

The Bacteriology of Oil Sardine (*Sardinella longiceps*) and Mackerel (*Rastrelliger kanagurta*) caught from Tropical Waters off Cochin. I - Quantitative Aspects*

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The total aerobic viable plate counts (TPCs) of skin, gills and intestine of newly caught oil sardine (*Sardinella longiceps*) and Indian mackerel (*Rastrelliger kanagurta*) at four different temperatures, namely $36 \pm 1^\circ\text{C}$, $28 \pm 2^\circ\text{C}$ (RT), $8 \pm 1^\circ\text{C}$ and $1 \pm 1^\circ\text{C}$, are reported. The total plate count at RT of the skin of oil sardine and Indian mackerel were in the range of 10^3 to 10^7 and 10^4 to 10^6 per cm^2 , that of gills in the range of 10^5 to 10^8 and 10^4 to 10^8 per g and that intestine in the range of 10^5 to 10^9 and 10^5 to 10^8 per g respectively. The TPCs were markedly affected by the incubation temperature. Incubation at $28 \pm 2^\circ\text{C}$ gave the highest count; at $36 \pm 1^\circ\text{C}$ and $8 \pm 1^\circ\text{C}$, the counts decreased by nearly 1-2 log. cycles from that at RT. Incubation at $1 \pm 1^\circ\text{C}$ registered the lowest count. The peak values for bacterial counts of these fishes occurred at different periods of the year.

Considerable amount of information is available on the quantitative and qualitative aspects of bacterial flora of fish from the Northern sea (Stewart, 1932; Liston, 1956; 1957 and Georgala, 1958), the North Atlantic (Reed & Spencer, 1929, Gibbons, 1934 and Dyer, 1947), and the Pacific (Liston & Colwell, 1963). Data regarding the fishes in tropical seas are rather limited. Wood (1940, 1950 and 1953) had studied the number and types of bacteria found in the marine fish caught in the warmer waters of Australia. In India, Venkataraman & Sreenivasan (1952, 1954) and Karthiayani & Iyer (1967, 1971) investigated the bacteriology of the fish caught in the waters off east and west coast of India. Though Karthiayani & Iyer (1971) made a detailed investigation on the bacterial flora of oil sardine caught off Cochin, their observations were limited to the mesophilic bacteria only. The present study was designed to systematically investigate all aspects of the bacterial population, both mesophiles and psychrophiles of the tropical fishes caught off Cochin, from the Arabian sea, with special reference to the seasonal changes.

Materials and Methods

Oil sardine and mackerel immediately after capture were transferred aseptically into wide mouthed sterile glass bottles and brought to the laboratory, keeping the bottles under ice (within 2-4 hours after catch).

Both nutrient agar (NA) and sea water agar (SWA) were used to determine the total aerobic viable plate count (TPC). Nutrient agar was a distilled water based medium prepared as per Salle (1954). Sea water agar consisted of 10 g bactopectone (w/v), a trace of ferric phosphate, and 15 g agar powder (Difco), dissolved in one litre aged sea water, the pH being 7.2, sterilized at 1.05 kg per cm^2 for 15 min.

Four to five fish were used for determination of bacterial count. For bacterial count of the skin, a fixed area on each side of the fish was aseptically swabed and the swab was well agitated with 10 ml of sterile aged sea water or sterile saline (0.85% NaCl in distilled water). Proper serial dilutions from this were pour-plated or spread-plated using SWA and NA.

For bacterial count of gills, 2-3 g of the gill tissue was aseptically cut and homogenised with 100 ml sterile sea water and

*Part of Ph.D. Thesis (of the first author) approved by the University of Kerala, Trivandrum

appropriate dilutions were used for plating either by pour plate or spread plate technique using SWA and NA. For bacterial count of intestines, 1–2 g of the gut with contents were aseptically removed from 4–5 fish and were homogenised with 100 ml sterile sea water. Appropriate dilutions were plated with SWA and NA. The plates were incubated at $1 \pm 1^\circ\text{C}$ for 21, $8 \pm 1^\circ\text{C}$ for 10, $28 \pm 2^\circ\text{C}$ (RT) for 3 and $36 \pm 1^\circ\text{C}$ for 3 days. After incubation, counts were taken using a Qubec colony counter, equipped with a guide-plate ruled in square centimetres. Counts falling between 30 and 300 at the particular temperature of incubation were used for calculation of TPC. Colonies on crowded plates and on plates with spreading colonies were estimated by the method of APHA (1962).

