SEASONAL VARIATIONS OF BACTERIAL FLORA OF FRESH OIL SARDINES (Sardinella longiceps)

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An elaborate study was made on the qualitative and quantitative seasonal variations in the bacterial flora of fresh oil sardines and their biochemical reactions. It was observed that the total bacterial loads and their phosphorescent and biochemical characters were influenced by changes in seasons. During monsoon season total bacterial count was high. Mesophiles predominated during summer, but phosphorescent bacteria were less. Winter favoured the selection of biochemically less active groups of bacteria.

Introduction

Qualitatively, the bacterial flora of marine environments in different parts of the world show some differences. Much work has not been done on the effect of seasonal and environmental differences on the bacterial flora of fish. Shewan (1966) has reported some quantitative and qualitative variations in the bacterial flora of North Sea fish, depending upon season. The Food Investigation Board (1950) has reported that luminiscent bacteria are present in the slime of halibut in appreciable numbers from April to October. Preliminary experiments with oil sardines in this laboratory for a period of one year had suggested seasonal variations in the quantitative and qualitative pattern of bacterial flora. Bacterial counts of the skin with muscle recorded high peaks during the months of June and September and those of intestine in June and October (Karthiayani and Iyer, 1967). Also, the microorganisms present were found to be biochemically less active during December and January. The present study was undertaken to obtain more information regarding the effect of seasons upon the bacterial flora of oil sardines caught off Cochin.

Materials and methods

Studies were carried out for three years. Fresh oil sardines caught off Cochin were transferred to sterile bottles and brought to the laboratory without delay. Skin with muscle, gills and intestines were pour plated with sea water agar. The plates were incubated at room temperature (30°C) and at 37°C for 48 hours and total

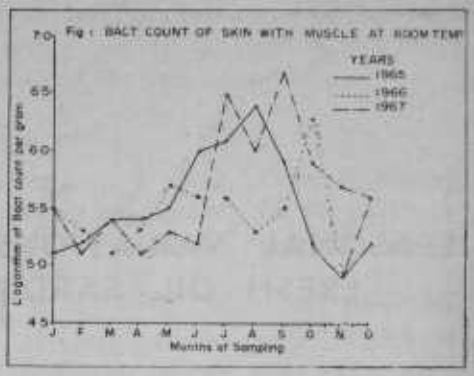
plate counts were taken. The counts of luminiscent bacteria were taken in a dark room.

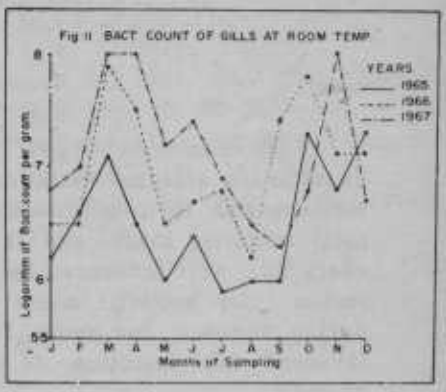
Bacterial colonies were picked at random from plates incubated at room temperature and transferred to sea water peptone. Biochemical characteristics of the organisms, like reduction of nitrate to nitrite, gelatin liquefaction, indole production, acid and gas production from glucose, lactose, sucrose, mannitol and maltose were studied.

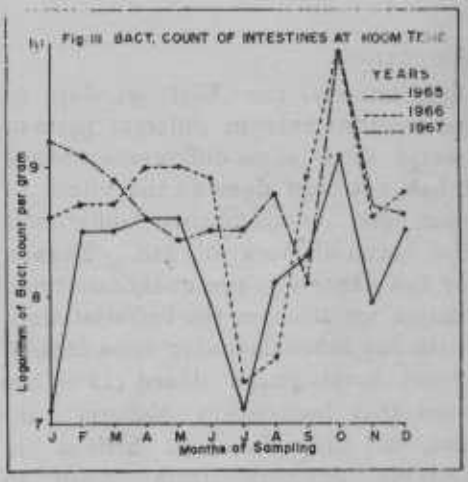
Results and discussion

Figures I, II and III respectively represent the logarithms of total bacterial counts of skin with muscle, gills and intestines at room temperature for three years. Peak values are obtained during July–October for skin with muscle, March–April and September–November for gills and October for intestines. Georgala (1958) reported such high peaks in June and October for the counts of skin for North Sea cod. The higher bacterial counts during the July–October season may be attributed to the effect of monsoon.

