The Bacteriology of Oil Sardine (Sardinella longiceps) and Mackerel (Rastrelliger kanagurta) from Tropical waters off Cochin. II. Qualitative Aspects*

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80% of the flora of skin, gills and intestines of oil sardine and mackerel at isolation temperature 28 ±2°C consisted of Gram negative asporogenous rods or cocci, belonging to the genera *Vibrio, Pseudomonas, Moraxella, Acinetobacter* and *Flavobacteria/ Cytophaga*. Nearly 10% of the flora was constituted by Gram positives, *Micrococcus* and *Arthrobacter*. Incubation temperature of 36± 1°C recovered more *Vibrio* spp. and Gram positives, while at lower temperatures of 8±1°C and 1±1°C, more *Pseudomonas, Acinetobacter* and *Moraxella* spp. were recovered. Significant changes with respect to season were observed in the relative distribution of different genera.

Fish harbours heavy loads of bacteria on their skin and adhering slime, gills and intestines. The bacterial flora of fish show considerable variation with the environmental conditions in which the fish live. Spoilage of fish, post mortem, is mainly due to bacterial action. In order to develop suitable methods of control of bacterial spoilage, a thorough knowledge of the bacteriology of fish is imperative. Exhaustive studies were carried out on the bacterial flora of fishes from temperate waters (Liston, 1956, 1957, Georgala, 1958; Dyer, 1947, Liston & Colwell, 1963; Shewan, 1961). However, such informations pertaining to tropical fishes are rather very limited. Invsetigations of Wood (1940, 1950 & 1953) on the bacterial flora of the fishes caught in the warmer waters off Australia have showed a preponderence of Gram positive types. Some preliminary studies on the bacteriology of fish on the east and west coasts of India were made by Venkataraman & Sreenivasan (1952 & 1954). Karthiayani & Iyer (1967 & 1971) studied the flora of oil sardines caught off Cochin, but, their findings were limited to the mesophilic types only. In a previous communication on the bacteriology of oil sardine and mackerel (Surendran & Gopakumar, 1982) the quantitative aspects were reported and the present paper, deals with the qualitative aspects, with special reference to seasons.

Materials and Methods

Ocean fresh oil sardine and mackerel were transferred to large wide mouthed sterile glass bottles and immediately brought to the laboratory, keeping them under ice (within 2–4 h after catch.)

Total bacterial counts of the skin, gills and intestines were determined as described by Surendran & Gopakumar (1982). Cultures from sea water agar (SWA) plates were isolated. For isolation of cultures, usually all the colonies from the plate whose count fell between 30 and 60 were aseptically picked and transferred to sea water peptone (SWP) tubes. In cases where plates with a count less than 60 colonies were not available, about 30–60 colonies were picked from each such plate, taking care to pick colonies of different colony morphology proportional to their relative distribution in the plate. The inoculated tubes were incubated at two different temperatures, those from plates at 1±1° C and 8±1° C were incubated at 8±1° C (for 7 to 10 days) and the ones from plates at 28±2 °C and 36±1 °C were incubated at 28±2 °C for 2 to 4 days. Cultures that showed positive growth after the incubation periods mentioned were transferred to SWA

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slants and incubated at the above two temperatures in the respective cases. Each isolate was re-streaked three times to ensure purity before their morphological and biochemical characteristics were studied. Pure cultures were maintained on SWA slants at 8±1 °C for subsequent studies. Morphological, biochemical and cultural characteristics of the cultures were determined and the cultures were differentiated upto the generic level, as described by Surendran (1980) and Surendran & Gopakumar (1981).

Results and Discussion

1. *Generic distribution of bacteria*

Tables 1 to 6 present typical data on the generic distribution of bacteria on the skin and in the gills and intestines (with contents) of oil sardine and mackerel, at four different temperatures. Majority of the flora of oil sardine consisted of Gram negative asporogenous rods or cocci, belonging to *Vibrio, Pseudomonas, Moraxella, Acinetobacter* and *Flavo-bacteria/Cytophaga* (Tables 1, 2 and 3) and constituted more than 80% of the flora of skin, gills and intestines, at the isolation temperature of 28±2°C. *Vibrio* accounted for 30% of the flora on skin, followed

Table 1. *Generic distribution of bacteria on the skin of newly caught oil sardine*