Results and Discussion

1. Total bacterial count

The total aerobic plate counts (TPC) of the skin, gills and intestines (with contents) of newly caught sardine and mackerel at four different temperatures on two different plating media are given in Tables 1 and 2.

The TPC per sq. cm of skin of oil sardine, on SWA ranged between 2.15×10^3 and 2.50×10^7 and that of Indian mackerel between 4.63×10^4 and 8.35×10^6 at room temperature (RT, $28 \pm 2^\circ\text{C}$). The TPC/g of gills at RT were in the range of 2.42×10^5 to 8.76×10^8 and 6.81×10^4 to 3.14×10^8 in the case of oil sardine and Indian mackerel respectively. The counts/g at RT of intestine with contents of oil sardine and Indian mackerel respectively were in the range of 7.12×10^5 to 5.34×10^9 and 3.51×10^5 to 9.86×10^8 .

These data show that the bacterial density is the highest in the intestine and the lowest on the skin, that of the gills being intermediate in the case of both the fishes. This observation is in agreement with the results of Liston (1956), who had recorded a total bacterial count of 10^3 to 10^5 per sq.cm of skin and 10^3 to 10^7 per gram of the guts of flat fish from North Sea. Similar results were also obtained by Shewan (1962) and Georgala (1958) in North Sea cod.

The effect of incubation temperature on the plate count is very significant as observed from the Tables 1 and 2. Change in the incubation temperature either to the higher side or to the lower side from RT resulted in a decrease in the counts of skin, gills and intestines of both oil sardines and mackerel. The decrease by 1 to 2 log cycles in the TPCs at $36 \pm 1^\circ\text{C}$ compared with those at RT, implies that a significant portion of the bacteria recovered at RT is not mesophilic. Similarly, incubation at $8 \pm 1^\circ\text{C}$ caused a decrease of 1 to 2 log. cycles from the count at RT, indicating that lower temperature eliminated at least a portion of the mesophiles that are recovered at RT. Incubation at still lower temperature resulted in very considerable lowering of count. The bacterial counts at $1 \pm 1^\circ\text{C}$ were only 0.02 to 2.2% and 0.08 to 0.28% of the TPC at RT in the case of the skin of oil sardine and mackerel respectively. The corresponding counts of gills were only 0.06 to 0.29% and 0.10 to 0.32% of the counts at RT and of intestines only 0.001 to 0.02% and 0.0006 to 0.03% of the counts at RT. This indicates that only a very small proportion of the bacteria of fish caught from tropical waters is capable of growth at $1 \pm 1^\circ\text{C}$. This observation is different from that of Liston (1956) who reported that although the counts obtained by incubation at 0°C was lower than that obtained at 20°C , such difference was not excessively great in the case of fish caught from North Sea. In the case of fish from northern waters, where the temperatures of water in which fish are caught range from -2° to $+12^\circ\text{C}$, the viable counts at 37°C was only 5% of the counts at 0°C and 20°C , which were approximately equal (Shewan, 1949; Georgala, 1957 b, 1958). Whereas, in tropical waters off India, the temperature of sea water ranges from 20°C to 32°C and naturally one should expect the observed phenomena in the bacterial count of the fish caught from those waters. The results of Karthiayani & Iyer (1967 and 1971) on the bacterial count of oil sardine, caught off Cochin, at RT and 8°C compared well with this observation. However, they have not studied the TPCs at 1°C and $36 \pm 1^\circ\text{C}$.