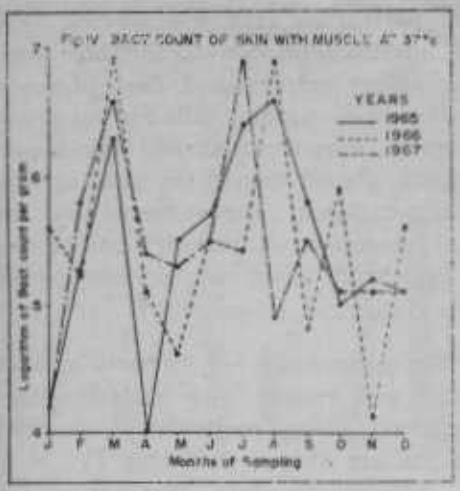
Figures IV, V and VI represent the logarithms of total bacterial counts of skin with muscle, gills and intestines respectively at 37°C for three years. Peak values are obtained during March and July–August for skin with muscle, March–May and August–November for gills and March–April and October for intestines. Peak values during July–August, August–November and in October may be attributed to the fact that peak values for total bacterial counts at room temperature were also obtained during the above seasons. The common peak values for skin with muscle, gills and intestines during March show the presence of greater numbers of mesophiles. This may be due to the effect of summer.

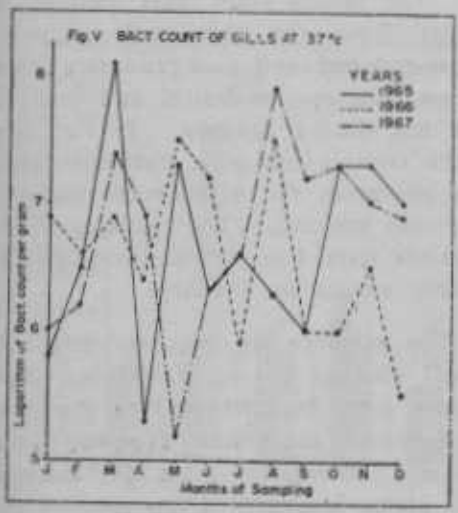






Tables I, II and III represent the total phosphorescent bacterial counts on skin with muscle, gills and intestines respectively for three years. From the data it is evident that phosphorescent





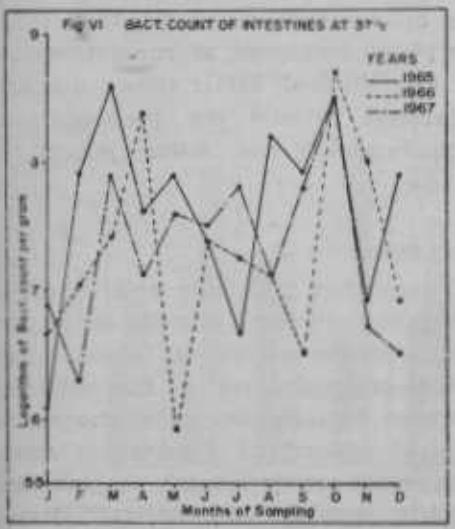


Table I Total count of phosphorescent bacteria on skin with muscle for three years.

Months of sampling	Bacterial counts per gram. Year 1965	Year 1966	Year 1967
January	0	1.2×10^{3}	9.0×10^{3}
February	0	1.8×10^{3}	0
March	0	0	0
April	0	0	0
May	0	0	0
June	0	0	0
July	0	3.0×10^{3}	0
August	1.4×10^{4}	0	0
September	0	0	7.0×10^{3}
October	1.1×10^{4}	2.1×10^{4}	0
November	1.7×10^{3}	0	0
December	4.1×10^{4}	1.0×10^{3}	1.2×10^{3}

Table II Total count of phosphorescent bacteria on gills for three years

Months of sampling	Bacterial counts per gram. Year 1965	Year 1966	Year 1967
January	9.0×10^{4}	3.0×10^{4}	1.5×10^{4}
February	1.1×10^{5}	1.2×10^{4}	0
March	0	1.5×10^{5}	0
April	0	0	0
May	0	0	0
June	0	0	0
July	0	4.0×10^{4}	0
August	8.3×10^{5}	0	0
September	6.0×10^{5}	2.0×10^{4}	0
October	0	3.2×10^{4}	0
November	0	0	0
December	6.0×10^{5}	1.1×10^{5}	3.8×10^{6}

bacteria are not present on skin with muscle during March to June and on gills from April to June. In the case of intestines, phosphorescent bacteria are present almost throughout the year and peak values are obtained during July to October. The absence of phosphorescent bacteria on skin with muscle and gills from March to June may be due to the