	Percentage of total isolates from SWA plates			
	Temperature of isolation			
	28± 2°C	8± 1°C	36± 1°C	1± 1°C
Vibrio	30	12	48	8
Pseudomonas	22	36	6	42
Moraxella	8	12	6	14
Acinetobacter	20	24	12	22
Flavobacteria/ Cytophaga	2	3	4	2
Arthrobacter	4	1	7	1
Micrococcus	6	8	12	5
Bacillus	1	0	1	0
Aeromonas	1	1	0	0
Photobacterium	2	0	0	0
Unidentified	4	3	4	5
Number of colonies identified	86	78	69	82

Table 2. *Generic distribution of bacteria in the gills of newly caught oil sardine*

	Percentage of total isolates from SWA plates			
	Temperature of isolation			
	28± 2°C	8± 1°C	36± 1°C	1± 1°C
Vibrio	18	10	38	6
Pseudomonas	16	36	10	50
Moraxella	17	14	12	15
Acinetobacter	28	24	16	19
Flavobacteria/ Cytophaga	4	5	2	4
Arthrobacter	3	0	4	0
Micrococcus	10	8	12	5
Bacillus	0	0	1	1
Aeromonas	1	1	0	1
Photobacterium	1	1	0	0
Unidentified	2	1	5	0
Number of colonies identified	110	92	88	102

Table 3. *Generic distribution of bacteria in the intestines with contents of newly caught oil sardine*

	Percentage of total isolates from SWA plates			
	Temperature of isolation			
	28± 2°C	8± 1°C	36± 1°C	1± 1°C
Vibrio	16	9	24	8
Pseudomonas	28	40	16	56
Moraxella	24	18	20	16
Acinetobacter	6	8	8	9
Flavobacteria/ Cytophaga	12	10	4	5
Arthrobacter	2	0	6	0
Micrococcus	8	9	12	5
Bacillus	0	0	1	0
Aeromonas	1	1	0	0
Photobacterium	1	0	0	0
Unidentified	2	6	9	1
Number of colonies identified	128	103	92	98

Table 4. *Generic distribution of bacteria on the skin of newly caught mackerel*

	Percentage of total isolates from SWA plates			
	Temperature of isolation			
	28± 2°C	36± 1°C	8± 1°C	1± 1°C
Vibrio	36	43	21	12
Psedomonas	16	7	36	51
Moraxella	8	6	11	7
Acinetobacter	16	18	14	10
Flavobacterium/ Cytophaga	4	2	4	5
Arthrobacter	2	4	1	0
Micrococcus	6	10	7	8
Bacillus	5	6	1	0
Aeromonas	1	0	1	1
Photobacterium	0	0	0	1
Yeast	4	2	3	2
Unidentified	2	2	1	3
Number of colonies identified	90	78	94	110

Table 5. *Generic distribution of bacteria in the gills of newly caught mackerel*

	Percentage of total isolates from SWA plates			
	Temperature of isolation			
	28± 2°C	36± 1°C	8± 1°C	1± 1°C
Vibrio	24	38	16	8
Pseudomonas	32	18	46	56
Moraxella	8	7	10	8
Acinetobacter	10	10	14	12
Flavobacterium/ Cytophaga	5	2	5	3
Arthrobacter	3	5	2	0
Micrococcus	8	9	5	7
Bacillus	1	2	0	0
Aeromonas	2	0	1	1
Photobacterium	2	0	0	0
Yeast	2	1	0	0
Unidentified	3	6	1	5
Number of colonies identified	147	88	106	85

Table 6. *Generic distribution of bacteria in the intestines with contents of newly caught mackerel*

	Percentage of total isolates from SWA plates			
	Temperature of isolation			
	28± 2°C	36± 1°C	8± 1°C	1± 1°C
Vibrio	29	44	18	11
Pseudomonas	19	9	32	47
Moraxella	17	12	14	12
Acinetobacter	12	8	11	14
Flavobacterium/ Cytophaga	6	3	4	4
Arthrobacter	2	4	1	0
Micrococcus	8	12	10	8
Bacillus	0	1	0	0
Aeromonas	1	0	1	0
Photobacterium	2	0	0	0
Yeast	2	3	1	0
Unidentified	2	4	8	4
Number of colonies identified	85	110	98	76

