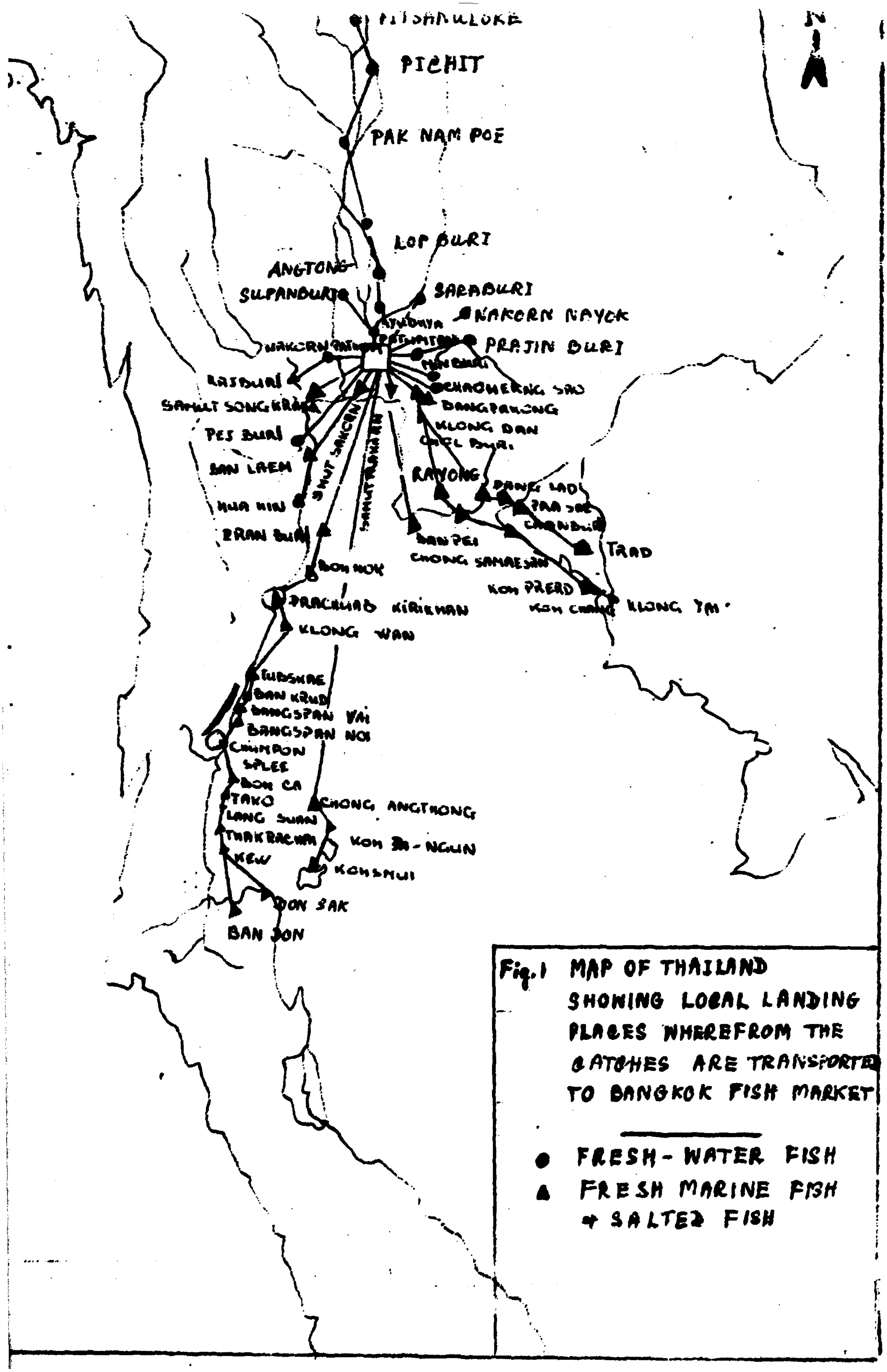

Fig. 1 MAP OF THAILAND SHOWING LOCAL LANDING PLACES