Selection of Suitable Diluents for Bacteriological Examination of Fishery Products

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For raw, iced and frozen samples of fish and prawn, significant difference was observed in total plate counts done with various diluents, the significance level ranging from 5% to 0.1%. For raw fish, N-saline, seawater and quarter strength Ringers' solution gave maximum total plate counts. In the case of iced-fish, n-saline yielded highest total plate counts. For frozen samples, however, peptone water and n-saline gave good recoveries. Trials with suitable ecombinations of diluents showed that though some of them were as good as the control, namely n-saline, none were superior in any way.

The primary objective in the quantitative assessment of microorganisms is to recover the surviving population and this in turn depends on the proper diluents used for obtaining manageable cell suspension. The suspending fluid is thought to exert some influence on microorganisms present in it. This is because a particular species or groups of organisms present in the food show varying degree of sensitivity to the inorganic ions due to hypo or hypertonic action. It has been shown that seawater possess a bactericidal effect on certain organisms such as *E. coli* (Carlucci & Pramer, 1959). On the other hand some marine bacteria show lytic tendencies when suspended in distilled water (Mac Leod, 1965)'

The effect of suspending fluid on the total viable population has been studied by many workers for a variety of materials. Butterfield (1932) studied the recovery of bacteria from riverwater using different diluents and observed better survival with phosphate buffer. Straka & Stokes (1957) claimed that the number of surviving bacteria after 20 min. in distilled water were reduced by 40–60% and in phosphate buffer 20–30% where as 0.1% peptone water permitted nearly 100% recovery even after 1 h. Sinnhuber & Lee (1964) while studying the microbioflora surviving radiation pasteurization of sea foods noted that 0.2% peptone water was superior to distilled water, 0.067 M phosphate buffer and 0.1% peptone water. Later work by Lee & Harward (1970) claimed that Butterfield's phosphate buffer is superior to 0.2% peptone water for enumeration of microorganisms from frozen sea-foods and mixed vegetables. The present study was undertaken to determine the diluent effect on bacteria of fishery origin.

Materials and Methods

Two types of fish, sardine *(Sardinella longiceps)* and mackerel *(Rastrelliger kanagurta)* and two species of prawns *(Metapenaeus monoceros* and *Parapenaeopsis stylifera)* were used in the study. Part of the sample was iced (0 ± 1°C) for 2 days and used as iced samples. Another part frozen and stored at –22°C was used as frozen samples.

Diluents: Diluents included in the study were

1. Distilled water
2. Seawater (full strength)
3. Normal saline (0.85 w/v Nacl)
4. Phosphate buffer (Butterfield, 1932 and ICMSF, 1978 Media No. 90)
5. Peptone water (0.1% peptone in dist. water)
6. Ringer's solution (Quarter strength, ICMSF (1978) media no. 97)

Additionally the following 3 combinations of these diluents were also tried.

1. Peptone saline (P.S.), (ICMSF, 1978 media no. 83)
2. Peptone phosphate buffer (P.P.B.), (0.1% peptone in Butterfields phosphate buffer)
3. Phosphate buffered saline (P.B.S., (ICMSF, 1978 media no. 91).

Normal saline (N.S.) was used as control. Comparitive studies were made for raw, iced