Tables 1 and 2 also present data on the recovery of bacteria from oil sardine and mackerel, on distilled waterbased medium

Table 1. Total bacterial count (TPC) of newly caught oil sardine at different temperatures of incubation

Sample	Bacterial count on SWA				Bacterial count on NA			
	Temperature of incubation				Temperature of incubation			
	28±2°C	36±1°C	8±1°C	1±1°C	28±2°C	36±1°C	8±1°C	1±1°C
Skin (per cm ²)	2.15x10 ³	7.68x10 ³	6.24x10 ²	48	2.14x10 ²	1.78x10 ²	1.21x10 ²	11
	to 2.50x10 ⁷	to 8.14x10 ⁵	to 9.21x10 ⁵	to 6818	to 7.71x10 ⁵	to 3.42x10 ⁴	to 8.63x10 ⁴	to 864
Gills (per g)	2.42x10 ⁵	1.47x10 ³	8.18x10 ³	7.12x10 ²	6.46x10 ³	1.88x10 ¹	2.2x10 ²	1.12x10 ²
	to 8.76x10 ⁸	to 6.91x10 ⁵	to 5.07x10 ⁴	to 5.07x10 ⁴	to 5.93x10 ⁶	to 9.21x10 ³	to 2.32x10 ⁵	to 6.78x10 ²
Intestine with contents (per g)	7.12x10 ⁵	1.17x10 ³	2.35x10 ³	2.07x10 ²	9.28x10 ²	1.09x10 ²	2.42x10 ²	Nil
	to 5.34x10 ⁹	to 4.35x10 ⁷	to 4.35x10 ⁷	to 5.34x10 ⁴	to 8.42x10 ⁴	to 2.28x10 ⁴	to 7.99x10 ⁴	to 9.21x10 ²

Table 2. Total bacterial count (TPC) of newly caught Indian mackerel at different temperatures of incubation

	Bacterial count on SWA				Bacterial count on NA			
	Temperature of incubation				Temperature of incubation			
	28±2°C	36±1°C	8±1°C	1±1°C	28±1°C	36±2°C	8±1°C	1±1°C
Skin (per cm ²)	4.63x10 ⁴	8.22x10 ³	8.28x10 ³	128	6.28x10 ²	1.91x10 ²	3.02x10 ²	92
	to 8.35x10 ⁶	to 5.37x10 ⁵	to 2.02x10 ⁵	to 7137	to 5.38x10 ⁴	to 6.21x10 ⁴	to 1.19x10 ⁴	to 4064
Gills (per g)	6.81x10 ⁴	2.47x10 ³	1.13x10 ³	2.17x10 ²	3.03x10 ³	2.49x10 ²	1.09x10 ²	1.21x10 ¹
	to 3.14x10 ⁹	to 3.47x10 ⁵	to 3.28x10 ⁶	to 32.7x10 ⁴	to 9.28x10 ⁶	to 4.54x10 ⁴	to 6.93x10 ⁴	to 4.73x10 ²
Intestines with contents (per g)	3.51x10 ⁵	2.11x10 ⁴	9.21x10 ³	1.07x10 ²	7.20x10 ³	6.72x10 ²	3.21x10 ²	3.07x10 ¹
	to 9.86x10 ⁸	to 6.04x10 ⁷	to 6.44x10 ⁶	to 6.27x10 ⁴	to 822x10 ⁶	to 6.74x10 ⁵	to 8.62x10 ⁴	to 9.04x10 ³

(NA). The counts of skin, gills and intestine, at all the four temperatures namely, $36 \pm 1^\circ$, $28 \pm 2^\circ$, $8 \pm 1^\circ$ and $1 \pm 1^\circ$ were lower from the corresponding count on SWA. This decrease was of the order of 1 to 2 log cycles in the case of skin, 2 log cycles in the case of gills and 3 to 5 log cycles in the case of intestines. The decrease in count implies that the majority of the bacteria associated with marine fish required higher salt content in the media. The more pronounced decrease in the count of intestines, indicated the true marine nature of the bacteria of the intestines, in that they seem to be nutritionally exacting regarding the requirement of sea water for growth.

Karthiayani & Iyer (1967) have recorded similar observations on the recovery of skin and gut bacteria of oil sardine on SWA and distilled water agar (DWA). They reported that in 50% of platings, there was no growth in the DWA at RT in the case of gut bacteria, though there was growth in SWA.