Table III Total count of phosporescent bacteria on intestines for three years

Months of sampling	Bacterial counts per gram. Year 1965	Year 1966	Year 1967
January	0	1.9×10^{6}	7.0×10^{5}
February	4.5×10^{6}	1.7×10^{7}	2.0×10^{5}
March	1.8×10^{4}	2.8×10^{4}	0
April	0	2.0×10^{4}	4.2×10^{3}
May	1.2×10^{4}	4.1×10^{4}	9.0×10^{4}
June	1.8×10^{3}	1.2×10^{4}	6.8×10^{4}
July	4.0×10^{5}	1.3×10^{6}	2.1×10^{7}
August	2.7×10^{6}	1.7×10^{6}	2.0×10^{7}
September	5.0×10^{7}	1.5×10^{7}	1.0×10^{7}
October	1.4×10^{7}	2.0×10^{8}	3.1×10^{5}
November	1.5×10^{5}	8.3×10^{6}	5.8×10^{6}
December	2.0×10^{6}	1.5×10^{7}	5.0×10^{6}

fact that phosphorescent character of bacteria is lost by the high temperature of the season. This is supported by our finding that phosphorescent cultures lost that character when grown at 37°C and also by the lesser phosphorescent bacterial counts on plates incubated at 37°C. The presence of phosphorescent bacteria on intestines from March to June may be due to the fact that complete destruction of phosphore-cent bacteria is not effected because of the high initial load.

Figures VII, VIII and IX represent the percentages of *Pseudomonas* present on skin with muscle, gills and intestines respectively for three years. High percentages are obtained during May for skin with muscle, during September for gills and during March and September for intestines. Figures X, XI and XII show the percentages of *Vibrios* present on skin with muscle, gills and intestines, respectively for three years. *Vibrios* predominate in June in the case of skin with muscle, in March and June in the case of gills and in June and October in the case of intestines. Figures XIII, XIV and XV show the percentages of *Achromobacters* present on skin with muscle, gills and intestines respectively for three years. Peak values are obtained during August for skin with muscle, gills and intestines. Generally, *Pseudomonas* and *Vibrios* predominate almost during the same seasons of the year, viz, the summer and the end of monsoon, whereas *Achromobacters* are present in great numbers only during August, the end of monsoon.

The percentages of bacteria present on skin with muscle, gills and intestines respectively giving particular biochemical reactions are shown in tables IV, V & VI. The results show that among the isolates from the skin with muscle, nitrate-reducers and acid producers from glucose, sucrose, mannitol and maltose were less during January. In the case of the isolates from gills, much variations with season in the biochemical reactions were not noticed, The isolates from intestines were less active biochemically towards sugars in January.

The presence of biochemically less active groups during December and January may be attributed to the low temperature of the winter. This, combined with our observation that the isolates from plates incubated at low temperature were biochemically less active than those from plates incubated at room temperature (unpublished data), shows that low temperature favours the preponderance of biochemically less active groups of bacteria.

Conclusion

Season has a definite role in determining not only the population of the bacteria present on marine fish, but also on the preponderance of the different genera of bacteria and their phosphorescent and biochemical characters. Generally, monsoon season favours the presence of greater numbers of bacteria. Though