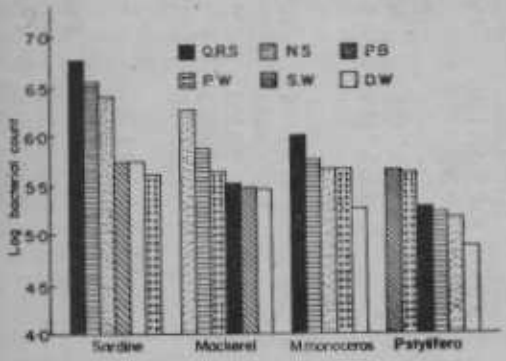

Fig. 1 Effect of single diluents of TPC of raw fish

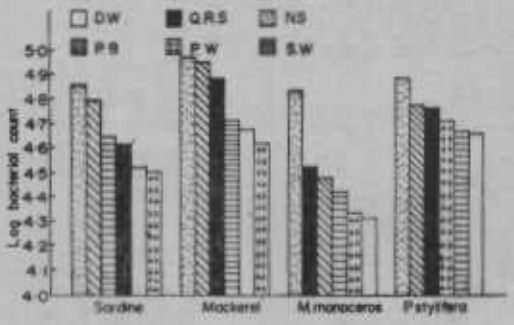

Fig. 2 Effect of single diluents on the TPC of iced-fish

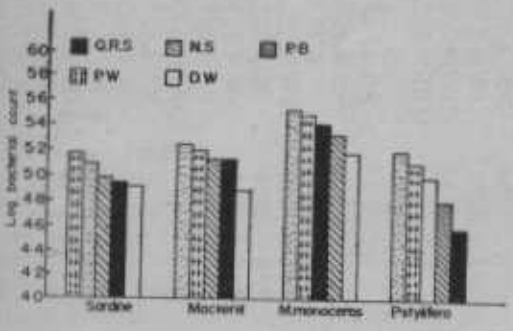

Fig. 3 Effect of single diluents on the TPC of frozen fish

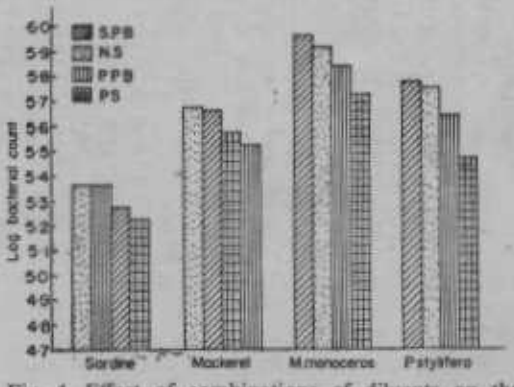

Fig. 4 Effect of combinations of diluents on the TPC of raw fish

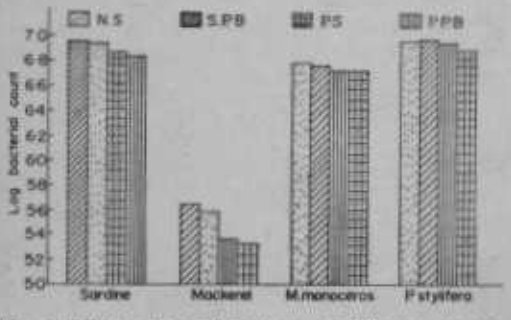

Fig. 5 Effect of combinations of diluents on the TPC of iced-fish

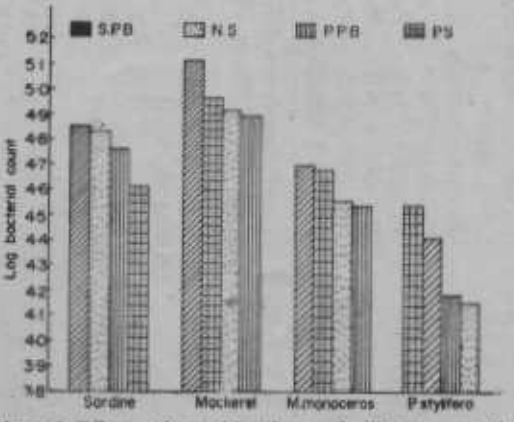

Fig. 6 Effect of combinations of diluents on the TPC of frozen fish

Table 1. *Summary of the level of significance of variance ratio of bacterial counts between diluents and between samples from the ANOVA*

	Raw fish				Iced fish				Frozen fish			
	Single		Combination		Single		Combination		Single		Combination	
	Diluent	Sample	Diluent	Sample	Diluent	Sample	Diluent	Sample	Diluent	Sample	Diluent	Sample
Sardine	P<.001	NS*	P<.01	NS	P<.01	NS	P<.001	NS	P<.05	NS	P<.05	NS
Mackerel	P<.05	NS	P<.001	NS	P<.001	NS	P<.01	NS	P<.001	P.05	P<.001	NS
M. monoceros	P<.001	NS	P<.001	NS	P<.05	NS	P<.001	P.01	P<.001	NS	P<.01	NS
M. stylifera	P<.05	NS	P<.001	NS	P<.05	NS	P<.01	NS	P<.001	NS	P<.05	NS

*NS = Not significant at 5% level.