As regards the total bacterial count, the results reported here (Tables 1 and 2) show comparatively higher values in the case of the TPCs of skin, gills and intestines of both oil sardine and Indian mackerel. The corresponding bacterial load of fish from temperate water is lower. The bacterial counts of North Sea fish were 10^2 – 10^5 /cm² of skin, 10^3 – 10^7 /g of gills and 10^3 – 10^8 /g of gut contents (Shewan, 1962). In the case of Japanese flat fish, Simudu *et al.* (1969) have reported a bacterial count of 10^4 /cm² of skin, 10^3 – 10^6 /g of gills and 10^3 – 10^7 /g of intestine. Karthiayani & Iyer (1967), in the case of the oil sardine caught from tropical waters, have obtained 10^3 – 10^7 organisms/g of skin with muscle and 10^4 – 10^8 /g of guts. Hence, the bacterial loads in the fishes from tropical and sub-tropical waters, are higher than the bacterial population of the fishes from colder waters. According to Shewan (1977) the flora of fish appears to be a function of the environment. "The somewhat higher loads on marine fish from tropical and sub-tropical areas appear to confirm Kriss' finding (1971) of greater numbers in waters from hotter areas than in the colder regions" (Shewan, 1977).

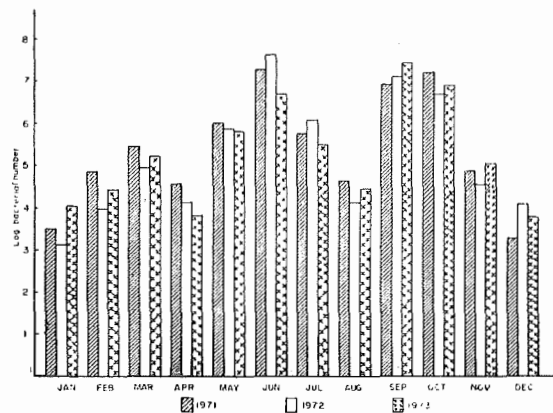


Fig. 1. Seasonal variation in the bacterial count on the skin of newly caught oil sardine at $28 \pm 2^\circ$ C on SWA

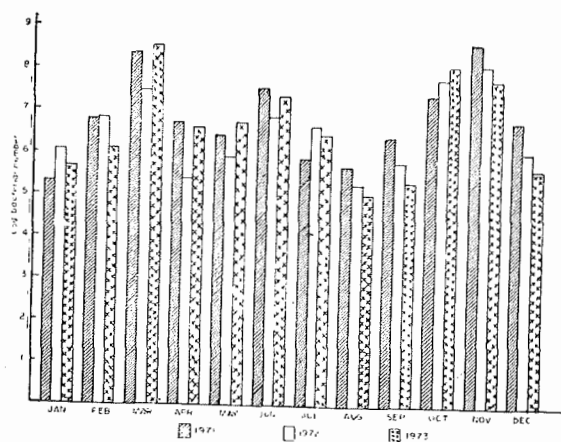


Fig. 2. Seasonal variation in the bacterial count of the gills of newly caught oil sardine at $28 \pm 2^\circ$ C on SWA

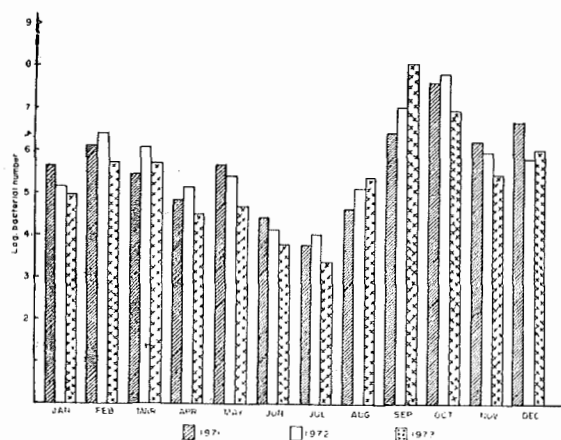


Fig. 3. Seasonal variation in the bacterial count of intestines (with contents) of newly caught oil sardine at $28 \pm 2^\circ$ C on SWA

2. Seasonal variations in the bacterial counts

Figures 1 to 3 show the seasonal variations in the bacterial counts at $28 \pm 2^\circ \text{C}$ (RT) of the skin, gills and intestine (with contents) of oil sardine. Similarly, figures 4 to 6 represent the seasonal variations in the bacterial counts at RT of the skin, gills and intestine (with contents) of Indian mackerel.

Figures 7 to 9 and 10 to 12 show the seasonal variations in the TPCs at $8 \pm 1^\circ \text{C}$, of skin, gills and intestines with contents of oil sardine and Indian mackerel respectively.