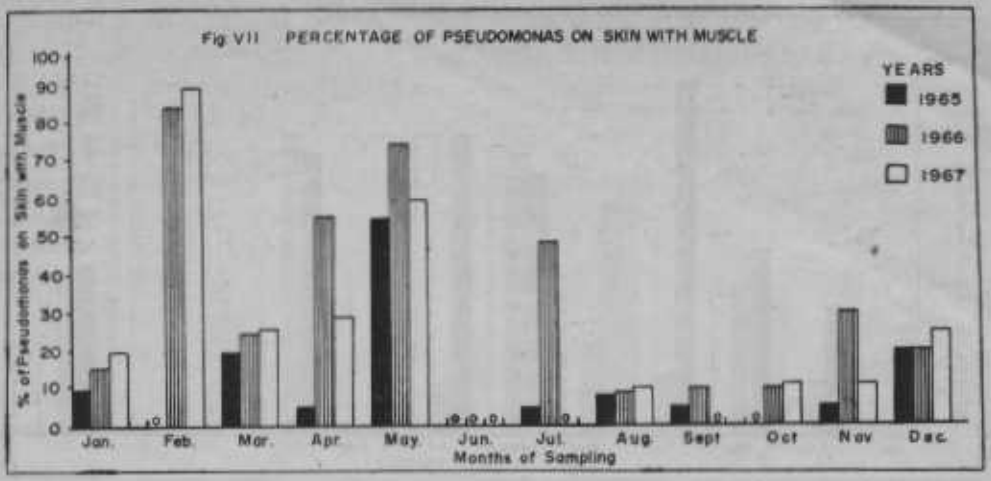


Fig. VII PERCENTAGE OF PSEUDOMONAS ON SKIN WITH MUSCLE
% of Pseudomonas on Skin with Muscle
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sept Oct Nov Dec.
Months of Sampling

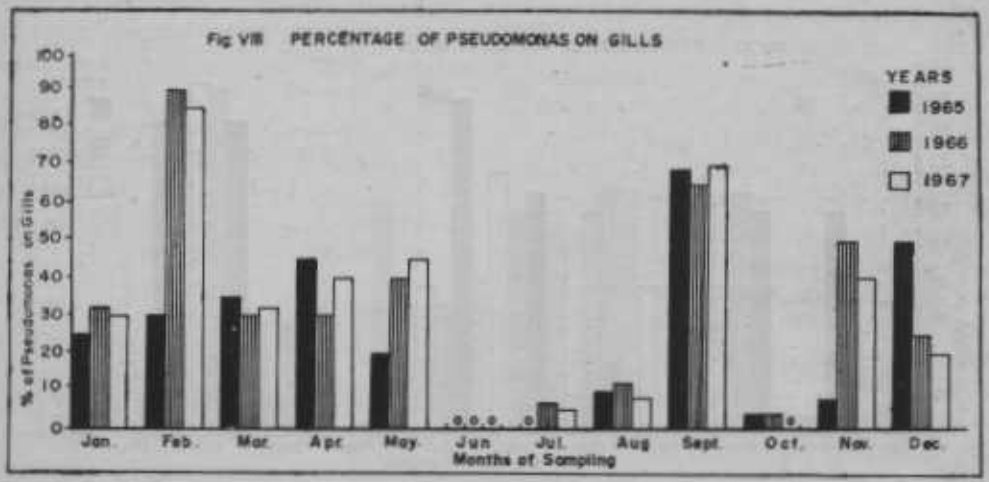


Fig. VIII PERCENTAGE OF PSEUDOMONAS ON GILLS
% of Pseudomonas on Gills
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May Jun. Jul. Aug Sept. Oct. Nov. Dec.
Months of Sampling

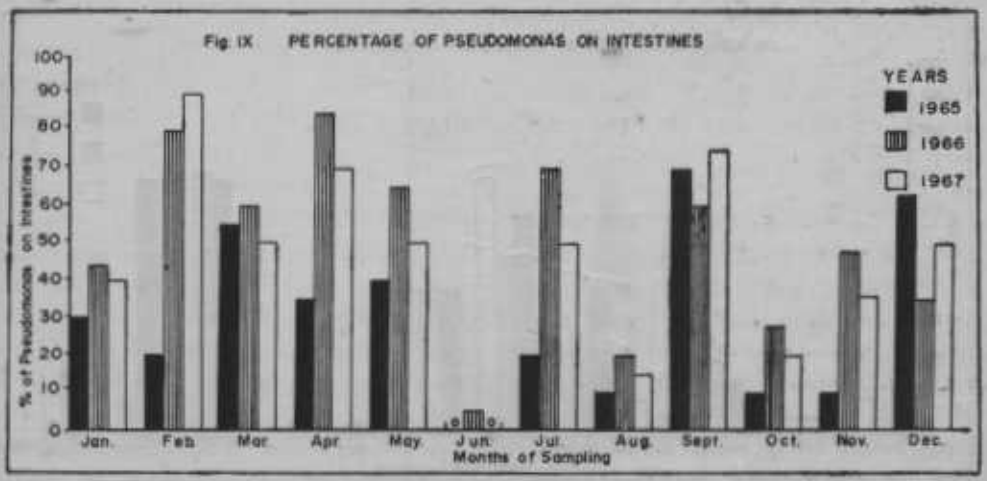


Fig. IX PERCENTAGE OF PSEUDOMONAS ON INTESTINES
% of Pseudomonas on Intestines
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sept. Oct. Nov. Dec.
Months of Sampling