Table 2. *Least significant difference (LSD) at 5% level and mean logarithmic bacterial count arranged in ascending order in different diluents, for raw iced and frozen fish*

		Raw fish		Iced fish		Frozen fish	
		Single	Combined	Single	Combined	Single	Combined
	LSD at 5% level	0.4834	0.0734	0.1533	0.0245	0.0595	0.1522
		PW 5.6032	PS 5.2299	PW:4.5032	PPB:6.8542	QRS 4.9081	PS:4.6120
		PB 5.7089	PBS 5.2394	DW:4.5133	PS:6.8627	DW:4.9539	PPB:4.7608
		DW 5.7250	NS 5.3609	QRS:4.6197	PBS:6.9459	PB:4.9786	PBS:4.8343
Sardine		NS 6.4117	PPB 5.3613	SW:4.6437	NS:6.9401	NS:5.1777	NS:4.8545
		SW 6.5533		PB:4.7932			
		QRS 6.7931		NS:4.8477			
	LSD at 5% level	0.4154	0.0219	0.0822	0.1130	0.0624	0.0489
		PB 5.4606	PPB 4.5184	PW:4.6130	PS:5.3251	DW:4.8997	PPB:4.8909
		DW 5.4719	PS 4.5258	DW:4.6735	PPB:5.3791	QRS:5.1110	PBS4.9124
Mackerel		QRS 5.5006	PBS 5.6642	SW:4.7083	NS:5.5929	PB:5.1514	
		PW 5.6263		QRS:4.8822			PS:4.9680
		SW 5.8530	NS 5.6739		PBS:5.6190	PW:5.2131	NS:5.0531
		NS 6.2472		PB:4.9471		NS:5.2619	
				NS:4.9749			
	LSD at 5% level	0.2314	0.0400	0.2927	0.0200	0.0821	0.0894
		DW:5.2331	PS:5.7210	DW:4.3117	PS:6.7236	DW:5.2105	PPB4.5328
		PW:5.6645	PPB:5.8289	PW:4.3375	PPB:6.7264	PB:5.3450	PBS:4.5435
		NS:5.6978	NS:5.9075	SW:4.4224	PBS:6.7707	QRS:5.4308	PS:4.6765
M. monoceros		SW:5.7428	PBS:5.9696	PB:4.4877	NS:6.7911	PW:5.4962	
		QRS:5.9937		QRS:4.5378		NS:5.5491	NS:4.6994
				NS:4.8373			
	LSD at 5% level	0.4156	0.0580	0.1026	0.0303	0.1384	0.2252
		DW:4.8717					
		NS:5.1889	PS:5.4709	DW:4.6545	PS:6.8730	QRS:4.5853	PBS:4 1079
		SW:5.2022	PPB:5.6472	SW:4.6645	PPB:6.9359	PB:4.8018	PPB:4.1996
		SW:5.2022	NS:5.7573	PW:4.7070	NS:6.9524	DW:5.0040	NS:4.3988
P. stylifera		QRS:5.2811	PBS:5.7788	PB:4.7417	PBS:6.9532	PW:5.0970	PS:4.5252
		PW:5.6269		QRS:4.7610		NS:5.2056	

and frozen fish and depending on the bacterial flora expected in the products, some minor changes were made in the selection of diluents. For raw fish and iced ones which harbour marine flora, seawater was included in the study. For frozen fish which are expected to carry more terrestrial flora than marine, seawater was replaced by phosphate buffer.

