PRELIMINARY STUDY OF TOTAL BACTERIAL PLATE COUNT METHOD FOR FISHERY PRODUCTS

By R. Paul Elliott*

INTRODUCTION

The total bacterial plate count[1] is a valuable test in the study of the spoilage of fishery products. This test is an index of the degree of contamination to which the fish have been subjected, and when used in conjunction with other freshness tests is an index of keeping quality.[2] Counts are especially useful in determining the sources of contamination in a processing line for filleting, canning, salting, etc.

An extensive study of the plate count for fishery products has never been published. Numerous investigators have described the methods they used, but in most cases, their use of a given method was based on limited work. Others have borrowed methods meant for use on other food products such as milk, meat, or water.

The work reported here is a study of methods recommended for or used on fish products by other bacteriologists in the past. There were several variables involved in the plate count method, only one of which could be studied at a time. Thus, sampling, shaking, choice of diluent, pH of the medium, choice of medium, incubation temperature, and incubation time all had to be studied individually yet were all interrelated. Since the choice of a given procedure was based on the treatment which gave the maximum plate count from a given fish sample, a step in the procedure which killed off or inhibited a portion of the flora would affect the results of the study on another step in the procedure. For this reason some portions of the work had to be repeated. A literature survey on the spoilage of fishery products was made in order to avoid such pitfalls. A portion of this survey has been published (Elliott, 1946).

EXPERIMENTAL

Mechanics of Sample Preparation

Preliminary tests showed that blending a fish sample with the Waring-type blender gave more reproducible colony counts than grinding and shaking. Parallel tests showed that there was no significant difference between the coefficients of variation of replicate plates when fresh and spoiled samples were compared. This implied that changes in flora which undoubtedly occurred had no effect on reproducibility.

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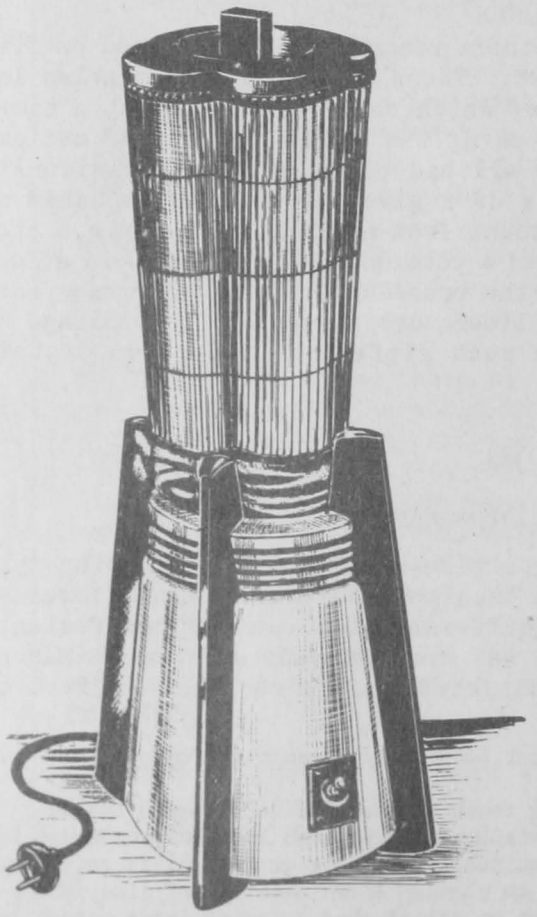
1/ The object of the plate count is to determine the total number of live bacteria in the sample. The bacteria, so small that they are invisible to the naked eye, are suspended in a nutrient "medium" in which all conditions are suitable for their growth and reproduction, and in which they cannot move readily from plate to plate. After a period of time, the individual bacteria so suspended reproduce to such an extent that colonies large enough to be seen form in the medium. Knowing the number of colonies and the extent of dilution of the fish material, it is then an easy matter to calculate the number of live bacteria which were present in the original fish material.

2/ See Griffiths and Stansby (1934), Fitzgerald and Conway (1937), Dyer and Dyer (1947), and Castell (1948).

It was found that most fishery products could be blended adequately only upon the addition of an equal amount of diluent in the blending jar. When less diluent was added to fish, "tunnelling" occurred in the mixing process. While watery flesh did not require as much diluent, an equal amount of diluent was added in such cases in order to standardize the treatment of samples.

While the material was being blended, a distinct rise in temperature occurred. This should be avoided, since marine bacteria are quite sensitive to heat. Zobell and Conn (1940) found that when marine muds were held for 10 minutes at 30° C. (86° F.), 25 percent of the bacteria were killed. In the present case, when 150 grams of fish sample and 150 cc. of diluent were blended for two minutes in the Waring jar 17244, the temperature rose from 22° C. (72° F.) to 30° C. (86° F.). When both fish sample and diluent were chilled to 3° C. (37° F.) in a refrigerator, the blending caused the temperature to rise as follows:

Blending time in minutes		0	1	2	3	4	5	6
Temperature of mixture	Degrees Centigrade	3	9	13	17	21	24	28
	Degrees Fahrenheit	37	48	55	63	70	75	82



BLENDING DEVICE

Thus when the material is chilled adequately, the rise in temperature should offer no difficulty. A three-minute blend of the chilled diluent and sample was adopted in the subsequent studies.

It was found impossible to pipet the 1 to 1 blended sample because it was too thick. Increase in the proportion of diluent resulted in a mixture easy to draw into the pipet, but with this increased amount of diluent, blending caused the whole mass to become a foam containing about 20 percent air. Twenty grams of the blended 1 to 1 dilution were therefore weighed into dilution bottles containing 80 cc. of the diluent. This gave a 1 to 10 dilution. Subsequent dilutions were made by pipetting 10 cc. of suspension to 90 cc. of diluent.