by *Pseudomonas* (22%). *Acinetobacter* (20%), *Moraxella* (8%) and *Flavovacteria/Cytophaga* (2%). Of the Gram positives, which formed 11% of the flora of the skin, *Micrococcus* constituted 6%, followed by *Arthrobacter* (4%). The relative distribution of different genera is influenced by the temperature of incubation. At higher temperature, namely, 36±1°C, the proportion of *Vibrio* increased considerably, while both *Pseudomonas* and *Acinetobacter* decreased greatly. Further, the Gram positives increased to 20%, *Micrococcus* accounting for 12% and *Arthrobacter* 7%. When the incubation temperature was lowered, the proportion of *Pseudomonas* increased very much, accounting for 36% of the flora at 8±1° C and 42% at 1±1° C. Similarly the proportion of *Acinetobacter* and *Moraxella* also increased. Thus, *Acinetobacter* constituted 24% of the flora at 8±1° C and 22% at 1±1° C and *Moraxella* 12% of the flora at 8±1° C and 14% at 1±1 C. But, there was a significant fall in the proportion of *Vibrio* at lower incubation temperatures, constituting only 12% of the flora at 8±1° C and 8% at 1±1° C.

The proportions of *Vibrio* in the flora of gills and intestines of oil sardines at 28±2 °C were less, compared to that of the skin flora, whereas, *Acinetobacter* and *Moraxella* were more in the gills and *Pseudomonas* and *Moraxella* in the intestines. In the flora of gills, *Acinetobacter* constituted 28%, *Pseudomonas* 16%, *Moraxella* 17% and *Vibrio* 18%. 28% of the flora of intestines was composed of *Pseudomonas,* 24% by *Moraxella,* 16% by *Vibrio* and only 6% by *Acinetobacter.* But 12% of the flora was comprised by *Flavobacter/Cytophaga.* The effect of incubation temperatures on the proportion of different genera was similar to that of the flora of skin. Higher incubation temperature was favouring the recovery of *Vibrio* and Gram positives while lower incubation temperature recovered more of *Pseudomonas.* It is significant to note that at 1±1 °C, 42%, 50% and 56% of the flora of the skin, gills and intestines respectively of oil sardines, were constituted by *Pseudomonas* alone.

As in oil sardines, more than 80% of the flora of skin, gills and intestines of mackerel was comprised of Gram negative, asporogenous rods or cocci at 28±2 °C. These were constituted by *Vibrio, Pseudomonas, Moraxella, Acinetobacter* and *Flavobacteria/ Cytophaga.* The share of Gram positives was nearly 10% of which *Micrococcus* formed the major constituent. Vibrio accounted for 36% of the flora of the skin, 24% of the gills flora and 29% in intestines with contents. *Pseudomonas* constituted 32% of the flora of the gills, but only 16% and 19% of the flora of skin and intestines respectively. The proportion of *Moraxella* was more in intestines (17%), compared to skin and gills (8% each). *Acinetobacter* accounted for 16%, 10% and 12% respectively of the flora of skin, gills and intestines. *Flavobacteria/Cytophaga* remained at 4 to 6% in them. The effect of incubation temperature on the selection of various genera of bacteria from skin, gills and intestines of mackerel was more or less similar to that observed in oil sardines, higher temperature (36±1°C) favouring the selection of *Vibrio* and Gram positives and lower temperatures favouring *Pseudomonas.* At 36±1°C, *Vibrio* accounted for 43% of the skin flora, 38% of the gills flora and 44% of the intestinal flora. The proportions of *Pseudomonas* at 8±1°C were 36%, 46% and 32% of the flora of skin, gills and intestines respectively. At 1±1°C the proportion of *Pseudomonas* increased to 51%, 56% and 47% respectively in skin, gills and intestines.