WHEREFROM THE CATCHES ARE TRANSPORTED TO BANGKOK FISH MARKET

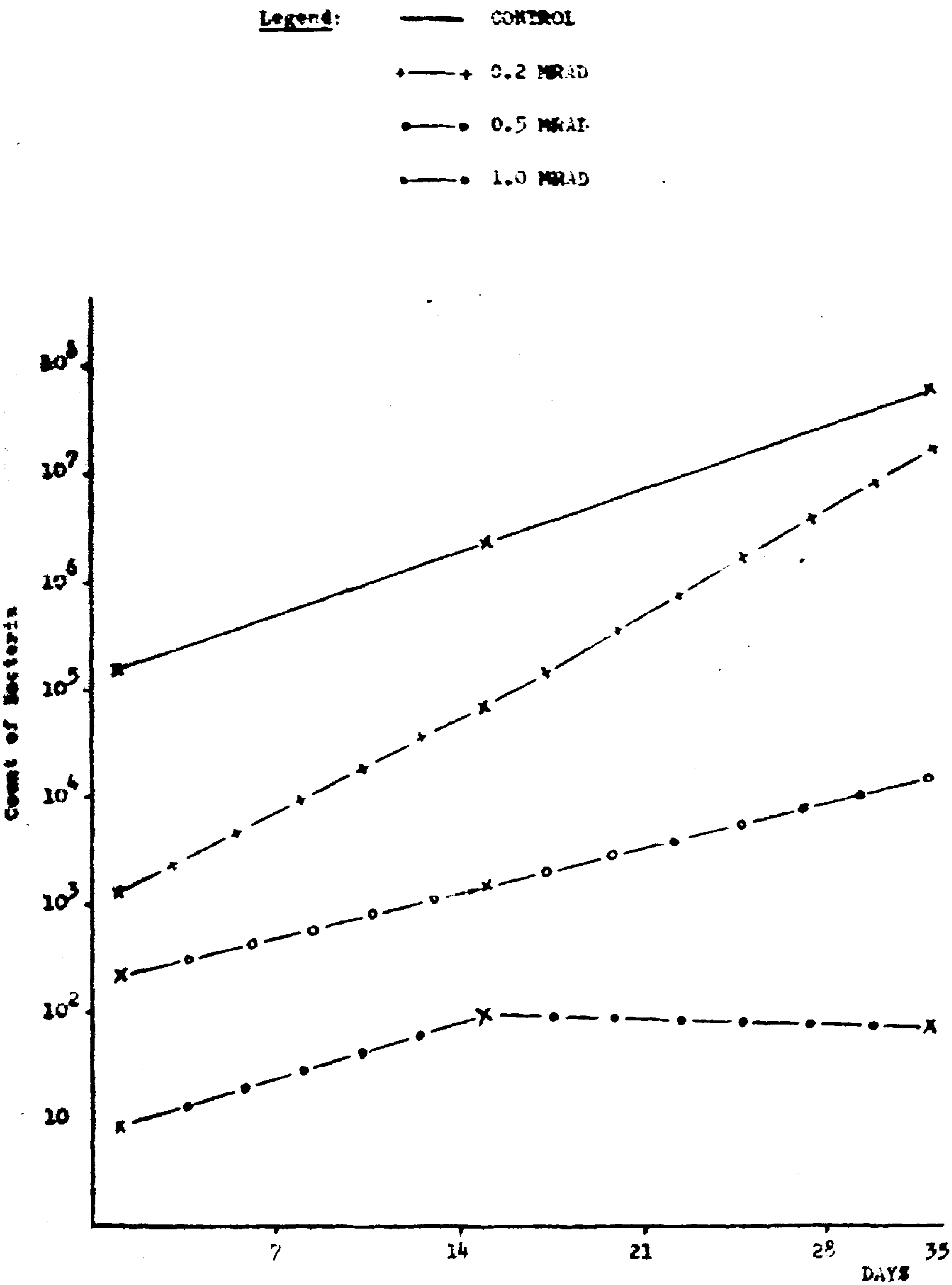