Shaking Time and Settling Time

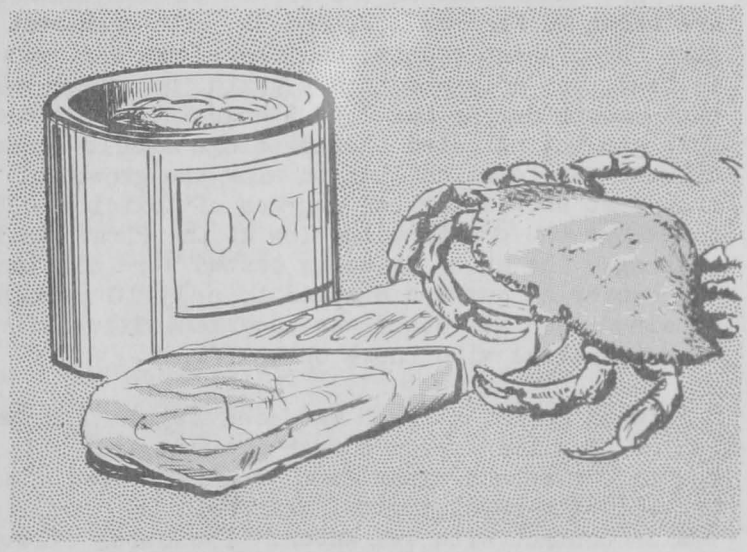
A shaker with a 4 inch stroke, 150 strokes per minute, was used in the experiments. Examination of reproducibility of replicate pipettings revealed that a 2 to 4 minute shaking period was satisfactory. Settling of the material was not a serious difficulty even though the dilutions were not plated immediately, because the supernatant liquid gave the same count as the homogenous mixture. Only after an hour's settling was any inconsistency noted; and even then the material could be mixed sufficiently by shaking it a few seconds by hand.

Diluent

When a diluent is used to dilute a suspension of material so that the concentration of bacteria is in the right range for plating, it is always assumed that the bacteria remain dormant during this time, and that the diluent does not kill any of the organisms in the sample. Since it was thought that this might not always be the case, a study was made of the toxicity of various diluents. Those examined were:

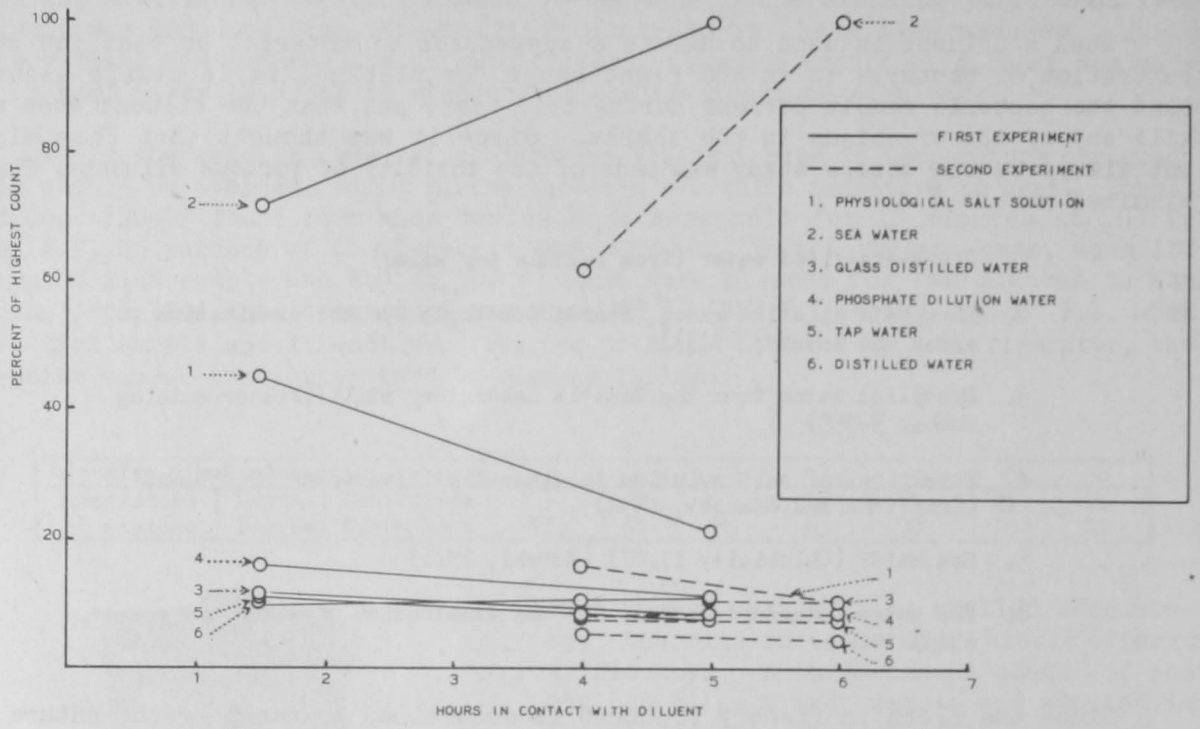
1. Glass-distilled water (from Seattle tap water)
2. Phosphate dilution water (Standard methods for the examination of water and sewage, 1943)3/
3. Distilled water from the Seattle laboratory still (Fisher catalog number 9-055)
4. Physiological salt solution in glass-distilled water (0.85% NaCl) (Griffiths and Stansby, 1934)
5. Sea water (Chlorinity 17.07) (Zobell, 1941)
6. Tap water (Standard methods for the examination of water and sewage, 1943)

Since the flora in fishery products is determined somewhat by the nature of the product (Elliott, 1946), three types of fishery products were used in the evaluation of the diluents. Samples of Pacific oysters (Ostera gigas), meat of the Dungeness crab (Cancer magister) and Pacific rockfish (Sebastodes sp.) were stored at 1° C. (34° F.) in a refrigerator for 8 days. This treatment was used to make certain that the flora present was that responsible for spoilage of the product, because at 8 days, spoilage was incipient. Each sample was blended, and replicate samples were weighed into the respective autoclaved, cooled diluents under study. These suspensions were shaken, and further dilutions were made with the diluents under tests, and then were plated in Difco nutrient agar made up with sea water. After a fixed holding period at room temperature, these suspensions were again plated. Colony counts were made after the plates had been incubated for 5 days at 25° C. (77° F.).



3/ The formula for phosphate dilution water is as follows: Dissolve 34 grams of KH_2PO_4 in about 500 ml. of (glass-) distilled water. Add 1 N. NaOH until a pH of 7.2 is reached. Dilute to 1 liter. Add 1.25 ml. of this stock buffer solution to each liter of aerated, (glass-) distilled water.