A comparison of the flora of oil sardines and mackerel shows that there is virtually no difference in the qualitative composition but small differences in the relative proportions of the different genera may be visualised. Their flora were found to be very similar to the flora of the fishes from temperate waters, in that the majority of the flora was comprised of Gram negative asporogenous rods. The flora of North Sea skate and lemon sole consisted mainly of *Pseudomonas, Achromobacter (Acinetobacter-Moraxella* group), *Flavobacteria* and coryneforms (Liston, 1957). The skin flora of North Sea cod constituted mainly *Pseudomonas, Achromobacter, Flavobacteria, Vibrio* and coryneforms (Georgala, 1958). Aschehong & Vesterlius (1943) reported the flora of Norwegian winter herring to be comprised of *Pseudomonas, Achromobacter, Flavobacteria* and *Micrococcus.* Although, the bulk of the flora of the fishes from temperate seas and the flora of oil sardines and mackerel, caught off Cochin, is constituted by gram negative asporogenous rods and cocci, they differ very much in qualitative composition. The results reported in Tables 1–6 show that nearly 20 to 35% of the flora of oil sardine and mackerel at 28±2 °C is composed of *Vibrio,* whereas in North Sea and Norwegian fishes, only a small proportion is constituted by *Vibrio.*

The tropical fishes are reported to harbour chiefly Gram positive bacteria (Shewan, 1961). Thus, Wood (1953) found the skin flora of Australian teleosts to have 60% of *Micrococcus* followed by *Pseudomonas* (16%), coryneforms (12%), *Bacillus* (8%) and *Vibrio* (4%). Venkataraman & Sreenivasan (1952) reported that 55% of the skin flora and 85% of the gill flora of mackerel was constituted by *Bacillus. Achromobacter* accounted for a small portion. Karthiyani and Iyer (1967) found that the skin flora of oil sardines was comprised of *Achromobacter* (32%), *Vibrio* (27%), *Pseudomonas* (14%) and *Flavobacteria* (11%) and that the gut flora, mainly comprised of *Achromobacter* (30%), *Vibrio* (15%) and *Pseudomonas* (30%).

According to Shewan (1961), the marine environment does affect the flora of the fish and that in warmer waters off India, east coast of South Africa and Australia, a greater percentage of Gram positives (mesophilic groups like *Bacillus* coryneforms and *Micrococcus)* should occur and that the colder waters favoured Gram negatives, particularly the psychrophilic types of *Pseudomanas* and *Achromobacter* groups. But the results of the present study seems to be at variance from that of Venkataraman & Sreenivasan (1952) and is in agreement with the reports on fish from temperate waters. This also confirms the earlier reports of Karthiayani & Iyer (1967).

2. *Seasonal variations in the flora*

Figures 1 to 4 show the seasonal variations for three years, in the distribution of various genera of bacteria in skin, gills and intestines of oil sardines. Figures 5 to 8 represent the seasonal variations in the distribution of four important genera of bacteria in the skin, gills and intestines of mackerel.

Seasonal variations in the percentage of *Pseudomonas* present on the skin, gills and ntestines of sardines are shown in figure 1. High percentages as much as 50–70% of skin flora were obtained in February and May in the case of skin flora. Peak values in gill flora were obtained in April and September, whereas for intestines with contents, high proportion of *Pseudomonas* were in March, September and December.

Figure 2 represents seasonal changes in the distribution of *Vibrio* in the skin, gills and intestines of oil sardines. Peak values in skin, are obtained during June and September. In gills, peak values are in March, June–July and October–November. In intestines, high percentage of *Vibrio* were noticed in June and October–November.

Seasonal variations in the proportion of *Acinetobacter* in the skin, gills and intestines of oil sardines are presented in figure 3. Distinct peak values are seldom obtained and in most of the months, *Acinetobacter* formed nearly less than 20% of the total flora. Maximum of 30% was obtained in August in the case of skin. But for gills,

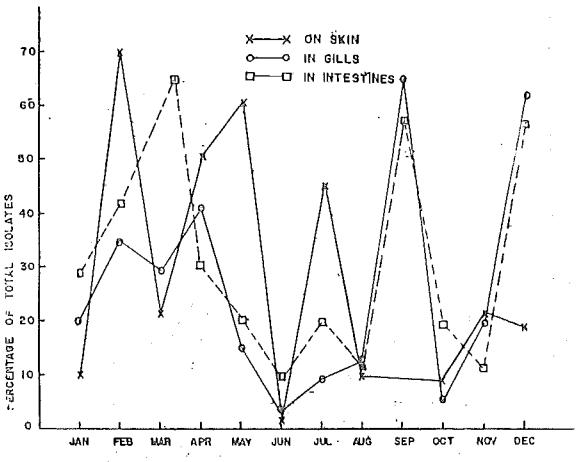


Fig. 1. Seasonal variations in the distribution of *Pseudomonas* spp. in newly caught oil sardines at 28±2° C

