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Bateriology of Chilled Water during the Preservation of Fish

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Inoduction

In920 a patent was issued to a Frenchman, E. Le Danois, for a process in which fish was stored in ched brine at a temperature of − 4°C. But little attention was paid to the prospect of commercial prervation of fish by this method until Huntsman in 1931 reported on the possibilities of chilled bre storage.

In an excellent study Hess (1933) showed that fish held at −1°C. kept twice as well as fish sted at +2° to +3°C. He also showed that the temperature of −1°C. could be easily achieved by ading crushed ice to sea-water. The advantage of Hess' process over that of Le Danois was that in tl former there was no risk of the undesirable slow freezing of the fish muscle. Further in the Le Inois process, sea-water has to be supplemented with salt to attain the specified temperature.

Within the last decade the practice of chilled water storage has been gaining currency eecially in Canada and the U. S. A. (Bloomberg, 1055 ; Pacific Fisherman, 1957). In Ceylon, two dled water tanks, each with about a capacity of 15 tons, have been operating very successfully at t Government Fisheries Factory, Colombo. In addition two exploratory fishing vessels have been fed with chilled water tanks and successfully operated (Lantz, 1956). There are many operationel ₰antages of chilled water storage, among them being, (*a*) the ease of storage and the discharge of t tanks, (*b*) uniformity of treatment of the fish, (*c*) ease of applying preservation additives such as ₰ibiotics, (*d*) the much improved storage rate (25 to 30%), and (*e*) avoidance of crushing. (Ann. ₱pt. Torry Research Station, 1958).

However apart from the engineering difficulties that have to be faced in the installation of dled water tanks in trawlers, the pattern of spoilage in chilled water storage also seems to be ferent from that in ice. Work at the Torry Research Station had indicated that under certain ıditions, anaerobic (micro-aerobic ?) spoilage may occur and that the spoilage flora is established ·lier than during the preservation in ice.

Much of the work on preservation of fish in chilled water has been done in Canada. But fortunately, except for an excellent study on the ionic imbalance between chilled sea water and ıscle tissue, its stabilization and the osmotic entry of water into fish muscle (Robert, Jonas and ₰Bride, 1960), much of the work published has been in the nature of preliminary reports (Tarr, 1947; ıntz, 1953 ; Roach and Harrison 1954 ; Harrison and Roach, 1954 ; 1955 ; Schmidt and Adler, 1955; ₰einer and Starr, 1955 ; Barker and Idler, 1955 ; McBride, Murray and McCleod, 1955 ; Roach ıd Harrison, 1958 ; Tarr, 1960).

Except for the preliminary studies at Torry in Scotland, no results have been reported on the ıccession of the bacterial flora during the storage of fish in chilled water. The present work was ndertaken to elucidate the dynamics of bacterial population changes in chilled fresh water under omparable conditions of storage in melting ice (+1° to +3°C.) which has been earlier studied by one f us (de Silva, 1960).

Materials and Methods

The experiments were conducted in a minature chilled water tank made according to a design by ₱antz (1955). The external dimensions of this apparatus were 58″×26″×26″ and holding 26 gallons

of water (4 cu. ft.). The cooling coils were placed internally. The chilling was done by a Freon air cooled refrigerator unit with automatic temperature control and powered by a ½ h.p. electric motor. The tank was heavily insulated and the temperature fluctuations of the water after stabilization did not exceed 1°C. The recirculation of the water was effected by means of a small centrifugal pump fitted to the top frame of the tank.

At the beginning of the experiment the tank was filled with fresh water and stabilized for about 15 days at 1°C. It was then filled with a mixed catch of small pelagic fish each about 8 to 10 cms. long. During Trial I the fish used were caught off the east coast of Ceylon and transported in ice. The fish in Trial II were from the north west coast and were also transported in ice. In both instances the fish were introduced into the tank within 12 hours of catching. Two samples of the chilled water were taken on each day of sampling and bacterial counts done on them using the roll tube technique and the pour plate technique. One set of roll tubes were incubated at 37°C. and the duplicate set at 20°C. They were counted after three days incubation.

For the qualitative studies 72 colonies were picked off from the plates which were incubated at 30°C., the colonies being selected at random from each set of plates at each point of sampling. The complete randomization of the picking off of the colonies was ensured by drawing numbered squares on the back of each plate and picking off those colonies in the squares which were chosen by reference to a table of random numbers. (Fisher and Yates, 1949).

The selected colonies (about 1,000) were identified and classified broadly according to the determinative scheme suggested by the Torry workers (Shewan, Hobbs and Hodgkiss, 1960).

Results

During the first trial the chilled water was sampled over 8 days. During the first three days of storage, samples were taken each day ; after that on alternate days. During the second trial the fish were stored for 14 days, the sampling being done on each alternate day.

The quantitative results shown in Fig. 1 indicated that after the first day of storage there was a gradual increase in the component of the flora which grew better at 20°C. That which grew preferentially at 37°C. remained nearly constant throughout storage or showed a slight increase. It was also found that while all the cultures that grew at 20°C. also grew at 30°C., many of those that grew at 30°C. did not grow at 20°C. The latter however thrived at 37°C.

The qualitative changes of the flora are illustrated in Figs. 2 and 3. During Trial I there were initially present a larger number of Gram positive groups than in Trial II. Among them the coryneforms showed a marked decrease while the micrococci showed a relative increase reaching a peak by about the third day of storage. After that they decreased in number during the second phase of spoilage. The decrease in numbers of the coryneforms, the micrococci and the flavobacterium-cytophaga group was accompanied by the marked increase in numbers of pseudomonads and the coliforms.