Preparation of inoculum: To maintain uniformity of the sampling material 50 g of the muscle was dry-grinded into a paste aseptically and 10g lots of this material was transferred to 5 sterile blenders and homogenised with 90 ml of five diluents under study. Further decimal dilutions were prepared in 9 ml aliquots of the respective diluents.

The serial dilutions were then pour plated and incubated at room temperature (29±1°C) for 48 h and bacterial counts estimated. Media and conditions used in this procedure were the same as that of Thampuran *et al* (1981).

Results and Discussion

To make a comparitive study of the total plate counts (TPC) of raw, iced and frozen fish the data were analysed by analysis of variance after converting bacterial counts into log values. The level of significance of variance ratios 'between samples' and 'between diluents' of the raw iced and frozen fish and prawn are summerized in Table 1. In raw, iced and frozen, samples significant difference existed in bacterial count 'between the diluents' in single and in combination, the significance level ranging from 5 to 0.1%. 'Between samples', no significant difference was noted at 5% level. Two exceptions to this were mackerel (single diluent) and *M. monoceros* (Combination of diluents).

The least significant difference (LSD) at 5% level and the mean logarithmic counts in ascending order are presented in Table 2.

Figures 1 to 6 show the effect of diluent on bacterial counts estimated on raw iced and frozen samples of two types of fish and two species of prawns. The values used in the histograms represent the average of three trials.

For reaching the final conclusion, the overall average of the log values of the bacterial counts of the two types of fishes and two species of prawn were taken. This is given in Table 3. This shows that for raw fish using single diluent, the highest recovery is obtained with Ringer's solution, followed by n-saline and seawater. Lowest count was obtained when distilled water was used. In the case of iced-fish, highest count was noted when n-saline was used. Frozen fish showed maximum count with n-saline and this was closely followed by peptone water and phosphate buffer.

Table 3. *Average log value of bacterial count*

Diluent	Raw fish		Iced fish		Frozen fish	
	S*	C*	S	C	S	C
D.W.	5.33	—	4.56	—	5.01	—
S.W.	5.84	—	4.62	—	—	—
N.S.	5.88	—	4.88	—	5.30	—
P.W.	5.62	—	4.54	—	5.25	—
P.B.	5.60	—	4.75	—	5.07	—
Q.R.S.	5.89	—	4.70	—	5.01	—
P.S.	—	5.24	—	6.45	—	4.69
P.B.S.	—	5.66	—	6.57	—	4.75
P.PB.	—	5.33	—	6.47	—	4.60
Control (N.S.)	—	5.67	—	6.57	—	4.60

S = Single diluent
C = Combination of diluents

The combinations of diluents were employed to see whether there was any synergistic effect in combining the ingredients of the single diluents. Table 3 indicates that for raw and iced fish, phosphate buffered saline came very near to the control n-saline in bacterial recovery. For frozen fish phosphate buffered saline showed a higher count than n-saline.

Thus it is clear that diluents exert a major role in the quantitative estimation of the bacteria. The variations in count obtained by using these diluents might be due to the difference in the background flora of the sample or due to sensitivity of species of the

bacteria to these diluents. Hoadley & Cheng (1974) while studying the recovery of *Pseudomonas aeruginosa, Streptococcus faecalis* and *Escherichia coli* showed that tap water was highly toxic to all the strains. While recovery of *P. aeruginosa* was most successful when phosphate buffer was used, it had no effect on improving the count of *E. coli* and *S. faecalis*. Gray *et al* (1977) suggested that the diluents effect was ionic and not osmotic. Cations enhanced the protective property of the diluent and divalent cations were superior in this respect.

It can be concluded that in qualitative studies where sensitivity rather than reproducibility of the results is the deciding factor, the selection of a most suitable diluent is very essential. It should be noted that n-saline and phosphate buffer uniformly gave good results with raw, iced and frozen fish. Hence in routine works such as follow up studies in a processing or production line, a diluent such as saline or phosphate buffer can be used provided the composition of the diluent is definite and other parameters like media, incubation temperature and period are strictly followed.

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