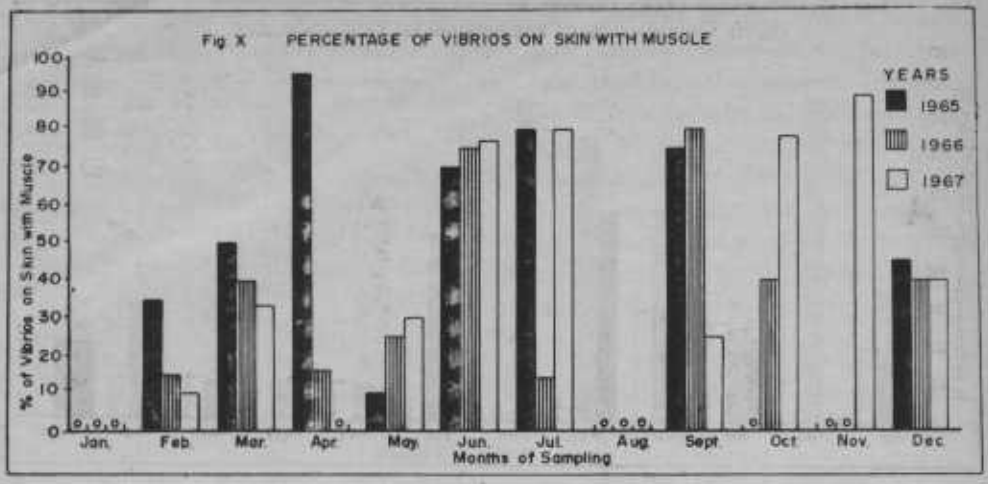


Fig X PERCENTAGE OF VIBRIOS ON SKIN WITH MUSCLE
% of Vibrios on Skin with Muscle
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May. Jun. Jul. Aug. Sept. Oct. Nov. Dec.
Months of Sampling

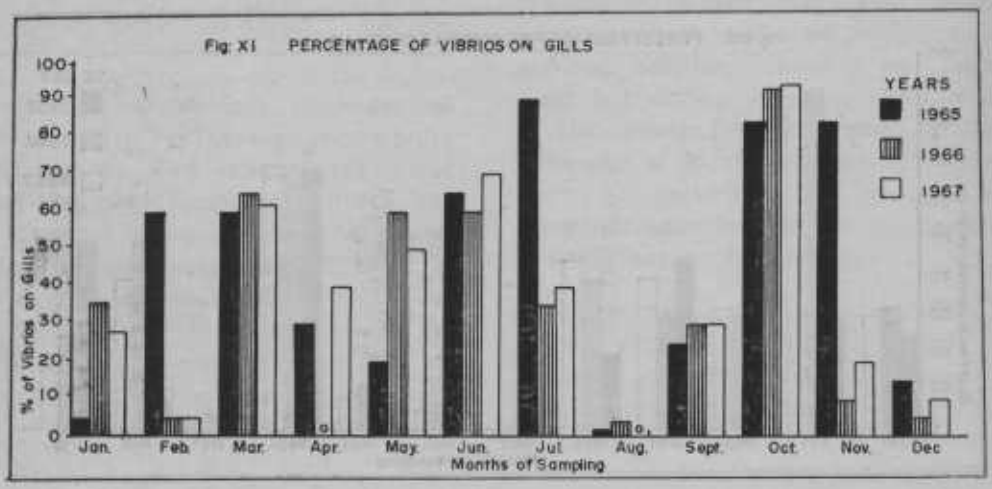


Fig XI PERCENTAGE OF VIBRIOS ON GILLS
% of Vibrios on Gills
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May. Jun. Jul. Aug. Sept. Oct. Nov. Dec.
Months of Sampling