FIG. 1 - EFFECT OF VARIOUS DILUENTS ON BACTERIAL PLATE COUNTS ON SPOILING DUNGENESS CRAB MEAT

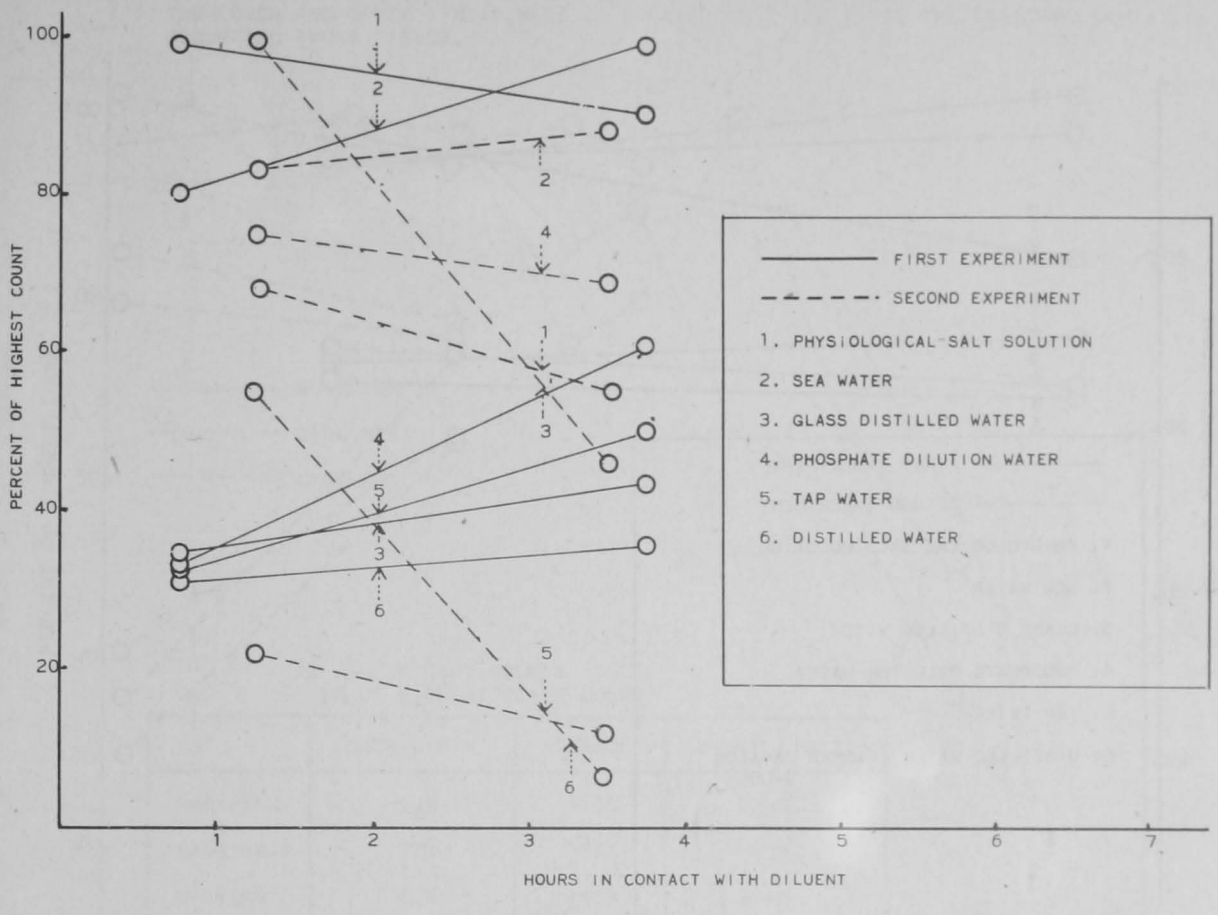


The experiment on the effect of the diluent was performed twice. Results are shown in Figures 1,2, and 3. With crab meat (Figure 1),sea water as a diluent gave the highest counts, and therefore was least toxic of any of the diluents. In fact, not only was it not toxic, but there was apparently growth of a significant amount during the 2 to 3 hour interval between the first and second plating. This growth indicated that platings should be as rapid as possible and that the diluent should be chilled to slow the growth of the bacteria. Other diluents for crab meat showed varying degrees of toxicity. Physiological salt solution showed toxicity, the counts being low at the first plating, and still lower at the second plating. The other diluents tested were distinctly toxic, for in each instance, the bacterial numbers dropped to only 10 percent or 20 percent of the numbers obtained when sea water was used as a diluent. Results on the second experiment were identical with those on the first.

When rockfish flesh was used as the sample (Figure 2), sea water was the choice of diluent,even though physiological salt solution gave equally high counts; for in the second experiment, counts showed a more distinct drop upon prolonged contact with physiological salt solution, while no drop occurred when the sample was in contact with sea water. The other diluents were grouped below these in both experiments. However, in the second experiment,(Seattle) tap water and distilled water from the laboratory still showed more distinct toxicity than was noted in the first experiment.

When oysters were used as the sample (see Figure 3), physiological salt solution and sea water were apparently of equal value, while glass distilled water was a close third. In the repeat experiment on oysters, however, phosphate dilution water was distinctly superior to the other diluents, and sea water and glass distilled water were next in value. This inconsistency between the two experiments makes a choice of diluent for oysters impossible without further work.