Fig.1- Total bacterial count

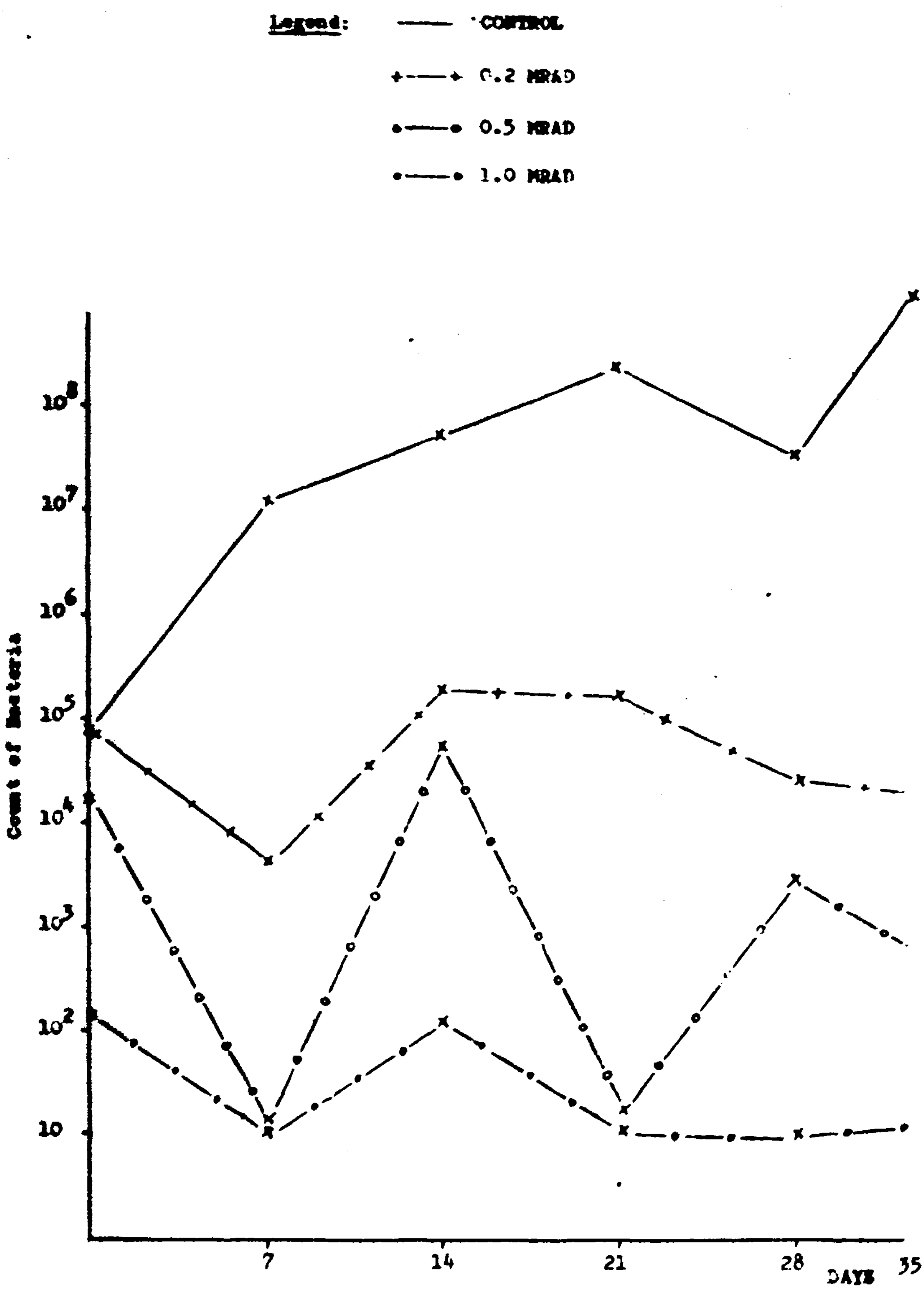

Fig.2- Total bacterial count