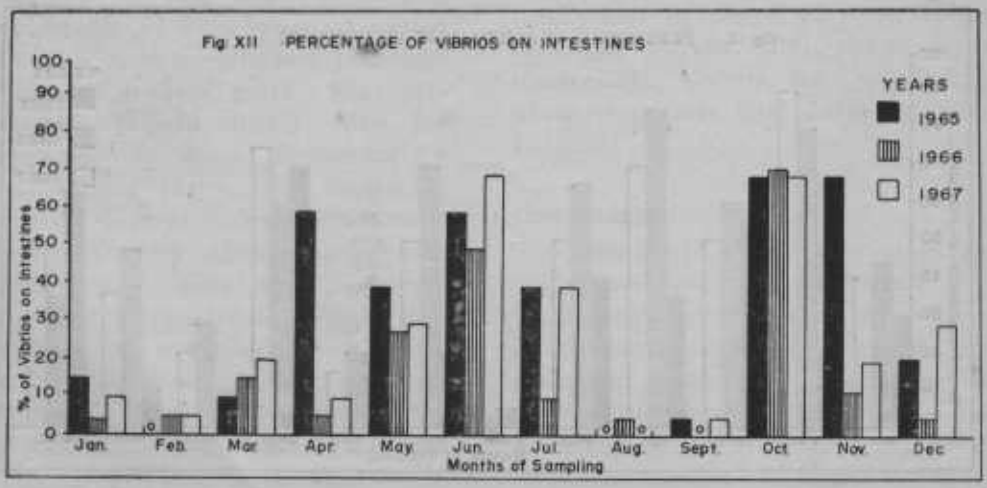


Fig XII PERCENTAGE OF VIBRIOS ON INTESTINES
% of Vibrios on Intestines
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May. Jun. Jul. Aug. Sept. Oct. Nov. Dec.
Months of Sampling

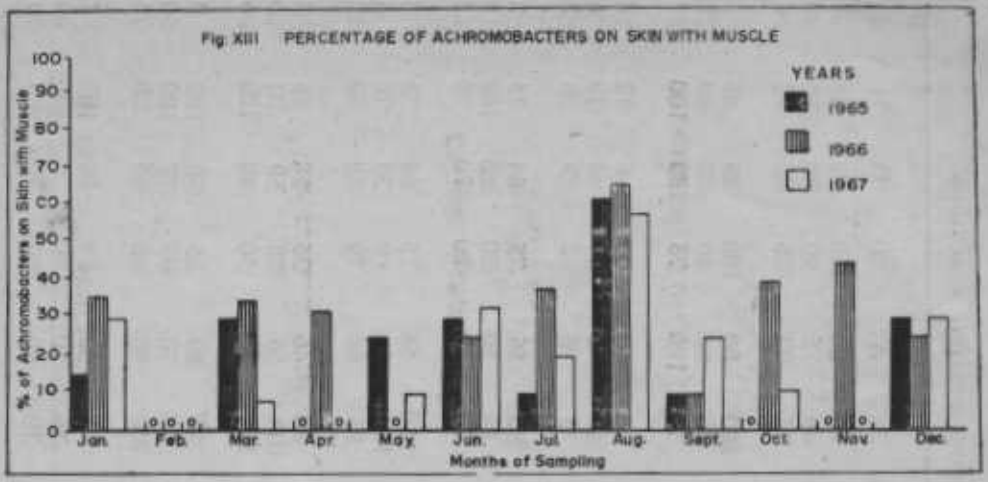


Fig. XIII PERCENTAGE OF ACHROMOBACTERS ON SKIN WITH MUSCLE
% of Achromobacters on Skin with Muscle
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sept. Oct. Nov. Dec.
Months of Sampling

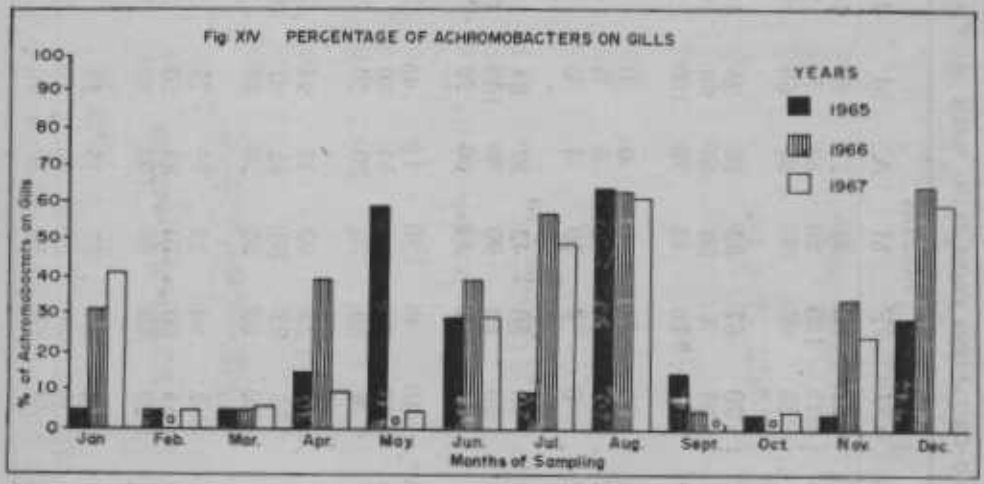


Fig. XIV PERCENTAGE OF ACHROMOBACTERS ON GILLS
% of Achromobacters on Gills
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sept. Oct. Nov. Dec.
Months of Sampling