FIG. 2 - EFFECT OF VARIOUS DILUENTS ON BACTERIAL PLATE COUNTS ON SPOILING PACIFIC ROCKFISH FLESH



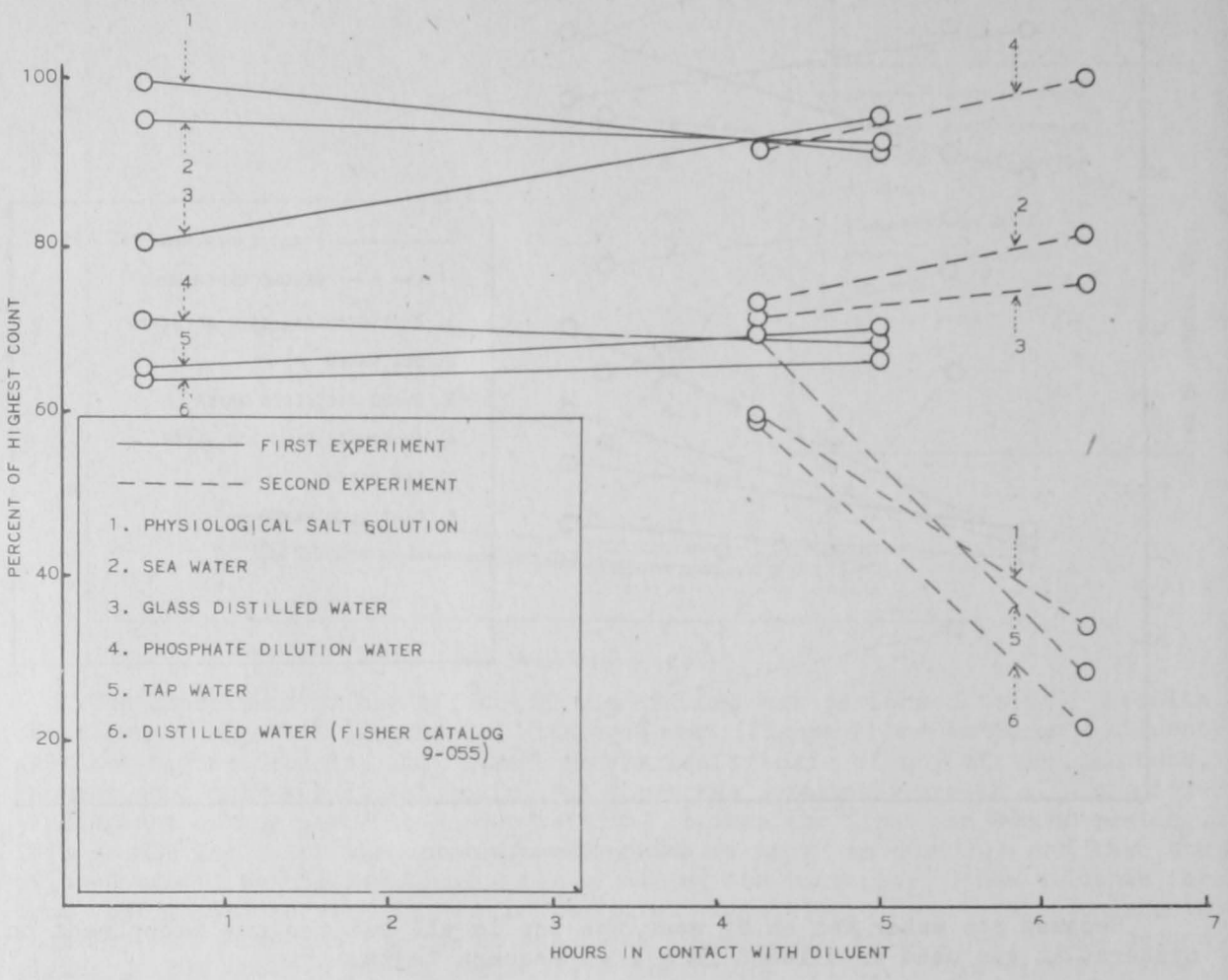
Because sea water was at or near the top in all but the one experiment on oysters, it was used as diluent in all subsequent tests.

pH of the Medium

Zobell (1941) suggested that a medium of pH 7.5 to 7.8 gave optimum conditions for marine bacteria. An experiment was undertaken to determine what effect the pH of the medium might have on the counts on fish products. A series of 10 batches of Zobell's medium number 2216 (Zobell, 1941) was made up, each at a different pH. The pH range of the media after sterilization was from pH 8.0 to 8.53. Suspensions of oysters, crab meat, and rockfish that had been held at 1° C. for 8 days were plated in each medium. In addition, direct microscopic counts were made on the fish suspension by the method described by Tarr (1943).

Results are shown in Figure 4. It is evident that media with pH values of 7.0 or lower were quite satisfactory. However, counts tended to drop markedly in alkaline media. The abruptness of this drop depended on the fishery product plated. When rockfish flesh was used, counts began to decrease at neutrality, but with oysters and crab meat, the drop occurred only when pH 8.0 was reached. It was tentatively concluded that for plate counts on fishery products, the pH of the medium should be neutral or slightly acid.