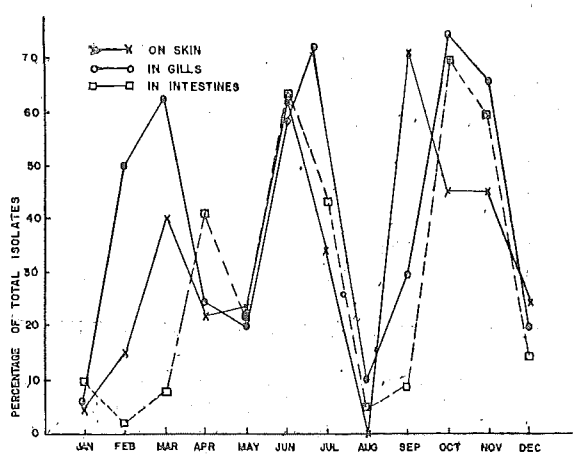
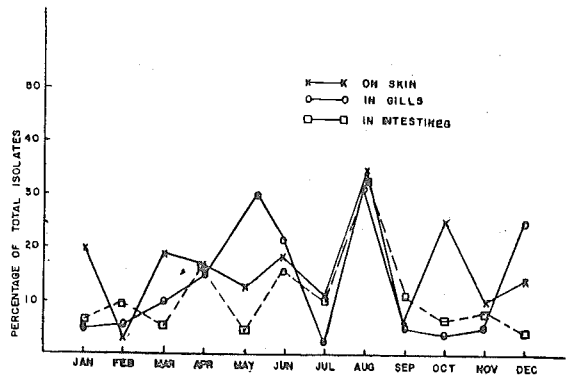


Fig. 2. Seasonal variations in the distribution of *Vibrio* spp. in newly caught oil sardines at at 28±2° C



Fig, 3. Seasonal variations in the distribution of *Acinetobacter* spp. in newly caught Oil Sardines at 28±2° C

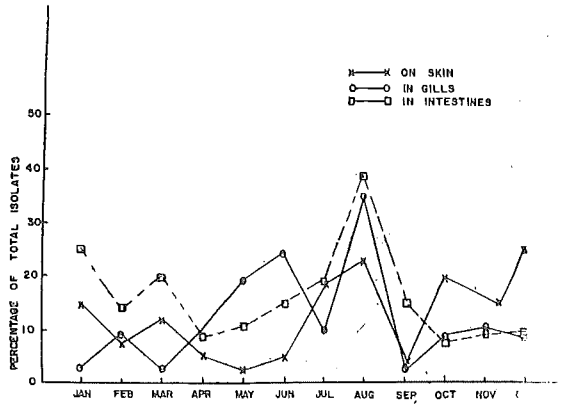


Fig. 4. Seasonal variations in the distribution of *Moraxella* spp. in newly caught oil sardines at 28+2° C

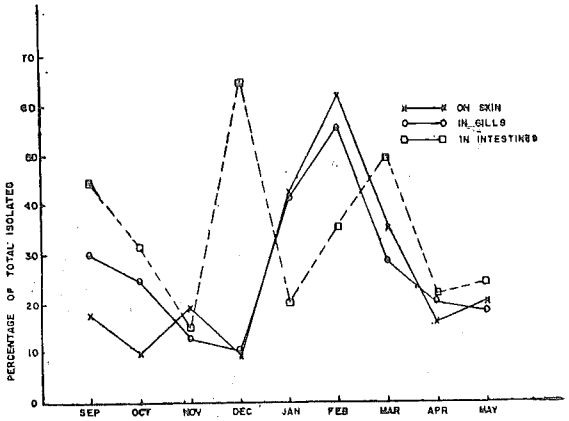


Fig. 5. Seasonal variations in the distribution of *Pseudomonas* spp. in newly caught Indian mackerel at 28±2° C

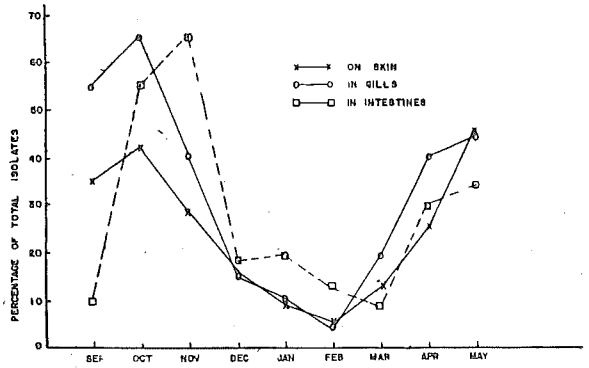


Fig. 6. Seasonal variations in the distribution of *Vibrio* spp. in newly caught Indian mackerel at 28±2° C

values as high as 30% were obtained in May, August and December. For intestines, peak value was in August.