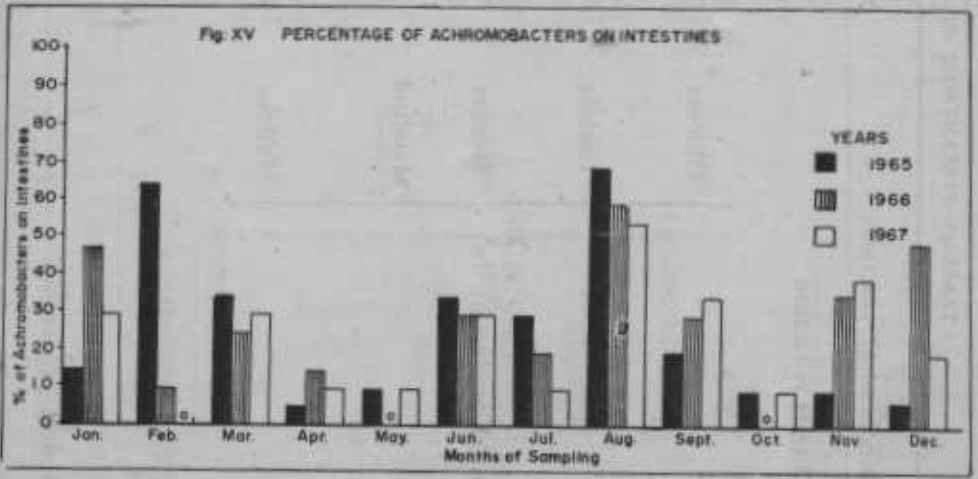


Fig. XV PERCENTAGE OF ACHROMOBACTERS ON INTESTINES
% of Achromobacters on Intestines
YEARS
1965
1966
1967
Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sept. Oct. Nov. Dec.
Months of Sampling

TABLE IV PERCENTAGES OF MICRO-ORGANISMS FROM SKIN WITH MUSCH GIVING POSITIVE REACTIONS

Biochemical reactions		Years	Months J	F	M	A	M	Jn	Ju	A	S	O	N	D
Nitrate reduction		1965	22	86	83	37	96	100	80	80	100	88	88	24
		1966	15	100	80	83	77	96	40	75	90	80	86	20
		1967	30	96	90	80	60	80	95	90	96	92	75	15
Acid production in	Glucose	1965	20	62	83	50	96	100	100	80	100	88	88	16
		1966	25	75	90	60	90	100	85	90	80	70	80	20
		1967	30	80	85	70	100	90	75	100	75	60	100	15
	Lactose	1965	6	2	0	0	0	0	20	0	25	6	12	4
		1966	0	4	2	6	0	0	10	0	10	4	10	6
		1967	0	8	10	4	0	2	5	0	2	5	8	2
	Sucrose	1965	10	50	42	37	84	100	80	56	75	64	75	8
		1966	20	60	90	70	100	100	80	50	70	60	100	10
		1967	10	100	80	60	90	80	70	100	80	70	80	15
	Mannitol	1965	10	46	50	12	46	100	80	56	75	64	75	8
		1966	12	95	60	33	40	80	100	50	60	35	70	6
		1967	8	80	70	20	50	90	85	60	70	50	60	12
	Maltose	1965	10	58	92	37	94	100	80	46	25	88	88	8
		1966	36	95	50	42	90	100	100	50	30	50	70	10
		1967	20	80	60	50	80	75	100	60	50	60	100	6
Gelatin liquefaction		1965	2	90	75	75	78	86	80	80	25	90	100	0
		1966	35	90	70	85	50	80	95	70	95	70	100	50
		1967	20	80	60	100	50	90	100	80	60	55	80	75
Indole production		1965	4	44	67	25	55	56	0	58	25	48	100	3
		1966	30	80	50	40	70	75	56	50	90	0	100	40
		1967	48	60	70	30	60	80	30	60	80	20	80	20