FIG. 3 - EFFECT OF VARIOUS DILUENTS ON BACTERIAL PLATE COUNTS ON SPOILING PACIFIC OYSTERS

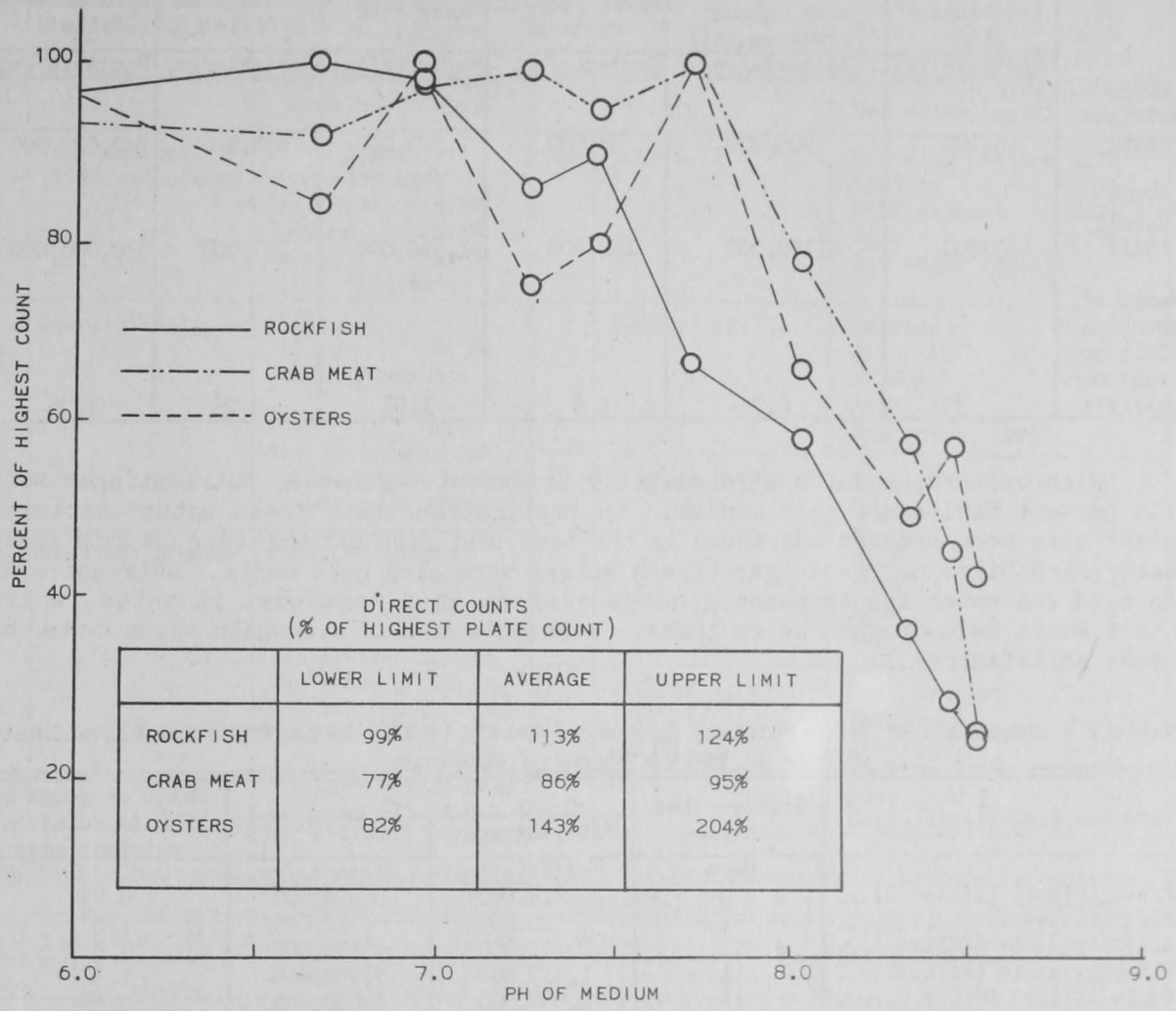


Medium

The work on the medium was essentially an evaluation of the media suggested by other workers. Preliminary work in which Zobell's 2216 medium (1941) was compared with nutrient agar showed that the former medium was distinctly superior. Results are shown in Table 1. Zobell's medium gave counts ranging from 1.03 times to 35 times as high as those obtained with the nutrient agar.

It was noted in the data of Table 1 that Zobell's medium gave a count 2.25 times greater than that on nutrient agar when a fresh sole fillet was plated, and only 1.26 times greater when a spoiled sole fillet was plated. Fresh clams and salmon gills gave even greater differentials. An experiment on the spoilage of sole was conducted in order to determine whether or not this inconsistency meant that Zobell's medium allowed a wide variety of marine forms to grow, but that the true spoilage bacteria grew nearly as readily on ordinary nutrient agar. It is evident on examination of the data in Table 2 that such was not the case, for Zobell's medium gave somewhat lower counts on the fresh sample, but considerably higher counts in all other cases. The low count on the fresh sample with

FIG. 4 - THE EFFECT OF PH OF THE MEDIUM ON TOTAL PLATE COUNTS ON FISHERY PRODUCTS. EACH POINT REPRESENTS THE AVERAGE COUNT ON THREE PLATES. IN THE DIRECT COUNT, THE LOWER AND UPPER LIMITS WERE CALCULATED AS 2 1/2 TIMES THE STANDARD DEVIATION OCCURRING AMONG FIELDS.



DIRECT COUNTS
(% OF HIGHEST PLATE COUNT)

	LOWER LIMIT	AVERAGE	UPPER LIMIT
ROCKFISH	99%	113%	124%
CRAB MEAT	77%	86%	95%
OYSTERS	82%	143%	204%

Zobell's medium in the data of Table 2 was probably an artifact. Zobell's medium 2216 is undoubtedly superior to nutrient agar for plate counts on fish products.

In a second experiment, the media listed in Table 3 were tested simultaneously with suspensions of oyster meat, crab meat, and rockfish flesh which had been held at 1° C. for 1 week. Results are shown in Table 4. There was some inconsistency but results were, in general, fairly uniform. Nutrient agar with 1.5 percent NaCl and nutrient agar prepared with aged[4/] sea water were considered of equally high value for rockfish and crab meat. Zobell's medium 2216 was next in value for these products, and probably quite satisfactory for most needs. Other media were grouped below these in value. Reuszer's (1933) peptone-glucose-phosphate medium was of distinctly inferior quality since it gave the lowest counts, and the colonies were small and difficult to count.

4/ Aging filtered sea water for 2 months in the dark reduces its organic content to a minimum, and thus gives sea water from widely different sources approximately the same composition. Such a treatment was necessary in a study such as this, so that the medium would be of reasonably constant composition. (See Zobell, 1941).

Table 1 - Comparison of Difco Nutrient Agar and Zobell's (1941) Medium 2216 for Plate Counts on Various Fish Products

Medium	Colony Count					
	Fresh live little neck clams	Gills from fresh pink salmon (river caught)	Fresh Pacific oysters	Fresh Dungeness crab meat	Fresh sole fillet	Spoiled sole fillet
	Bacteria/gm.	Bacteria/gm.	Bacteria/gm.	Bacteria/gm.	Bacteria/gm.	Bacteria/gm.
Difco nutrient agar	4,200	905,000	83,500	1,320,000	338,000	740,000,000
#2216 (Zobell, 1941)	147,000	4,150,000	134,500	1,360,000	760,000	930,000,000
Ratio of count on #2216 to count on nutrient	35	4.7	1.6	1.03	2.25	1.26

With oysters, results were slightly different. However, nutrient agar with 1.5 percent NaCl was a good medium. An implication that "fresh water" bacteria might have been present was found in the fact that fish extract agar (mostly fresh water) and Difco nutrient agar (fresh water) were also good media. Nutrient agar in aged sea water and tryptone glucose extract agar were next in value, while other media were grouped below these. Reuszer's medium was again shown to be the least satisfactory.

Table 2 - Comparison of Difco Nutrient Agar and Zobell's (1941) Medium #2216 for Plate Counts on Sole in Various Stages of Spoilage

Odor raw flesh[1]	Storage time in ice	Colony Count		Ratio of count on 2216 to count on nutrient agar
		Nutrient agar	Zobell's 2216	
	Days	Bacteria/gm.	Bacteria/gm.	
Fresh (rigor) (fillet 1)...	0	12,000	9,900	0.83
Flat (fillet 2)	7	15,400	18,600	1.2
Slightly stale (fillet 3)..	7	72,000	108,000	1.5
Slightly stale (fillet 4)..	11	1,510,000	2,050,000	1.36
Stale (fillet 5)	11	1,560,000	4,000,000	2.56
Putrid (fillet 6)	17	3,830,000	4,100,000	1.06
Putrid (fillet 7)	17	6,600,000	10,400,000	1.50

1/ Average opinion of 3 to 5 people.