In the case of oil sardine, the highest bacterial loads on skin, at $28 \pm 2^\circ \text{C}$ are obtained in June and September–October period; while peak values in the total plate counts of gills are obtained in March and October–November season. For intestines with contents, the highest bacterial counts are registered in September–October season.

At $8 \pm 1^\circ \text{C}$, peak values in the TPCs of skin of oil sardine are recorded in June to October season. In the case of gills, peaks are obtained in February–March, June and October–November seasons. For intestine with contents, highest counts are registered in May and October–November periods.

At RT, only one peak each is obtained in the case of the bacterial count of skin and gills of Indian mackerel, that is in January–February season. But, for intestines, highest bacterial counts are recorded in October and March–April period.

At $8 \pm 1^\circ \text{C}$, the peak values for the bacterial counts of skin of Indian mackerel are obtained in January, whereas for gills, significantly higher values are not found, even though during December–January–February period, small peaks are obtained. But in the case of intestines with contents, two peaks are registered, one in November–December period and the other in March–April season.

A comparison of the figures 1 to 3 with figures 7 to 9 shows that in the case of oil

sardine, the peak values for the total bacterial counts of skin, gills and intestines, at both $28 \pm 2^\circ \text{C}$ (RT) and $8 \pm 1^\circ \text{C}$, almost correspond to the same period of the year. Similarly, a comparison of figures 4 to 6 and 10 to 12 shows the peak values in the case of skin, gills and intestines of Indian mackerel at RT and $8 \pm 1^\circ \text{C}$, in more or less the same seasons of the year.

A full comparison of the data on oil sardine and Indian mackerel is not possible, because the fishery of Indian mackerel is limited to a period of nine months from September to the following May, while oil sardine is available throughout the year. However, it can be observed from a comparison of figures 1 to 3 with 4 to 6 and 7 to 9 with 10 to 12 respectively, that, even though caught from the same waters, the peak values for bacterial counts for oil sardine and mackerel, occur at different periods of the year. Thus, while the highest bacterial counts of skin of oil sardine are obtained in June and September–October season, the corresponding peaks for mackerel is in January–February period. Similarly, for the gills of oil sardine, peak counts are obtained in March and October–November period, while the peak values for the gills of mackerel are registered in January–February season. So also, in the case of intestines, oil sardine recorded the highest counts in February–March and September–December seasons and Indian mackerel registered peak values in November and March–April period.

According to Shewan (1961) the seasonal variations in the bacterial load on fishes, are a reflection of similar variations in the environment. Thus, in sole, skate (Liston, 1955, 1956) and cod (Georgala, 1957 a, 1958) caught off Aberdeen, two peak loads (at 0°C and 20°C) occurred during the year, in the late spring and autumn, each following at least 1 to 3 months interval after the spring and autumn plankton outbursts.

Peak bacterial loads are also said to coincide with maximum water temperature (Shewan, 1961). The higher counts at $28 \pm 2^\circ \text{C}$, recorded in the warmer months of March in the case of gills and May–June in the case of skin of oil sardine and March–April in the case of intestines of Indian

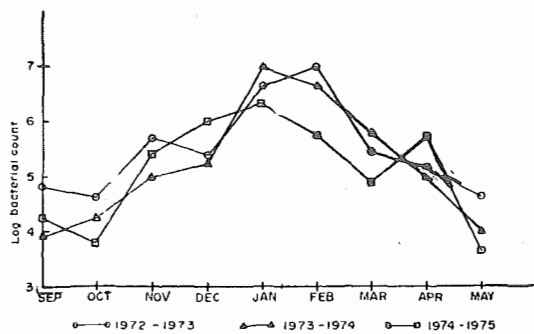


Fig. 4. Seasonal variation in the bacterial count on the skin of newly caught Indian mackerel at $28\pm 2^{\circ}\text{C}$ on SWA