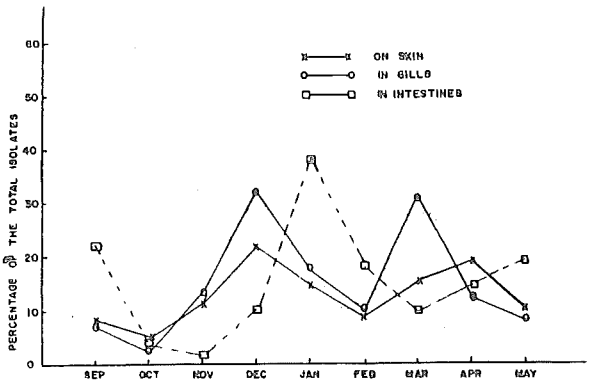


Fig. 7. Seasonal variations in the distribution of *Acinetobactor* spp. in newly eaught Indian mackerel at 28±2° C

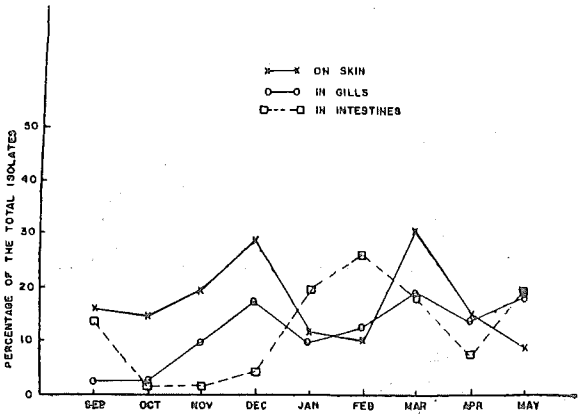


Fig. 8. Seasonal variations in the distribution of *Moraxella* spp. in newly caught Indian mackerel at 28±2° C

The seasonal variations in *Moraxella* in skin, gills and intestines of sardines, are not significant, in comparison with the seasonal changes in *Pseudomonas* and *Vibrio*. *Moraxella* comprised only 10–15% of the flora in most of the seasons. In the case of skin flora, only in August and December the highest value of 20% was obtained. For gills, peak values were in June and August and December and for intestines in January and August (Fig. 4).

The seasonal variations in the distribution of *Pseudomonas* in skin, gills and intestines of mackerel are given in Fig. 5. Wide fluctuations in the percentage of *Pseudomonas* with seasons were noticed. Peak values were in January–March season for the skin, September and January–March for gills and September, December and February–March period for intestines with contents.

Fig. 6 represents the seasonal changes in the relative distribution of *Vibrio* in mackerel. Considerable fluctuations in the distribution, varying from as low a value of 2% to as high as 72% were noticed. In the skin flora, peak values were in September–October and May. For gills, higher percentages were obtained in September–October and April–May and for intestines in October–November and April–May period.

In *Acinetobacter,* seasonal changes are not very appreciable (Fig. 7). The proportion of *Acinetobacter* in the skin flora is usally below 10–20%. But Peak values are obtained in December and April. The peak values in gill flora are given in December and March and in intestinal flora during September and January.

Moraxella is usually in the range of 2–30% of the total flora of skin, gills and intestines of mackerel (Fig. 8). Peak values for skin are obtained in December and March–April, for gills in December–January and March and for intestines in Frbruary.

From Fig. 1 to 8 it is evident that significant seasonal variations in the relative distribution of various genera of bacteria in oil sardines and mackerel, are exhibited by only *Pseudomonas* and *Vibrio.* It is interesting to note that peak values for *Pseudomonas* almost always correspond to the lowest values for *Vibrio* and vice versa. Peak values of *Pseudomonas* in the case of oil sardines are obtained in the late winter months and early summer. In mackerel, peak values of *Pseudomonas* are recorded only in the late winter months. Whereas, peak values for *Vibrio* are mainly during monsoon months in oil sardines, for mackerel peak values for *Vibrio* were obtained in monsoon and summer.