One of the most important decisions which must be made in making a plate count is the proper medium to use. From the first experiment in which Zobell's medium was compared with nutrient agar, it would seem that the former was the obvious choice. However, when in the second experiment these media were compared with several others, Zobell's medium was not the best. Nutrient agar with 1.5 percent NaCl was consistently good for oysters, crab, and fish muscle. However, nutrient agar made up with sea water was in some cases even better, and in all other cases nearly as good. Nutrient agar made up with aged sea water was chosen for the tentative method on the basis of these results. This medium differs from Zobell's medium 2216 only in the substitution of beef extract for ferric phosphate and in the reduction of the pH of the medium to below 7.0. It is probable that Zobell's medium might be equally as good if its pH were reduced to a value below neutrality (see Figure 4 in which the effect of reduction of its pH is shown.)

Table 3 - Media Tested in Parallel to Evaluate Acceptability for Plate Count

No.	References	Formula
1	Gibbons (1934)	Difco nutrient agar plus 1.5% NaCl
2	Newton (1924) Zobell (1941)	Difco nutrient agar made up with sea water aged in glass in the dark 2 months or more
3	Tarr and Bailey (1939) and others	Difco Bacto nutrient agar Beef extract 0.3% Peptone 0.5% Agar 1.5% Glass dist. water
4	Zobell (1941)	#2216 Peptone 0.5% Ferric Phosphate 0.01% Agar 1.5% Sea water aged in glass in the dark 2 months or more
5	Reed and Spence (1929)	Rockfish muscle 500 gm. Peptone 10 gm. Agar 15 gm. Sea water 100 cc. Tap water 900 cc. Infuse fish in water 12 to 15 hours. Boil 10 minutes. Filter. Add other ingredients.
6	Reed and Spence (1929) (modified)	Same as 5 but substitute sea water for tap water
7	Kiser (1944)	Bacto peptone 0.5% Yeast extract 0.2% Ferric phosphate 0.01% Bromthymol blue 0.0015% Sea water (aged)
8	Zobell (1941)	Proteose peptone 0.2% Peptone 0.3% Beef extract 0.2% Agar 1.5% Sea water (aged)
9	Reuszer (1933)	Peptone 0.1% Glucose 0.1% K_2HPO_4 0.005% Agar 1.5% Sea water (aged)
10	Bedford (1933)	Gelatin 1.0% Peptone 1.0% Glucose 1.0% NaCl 2.9% K_2HPO_4 0.025% Agar 1.5% Tap water
11	Gibbard, et. al. (1947)	Beef extract 0.3% Tryptone 0.5% Glucose 0.1% NaCl 1.0% Agar 1.5% Glass dist. water

Table 4 - Evaluation of Media Described in Table 3. Media Were Tested in Parallel and Are Listed in Order of Decreasing Count.

Oyster Meat			Crab Meat			Rockfish Flesh		
Medium No.	Average of 4 plates	Range	Medium No.	Average of 4 plates	Range	Medium No.	Average of 4 plates	Range
	Bact./gm.	Bact./gm.		Bact./gm.	Bact./gm.		Bact./gm.	Bact./gm.
1	158	139-170	1	116	92-148	2	204	185-217
5	130	121-141	2	108	99-130	1	197	183-208
3	115	78-171	4	42	28-55	4	183	178-190
2	92	76-109	3	40	33-46	8	173	138-208
11	82	69-100	7	40	21-55	7	159	143-177
8	73	54-96	8	40	23-56	11	155	140-167
6	67	53-92	5	38	31-46	3	145	140-150
4	62	55-71	6	38	30-49	10	142	136-149
7	56	49-72	11	35	30-42	6	138	125-151
10	47	39-50	10	33	24-41	5	131	118-145
9	22	17-25	9	16	14-18	9	111	102-119

Incubation Temperature

Various workers[5/] have shown that in the majority of instances, the temperature of optimum growth for fish spoilage and other marine bacteria lies between

5/ See Bedford (1933), Fellers (1926), Gibbons (1934), Hess (1934), Kiser (1944), Shewan (1942), Shewan (1945), Tarr (1941), Zobell (1941), Zobell and Conn (1940), and Zobell and Upham (1944).

20° C. (68° F.) and 25° C. (77° F.). Regardless of what fishery product has been examined, no one has ever found a bacterium which produces its most rapid rate of growth at a temperature lower than 20° C. Also, only a small proportion of such bacteria will grow at temperatures above 30° C. (86° F.). Shewan (1945) stated that in the slime and gills of fresh fish, only 0.5 percent of the bacteria growing at 20° C. would grow at 37° C., and that if this percentage increased, such increase indicated that contamination from human sources was high. In fact, Zobell and Conn (1940) found the sensitivity of marine bacteria to high temperatures to be such that plating with the medium at 45° C., which is the accepted temperature at which plates are generally poured, gave only 81 percent to 83 percent as many bacteria as plating at 30° C.

An investigation at this laboratory on the incubation temperature for total bacterial counts in fish products indicates (Table 5) that when spoiling sole is examined, plates incubated at 25° C. give much higher counts than those incubated at 37° C.

Table 5 - Comparison of 25° C. and 37° C. as Incubation Temperature for Plate Counts on Spoiling Sole

Sample number	Time fish were held in ice	Colony Count	
		Plates incubated 4 days at 25° C. (77° F.)	Plates incubated 2 days at 37° C. (99° F.)
	Days	Bacteria/gm.	Bacteria/gm.
A	10	585,000	3,000
	15	7,150,000	2,440
B	5	305,000	34,800
	10	5,700,000	3,000
	12	3,350,000	1,600

In another study, a test was made to determine whether or not temperature of storage during the spoilage period causes a variation in flora which would change the proportion growing at 37° C. as opposed to 25° C. Homogenous samples of rockfish were held at 3° C. and 25° C. Platings were made at intervals during the spoilage period, and replicate plates were incubated at 25° C. and 37° C. Table 6 gives the results of this study. For fish that spoiled at 3° C., plates held 18 days at 3° C. or 5 days at 25° C. gave equally high counts. On the other hand, 37° C. was a totally unsatisfactory incubation temperature, because in some instances counts actually fell as spoilage progressed. For fish that spoiled at 25° C., incubation of plates at 25° C. gave the highest count, as would be expected. Plates incubated at 3° C. gave somewhat lower counts, and those incubated at 37° C. also gave low counts, but, as would be expected, not nearly as low as with fish spoiling at 3° C.

In a third study, results exactly parallel to those described above were obtained. In this experiment, however, a sample of rockfish flesh was allowed to spoil at 37° C. as well as at the lower temperatures. It was found that when plates from fish spoiling at the higher temperature were incubated in parallel at 21° C. and 37° C., almost identical counts were obtained at the two temperatures. (See Table 7).