TABLE V PERCENTAGES OF MICRO-ORGANISMS FROM GILLS GIVING POSITIVE REACTIONS

Biochemical Reaction		Years	Months J	F	M	A	M	Jn	Ju	A	S	O	N	D
Nitrats reduction		1965	100	100	90	85	100	88	100	60	70	60	80	100
		1966	90	80	70	80	95	60	85	50	80	100	90	88
		1967	75	95	80	60	88	70	75	100	75	40	70	92
Acid production in	Glucose	1965	32	5	80	20	90	88	40	70	60	80	100	70
		1966	20	90	100	50	100	65	100	85	95	85	90	55
		1967	30	0	90	70	80	80	100	100	80	50	75	80
	Lactose	1965	0	15	0	0	0	2	0	0	0	10	0	0
		1966	0	6	8	0	4	0	0	4	0	8	0	6
		1967	0	4	2	0	0	10	0	6	0	2	0	0
	Sucrose	1965	48	8	80	25	88	80	45	70	60	80	75	95
		1966	40	86	98	50	95	60	85	50	65	90	70	85
		1967	20	10	90	80	75	75	100	80	70	60	80	60
	Mannitol	1965	16	0	60	30	70	96	45	50	100	100	70	60
		1966	20	90	50	20	75	70	100	70	88	90	60	50
		1967	15	20	70	50	80	80	80	60	65	50	80	40
	Meltose	1965	4	10	50	25	90	88	45	70	70	100	80	95
		1966	6	95	100	50	95	60	75	50	80	95	80	85
		1967	10	8	90	70	80	80	100	80	80	50	70	70
Gelatin liquefaction		1965	45	85	100	60	60	88	100	100	60	55	75	60
		1966	40	80	90	50	75	70	95	50	70	92	90	40
		1967	30	72	75	70	80	66	90	70	80	90	80	50
Indole production		1965	48	50	70	70	68	75	5	40	40	95	60	65
		1966	40	60	80	60	60	80	85	70	50	80	40	70
		1967	28	40	85	72	40	88	60	80	90	75	100	82

TABLE VI PERCENTAGES OF MICRO-ORGANISMS FROM SKIN WITH MUSCLE GIVING POSITIVE REACTIONS

Biochemical Reactions		Years	Months J	F	M	A	M	Jn	Ju	A	S	O	N	D
Nitrate reduction		1965	100	100	100	75	70	80	90	60	70	90	80	75
		1966	80	90	60	70	50	90	100	75	60	75	50	80
		1967	60	80	75	100	90	80	85	90	96	80	88	60
Acid production in	Glucose	1965	24	85	80	0	60	80	95	80	60	90	100	80
		1966	20	90	70	40	70	90	57	88	70	88	60	75
		1967	10	100	90	20	80	70	60	90	50	100	90	60
	Lactose	1965	0	10	0	0	0	2	0	10	2	5	0	0
		1966	0	8	2	4	8	0	18	8	8	4	0	0
		1967	0	6	0	6	0	4	0	0	6	0	0	0
	Sucrose	1965	40	25	80	0	60	75	30	70	60	45	100	88
		1966	20	30	60	30	65	90	27	60	70	80	50	70
		1967	25	10	90	10	80	70	30	75	45	90	80	70
	Mannitol	1965	36	15	50	0	60	100	25	80	60	70	100	98
		1966	30	5	40	10	50	70	15	50	65	88	70	80
		1967	10	10	30	20	70	60	30	75	50	98	80	88
	Maltose	1965	0	95	100	0	88	60	95	88	98	90	100	80
		1966	20	90	88	20	90	40	100	100	70	80	50	70
		1967	10	100	90	40	80	80	98	90	50	60	75	60
Gelatin liquefaction		1965	70	5	25	90	5	80	69	75	69	45	40	50
		1966	30	20	20	80	70	90	60	60	75	68	16	55
		1967	40	40	30	70	80	75	70	80	80	80	60	50
Indole production		1965	44	35	60	65	70	56	0	50	48	55	75	70
		1966	40	30	66	30	80	60	21	45	20	60	100	50
		1967	30	60	70	50	65	40	25	60	30	70	80	30

mesophiles predominate in the warmer months, the numbers of phosphorescent bacteria are less. Also it is noted that during winter, biochemically less active groups of bacteria are predominating in comparison with the other seasons.

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