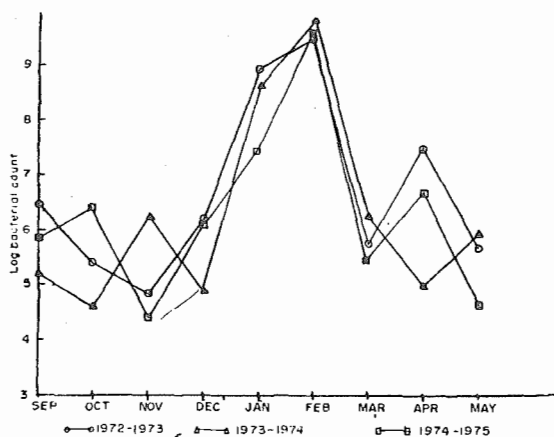


Fig. 5. Seasonal variation in the bacterial count of gills of newly caught Indian mackerel at $28\pm 2^{\circ}\text{C}$ on SWA

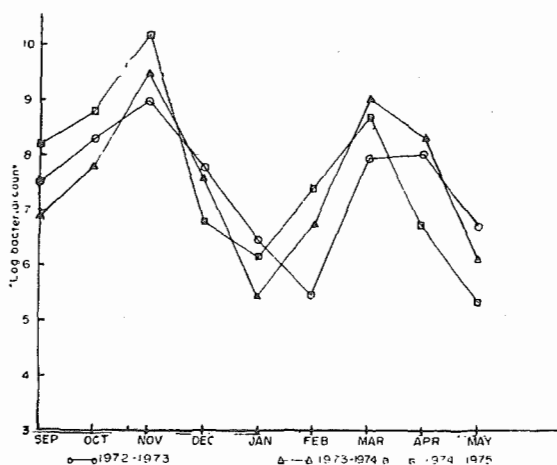


Fig. 6. Seasonal variation in the bacterial count of intestines (with contents) of newly caught Indian mackerel at $28\pm 2^{\circ}\text{C}$ on SWA

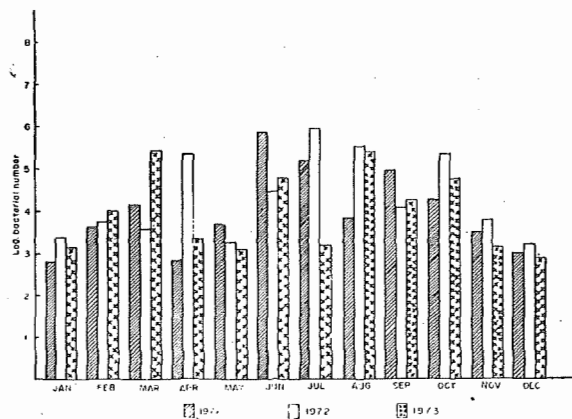


Fig. 7. Seasonal variation in the bacterial count on the skin of newly caught oil sardine at $8\pm 1^{\circ}\text{C}$ on SWA

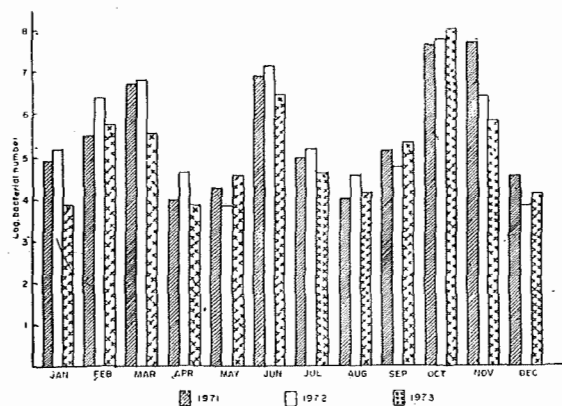


Fig. 8. Seasonal variation in the bacterial count of gills of newly caught oil sardine at $8\pm 1^{\circ}\text{C}$ on SWA

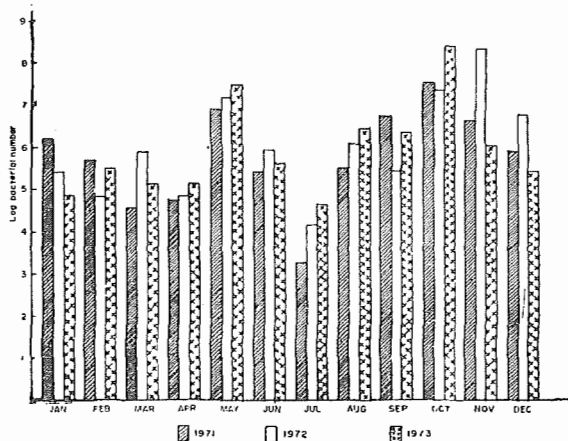


Fig. 9. Seasonal variation in the bacterial count of intestines (with contents) of newly caught oil sardine at $8\pm 1^{\circ}\text{C}$ on SWA