It is concluded from this study of incubation temperature that the temperature at which fish spoil determines to a large extent what types of bacteria predominate in the spoilage. This is indicated by the almost total absence of 37° C. growers in the fish spoiling at 3° C., by the presence of a fairly large proportion of these

Table 6 - Effect of Plate Incubation Temperature and Spoilage Temperature on Plate Counts of Rockfish Fillets[1/]

Sample	Spoilage Temp.	Storage Time	Plate Count		
			Incubation Temp. 3° C. (37° F.)	Incubation Temp. 25° C. (77° F.)	Incubation Temp. 37° C. (99°F.)
	Degrees C.	Days	Bacteria/gm.	Bacteria/gm.	Bacteria/gm.
A	25	0	1,715,000	1,170,000	148,500
	25	1	535,000,000	790,000,000	235,000,000
B	25	0	52,500	139,500	3,400
	25	1	41,450,000	89,500,000	51,000,000
A	3	0	1,715,000	1,170,000	148,500
	3	1	2,035,000	1,660,000	7,000
	3	2	191,000	1,310,000	14,050
	3	4	8,600,000	8,700,000	116,000
	3	7	53,000,000	56,000,000	38,500
B	3	0	52,500	139,500	3,400
	3	1	220,000	183,500	29,450
	3	2	340,300	324,500	2,000
	3	4	6,450,000	4,400,000	33,500
	3	7	69,000,000	72,000,000	8,000

1/ Samples A and B were homogenous ground mixtures held in sterile beakers at the two different spoilage temperatures after the initial sample was taken for count. The two samples were taken from widely separated fish filleting houses in Seattle.

organisms in the fish spoiling at 25° C., and by the predominance of 37° C. growers in fish spoiling at 37° C. It was further concluded that incubation temperatures of 20° C. to 25° C. are best for plate counts on unprocessed fish, since incubation in this range gave highest counts in all cases.

Table 7 - Effect of Incubation Temperature on Counts for Fish Spoiling at 37° C. (99° F.)

Storage time of fish at 37° C.	Plate Count	
	Incubation temp. 25°C.(70°F.)	Incubation temp. 37°C.(99°F.)
Hours	Bacteria/gm.	Bacteria/gm.
4	40,000	41,700
6	87,300	85,000
8	102,000	103,000
12	1,100,000	1,110,000

The following paragraph appears in Recommended procedure for the bacteriological examination of shellfish and shellfish waters (Gibbard et. al., 1947):

> "A standard colony count of shellfish samples has been found to be of value as an index of general sanitation and refrigeration."

In the method described in that publication, 37° C. is recommended as an incubation temperature. Because 37° C. appeared to be a relatively high temperature of incubation, a single experiment with shellfish was run to determine whether 37° C. or 25° C. gave the higher count. A sample of Pacific oysters which had been obtained freshly shucked at a commercial plant, and then held 8 days in the refrigerator was plated. All plating technics were according to the recommended method described in the above publication, but replicate groups of plates were incubated at 25° C. as well as at 37° C. Results in Table 8 show that plates incubated at 25° C. had 8.2 times as many colonies as those incubated at 37° C. These results indicate that this portion of the standard methods procedure should be investigated further.

Table 8 - Effect of Incubation Temperature on Plate Counts[1] of Pacific Oysters[2]

Incubation temperature		Incubation time	Plate count average of 4 plates
Centigrade	Fahrenheit		
degrees	degrees	days	Bacteria/gm.
25	77	7	82,000,000
37	99	2[3]	10,000,000

1/ Plate counts were made by the standard method described by Gibbard, et. al. (1947).
2/ Oyster meats were stored 1 week at 1° C. (34° F.)
3/ In an earlier experiment it was found that plates incubated at 37° C. for more than two days showed no increase in count up to 7 days at which time the medium had dried out markedly.

Time of Incubation

In order to determine the optimum incubation time for plates incubated at 25° C., one series was removed from the incubator at daily intervals for counting. To prevent contamination at the counting period, the lids of the plates were not removed. The count did not change perceptibly between 3 and 17 days' incubation. However, at 3 days many colonies were very small, which made counting difficult, and after 10 days, colonies often became so large that they fused together. Thus 4 to 7 days was considered best incubation time for bacterial counts on fish, and the 7-day period was preferred for ease of counting.

DISCUSSION

The data presented in this report are of a limited nature. More work must be done before any method for bacterial counts on fish products can be called "standard." The data are presented here merely as a basis for further work.

Shown below is the tentative method which has been developed from a consideration of these data. Because most of the work was done with fish flesh, the method is not necessarily recommended for shellfish.

> To 150 grams of chilled fish flesh or crab meat in a sterile blender jar, add 150 cc. of autoclaved chilled sea water. Blend for 3 minutes. Weigh 20 grams into a tared dilution bottle containing 80 cc. of chilled autoclaved sea water and shake for 3 to 4 minutes, but be certain that all clumps have been broken up. Transfer by pipet to subsequent dilutions in chilled, autoclaved sea water, and shake each dilution 3 to 4 minutes. Plate as promptly as possible, but if plating is delayed more than a few minutes, refrigerate the dilutions to be plated to prevent growth of the micro-organisms, and shake the suspensions slightly before plating. To plate, pipet 1 cc. of the suspension to petri dishes in triplicate and pour Difco nutrient agar made up with aged sea water, and with a pH between 6.0 and 7.0, into the plates and mix. The temperature of the medium should not be above 42° C. at the time of pouring. After the medium has solidified, invert the plates, incubate them 4 to 7 days (preferably 7 days) at 20 to 25° C., and count colonies. (Material of very low count may be weighed directly from the blended mass to the petri dishes without further dilution.)

SUMMARY

This report is a preliminary study of methods for determination of the total bacterial plate count on fishery products.

Blending a fish sample gives better reproducibility than does grinding and shaking. The temperature rise which occurs on blending the sample with the diluent might reduce the number of bacteria unless the sample and diluent are chilled

before being blended. Dilutions should be shaken 3 to 4 minutes. Settling after the shaking period is not a serious problem. Sea water was the most satisfactory diluent found, while many other commonly accepted diluents were distinctly toxic to fish spoilage organisms, some killing off more than 90 percent of the bacteria present. The pH of the medium should fall slightly below neutrality, since alkaline media reduce counts markedly. The choice of medium is difficult, but Difco nutrient agar made up with aged sea water proved satisfactory and is suggested for the tentative method.