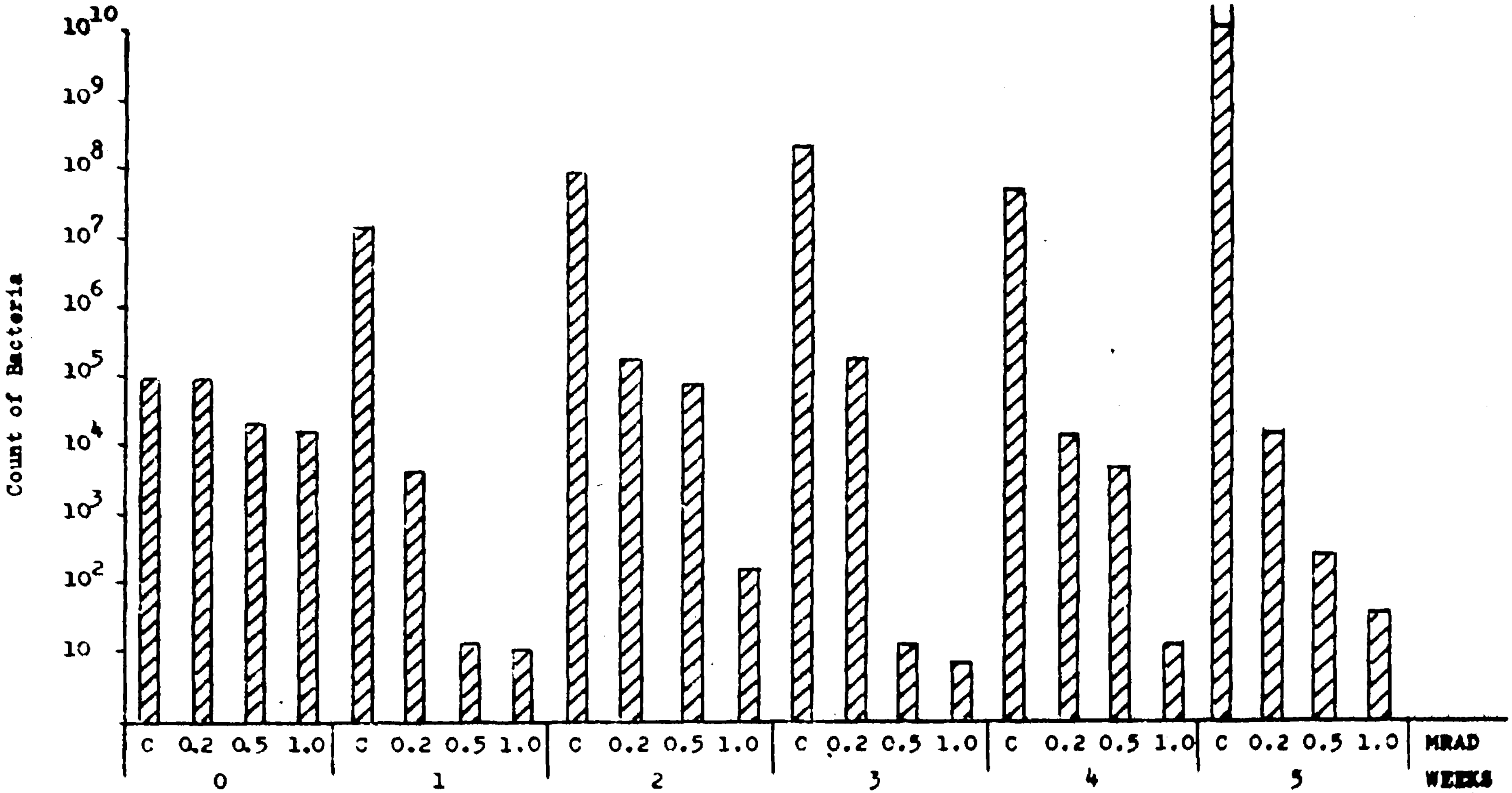

Fig.3- Data of microbiological investigations:

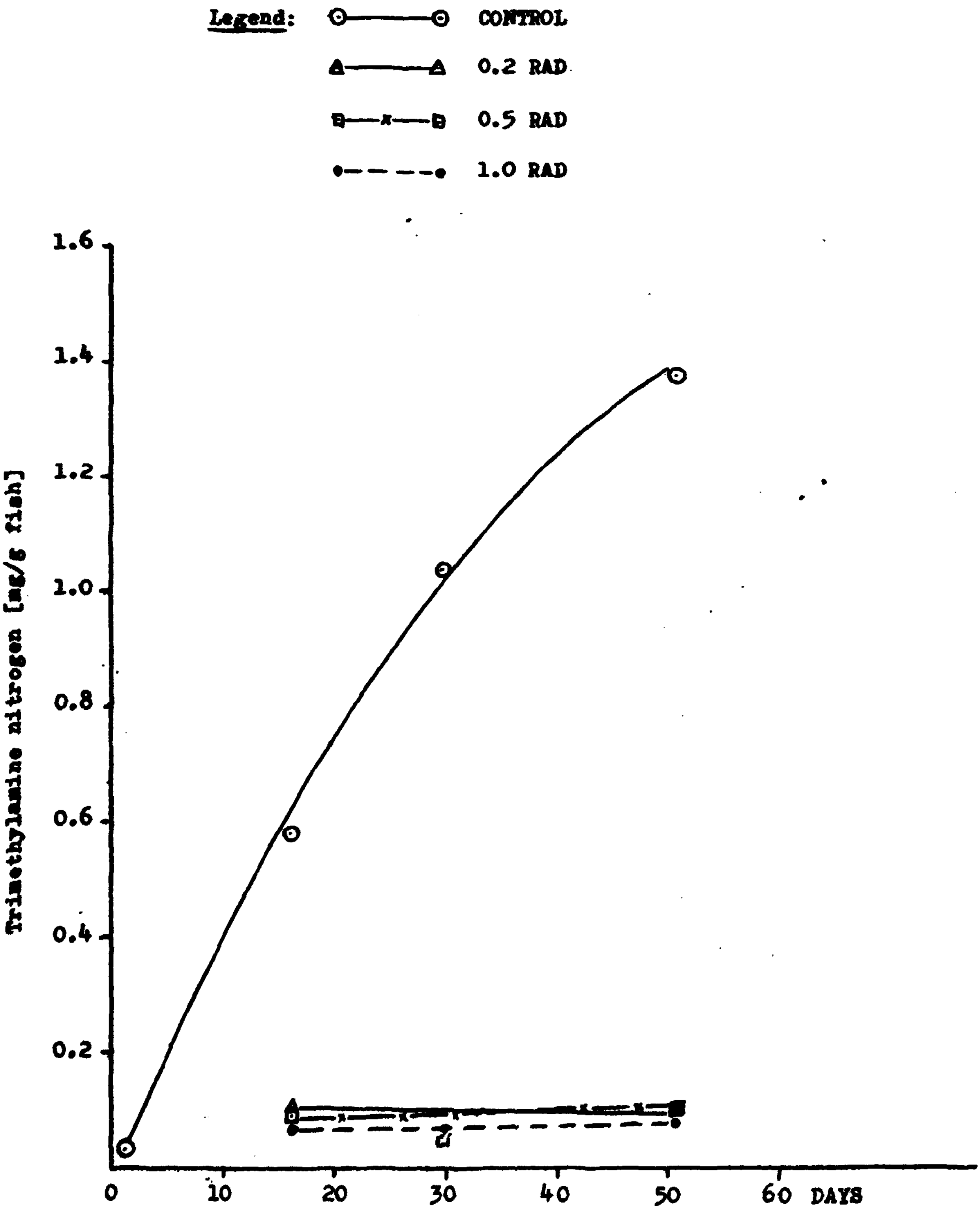

Fig.4- Trimethylamine content changes in fish during storage

Table. 1. Annual Catche in Thailand (1957-1968)

Kinds	Weight (ton)											
	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968
Marine Fish												
Mackerel	57,500	44,300	36,130	29,946	23,235	36,065	23,313	52,524	69,878	87,803	93,154	148,148
Shark	3,100	2,700	2,810	4,325	3,977	4,465	5,147	5,773	7,445	6,795	3,728	5,807
Miscellancous	34,100	37,100	42,100	44,861	102,608	126,763	189,998	327,154	338,944	384, 29	504,150	608,791
Prawns, shrimps and crabs	16,300	10,300	13,520	14,525	16,492	20,175	32,505	38,715	46,277	57,330	75,093	99,304
Mollmscus	59,900	50,600	53,210	52,814	86,963	82,241	72,411	70,030	66,939	98,808	86,063	142,162
Total	170,900	145,000	147,770	146,471	233,275	269,709	323,374	494,196	529,483	635,165	762,188	1,004,054
Fresh Water Fish												
Air Breathers	20,580	17,400	20,590	35,034	22,224	25,224	48,969	26,512	35,708	38,744	3[illegible],681	41,082
Carps	8,290	5,500	7,290	7,458	8,909	9,593	7,962	7,593	9,689	9,901	8,896	7,356
Miscellaneous	30,390	25,100	26,040	25,667	37,364	31,768	35,006	44,751	36,280	33,164	32,940	32,191
Prawns	4,410	3,300	3,100	4,415	3,303	3,494	3,374	3,834	3,960	3,308	3,738	4,539
Total	63,670	51,300	57,020	72,574	72,330	70,079	9[illegible],311	82,790	85,637	85,1[illegible]7	85,255	85,168
Grand Total	234,570	196,300	204,790	219,4[illegible]	305,605	339,788	418,685	576,986	615,120	720,2[illegible]	847,443	1089,222

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