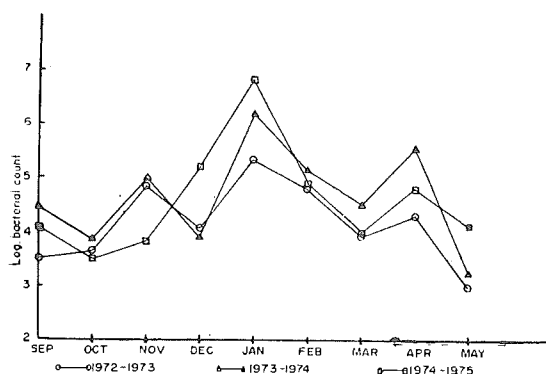


Fig. 10. Seasonal variation in the bacterial count on the skin of newly caught Indian mackerel at $8\pm 1^\circ\text{C}$ on SWA

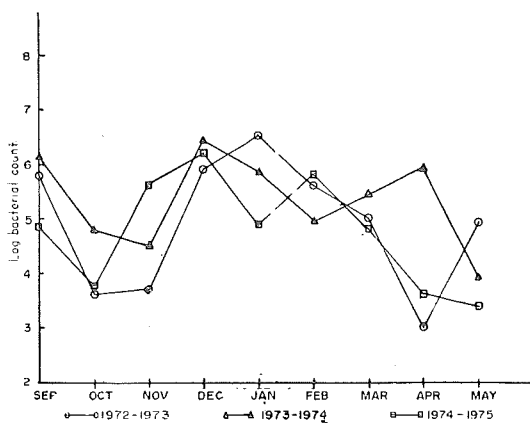


Fig. 11. Seasonal variation in the bacterial count of gills of newly caught Indian mackerel at $8\pm 1^\circ\text{C}$ on SWA

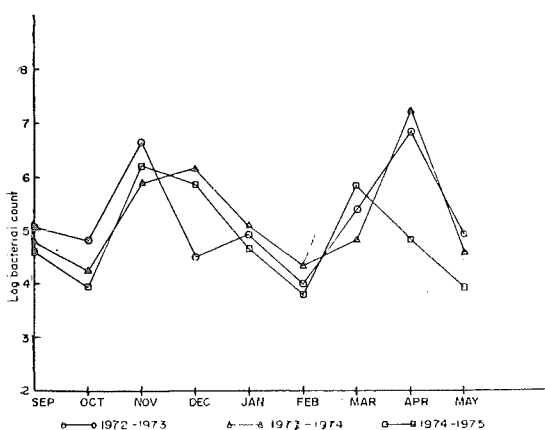


Fig. 12. Seasonal variation in the bacterial count of intestines (with contents) of newly caught Indian mackerel at $8\pm 1^\circ\text{C}$ on SWA

mackerel may be explained like this. Similarly, the lower counts obtained in December-January season in the case of intestine of mackerel may be attributed to the lower water temperature during winter. Such observations have been made by Georgala (1957 a, 1958) in the case of North Sea cod. Also Karthiayani & Iyer (1971) have reported peak values in the bacterial counts at 37°C of the skin with muscle, gills and intestines of oil sardines in the warmer months.

As explained earlier, though caught from the same waters, the peak counts of oil sardine and mackerel are obtained during different periods of the year. This cannot be explained on the basis of the changes in the environmental temperature alone, but a number of other factors-physical, chemical and biological-might influence the flora. The species of the fish might also affect the bacterial load (Shewan, 1961). Liston's observations (1955, 1956) that bacterial populations particularly of gills of sole and skate caught from the same area in the same time, were different, support this view. The micro-environments, say the constitution of the slime, for example, present in the particular fish might affect their bacterial load.

The authors express their sincere gratitude to the Director, Central Institute of Fisheries Technology, Cochin for the facilities and to the Scientist-in-Charge, Microbiology Division of the Institute for his co-operation.

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