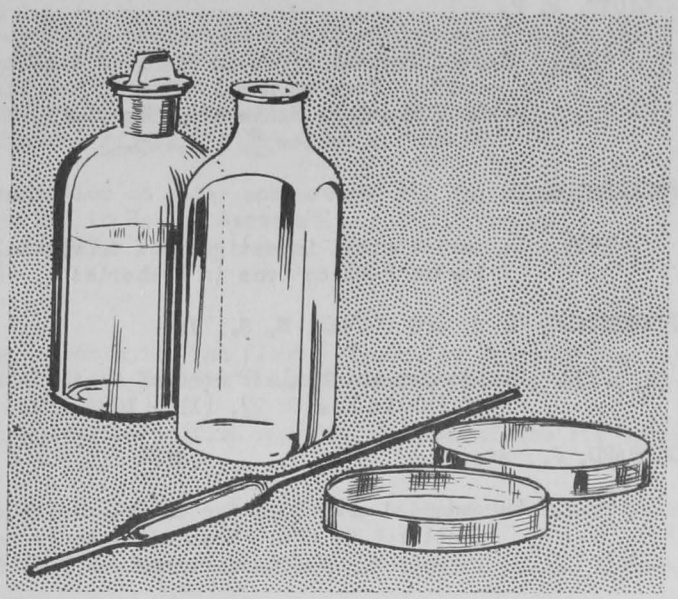


Plate incubation should be at 20° C. to 25° C. for 4 to 7 days. It was found that incubation in this temperature range gave higher counts than did 37° C. for fish materials spoiling in the range 1° C. to 25° C. This was true of oysters as well as fish. The 37° C. temperature recommended for plate incubation in the standard plate count for shellfish (Gibbard, et. al., 1947) should be investigated.

The temperature at which fish spoil determines to a large extent what bacteria are present.

A method tentative for determination of the total bacterial plate count in fish flesh is described.

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LITERATURE CITED

BEDFORD, R. H.

1933. Marine bacteria of the northern Pacific Ocean. The temperature range of growth. Contributions to Canadian Biology and Fisheries N. S. 7, 431-438.

CASTELL, C. H.

1946. Keeping time and quality of sea food fillets. Western Fisheries 35 (7), 34 and 36.

DYER, W. J. and DYER, F. E.

1947. Spoilage of freshly cut fillets. Fisheries Research Board of Canada, Progress Reports, Atlantic Coast Stns., No. 38, 10-14 (April).

ELLIOTT, R. P.

1946. Microbiological flora in fish. A bibliography with abstracts on 3 x 5 inch cards. Free on application to the Fishery Technological Laboratory, 2725 Montlake Boulevard, Seattle 2, Washington. Announced in Commercial Fisheries Review 8, (3), 25.

FELLERS, C. R.

1926. Bacteriological investigations on raw salmon spoilage. University of Washington Publications in Fisheries 1, (8), 157-188.

FITZGERALD, G. A. and CONWAY, W. S., Jr.

1937. Sanitation and quality control in the fishery industries. American Journal of Public Health 27, (11), 1094-1101.

GIBBARD, J., et. al.

1947. Recommended procedure for the bacteriological examination of shellfish and shellfish waters. Standard methods committee for the examination of shellfish. American Journal of Public Health 37, 1121-1129.

GIBBONS, N. E.

1934. A bacteriological study of "ice fillets." Contributions to Canadian Biology and Fisheries 8, 301-310.

GRIFFITHS, F. P. and STANSBY, M. E.

1934. Significance of the bacterial count and chemical tests in determining the relative freshness of haddock. Transactions of the American Fisheries Society 64, 401-407.

HESS, E.

1934. Cultural characteristics of marine bacteria in relation to low temperatures and freezing. Contributions to Canadian Biology and Fisheries N. S. 8, 459-474.

KISER, J. S.

1944. Effects of temperature approximating 0° C. upon the growth and biochemical activities of bacteria isolated from mackerel. Food Research 9, 257-267.

NEWTON, D. E.

1924. Marine spore forming bacteria. Contributions to Canadian Biology N. S. 1, 377-400.

REED, G. B. and SPENCE, C. M.

1929. The intestinal and slime flora of the haddock--a preliminary report. Contributions to Canadian Biology and Fisheries N. S. 4, 259-264.

REUSZER, H. W.

1933. Marine bacteria and their role in the cycle of life in the sea. III. Distribution of bacteria in the ocean water and muds about Cape Cod. Biol. Bull. 65, 480-497.

SHEWAN, J. M.

1942. Some bacteriological aspects of fish preservation. Chemistry and Industry 61, 312-314.

1945. The bacteriology of dehydrated fish. I Qualitative and quantitative studies of the drying process. J. Hygiene 44, No. 3, 193-209.

Standard methods for the examination of water and sewage, 8th Ed., Seventh printing (1943). American Public Health Association, 1790 Broadway, New York, N. Y., pp. 156 and 207.

TARR, H. L. A.

1941. A direct method for counting bacteria in fish flesh. Fisheries Research Board of Canada, Progress Report for the Pacific Coast Stations, No. 49, pp. 8-10.

1943. Rise and fall of bacterial populations in fish muscle. Journal of the Fisheries Research Board of Canada 6 (2), 119-128.

------ and BAILEY, B. E.

1939. Effectiveness of benzoic acid ice for fish preservation. Journal of the Fisheries Research Board of Canada 4 (5), 327-334.

ZOBELL, C. E.

1941. Studies on marine bacteria: The cultural requirements of heterotrophic aerobes. J. Marine Research 4, 42-75.

------ and CONN, J. E.

1940. Studies on the thermal sensitivity of marine bacteria. J. Bact. 40, 223-238.

------ and UPHAM, H. C.

1944. A list of marine bacteria including descriptions of sixty species. Bull. of Scripps Inst. of Oceanography 5, (2), 239-292.

FREEZING FISH AT SEA

One of the first large scale fishing operations involving the freezing of fish at sea was undertaken by a company organized in France with headquarters at Boulogne. This firm operated a fleet of several trawlers in the North Atlantic, North Sea, and Mediterranean. All were of similar size and construction and used the same freezing system, and each had a cold-storage capacity of about 150 tons of round fish. These vessels operated successfully in freezing fish at sea until 1940 when they were seized after France was occupied by the Germans.

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