However, the changes in the distribution of *Acinetobacter* and *Moraxella* in sardines and mackerel could not be correlated to a particular seasonal phenomenon. But, the peak values in oil sardines, for both *Acinetobacter* and *Moraxella* have been noticed during August, the middle of monsoon period. In mackerel, the peak values for *Acinetobacter* was in winter period and for *Moraxella* it was in late winter and early summer.

Karthiayani & Iyer (1971) made similar observations in the distribution of *Pseudomonas* and *Vibrio* in sardines, with respect to season. Seasonal differences in the relative proportions of *Pseudomonas, Achromobacter* and *Corynebacterium* were also recorded by Shewan (1953), Liston (1955) and Georgala (1957, 1958).

According to shewan & Hobbs (1967), seasonal differences in the relative proportions of the bacterial genera are related to temperature preference of various groups of bacteria. Also changes in salinity caused by heavy rains during monsoons may contribute to variations in qualitative composition of the flora. Further, seasonal variation within a single species from the same area was almost certainly related to such factors as temperature and the presence or absence of plankton (Liston, 1956).

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References

Aschehong, V. & Vesterlius, R. (1943) *Zentr. Bakteriol. Parasitenk Abt.* II. **106,**

Dyer, F. E. (1947) *J. Fish. Res. Bd. Can.* **7,** 128

Georgala, D. L. (1957) *J. appl. Bact.* **20,** 23

Georgala, D. L. (1958) *J. Gen. Microbiol.* **18,** 84

Karthiayani, T. C. & Iyer K. M. (1967) *Fish. Technol.* **4,** 89

Karthiayani, T. C. & Iyer, K. M. (1971) *Fish. Technol.* **8,** 69

Liston, J. (1955) *A Quantitative and Qualitative Study of the Bacterial Flora of Skate and Lemon Sole Trawled in the North Sea. Ph.D. Thesis, Aberdeen University, Aberdeen, Scotland*

Liston, J. (1956) *J. Gen. Microbiol.* **15,** 305

Liston, J. (1957) *J. Gen. Microbiol.* **16,** 205

Liston, J. & Colwell, R. R. (1963) *Host and Habitat Relationships of Marine Commensal Bacteria. In "Symposium on Marine Microbiology" pp.* 611–624 *(Ed.) C. H. Oppenheimer, Charless C. Thomas (Publisher), U. S. A.*

Shewan, J. M. (1953) *Atti. Congr. Intern. Microbiol. Roma.* 7, (xxii), 361

Shewan, J. M. (1961) *The Microbiology of Sea Water Fish. In 'Fish As Food'* (Borgstrom, G. Ed) Vol. 1, p. 487, Academic Press, New York & London

Shewan, J. M. & Hobbs, G. (1967) *The Bacteriology of Fish Spoilage and Preservation. In "Progress in Industrial Mocrobiology"* (Hocherhull, D. J. Ed.) p. 169, Ilifee Books, London

Surendran, P. K. (1980) *Chemical Preservatives in Relation to Control of Microbial Changes in Fishery Products.* Ph.D. Thesis, University of Kerala, Trivandrum

Surendran, P. K. & Gopakumar, K. (1981) *Fish. Technol.* **18,** 133

Surendran, P. K. & Gopakumar, K. (1982) *Fish. Technol.* **19,** 89

Venkataraman, R. & Sreenivasan, A. (1952) *Indian. J. Med. Res.* **40,** 529

Venkataraman, R. & Sreenivasan, A. (1954) *Proc. Nat. Inst. Sci. India.* **20,** 651

Wood, E. J. F. (1940) *Studies on the Marketing of Fresh Fish in Eastern Australia. Part III. The Bacteriology of Spoilage of Marine Fish. Council Sci. Industr. Res. Austr. Rept.* No. 3, Pamp. 100, 1-92

Wood, E. J. F. (1950) *Aust. J. Mar. Freshw. Res.* **1,** 129

Wood, E. J. F. (1953) *Aust. J. Mar. Freshw. Res.* **4,** 160