As regards the Gram negative component of the flora the establishment of the climax group took place much earlier than is usual in fish stored in ice. During the " lag phase " which lasted 1 to 2 days the Gram negative flora showed only slight changes. During the phase of interaction however, there was a dramatic increase in the numbers of pseudomonads, the achromobacters and the " coliforms " (fermentative Gram-negative rods). The final phase of spoilage was established by about the 10th day with the dominance of the pigmented and non-pigmented pseudomonads. (ps. I and II). The flavobacterium-cytophaga group gradually decreased in numbers and disappeared by about the 3rd day.

Among the pseudomonads the pigmented group (" fluorescens-putida " group, Klinge, 1960) showed a dramatic increase. The " coliform " group was mainly made up of the fermentative types which grew at 37°C. and not at 44°C. Over 75% of them belonged to *Es. coli* and strains of *Klebsiella*. Within the second group of *Pseudomonas* (ps. II) were also included a fair proportion (about 10 to 12%) of " yellow " pseudomonads which were described as an abberant group from North Sea Herring (de Silva 1960) but seeming to constitute a significant group on freshly caught tropical fish.

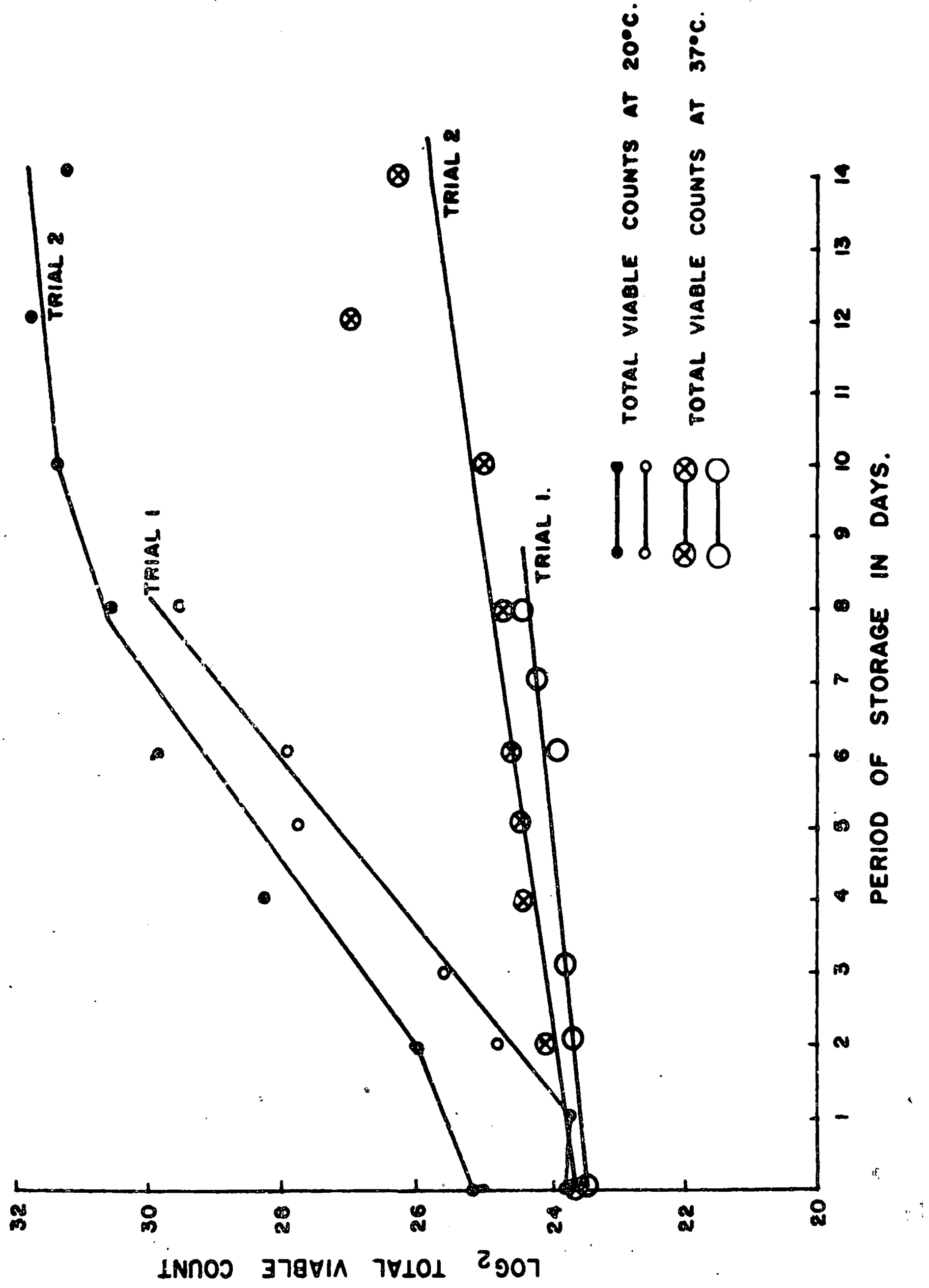


FIG. 1.

COMPARISON OF THE TOTAL VIABLE BACTERIAL COUNTS AT 20°C AND 37°C OF THE CHILLED WATER DURING PRESERVATION OF FISH.

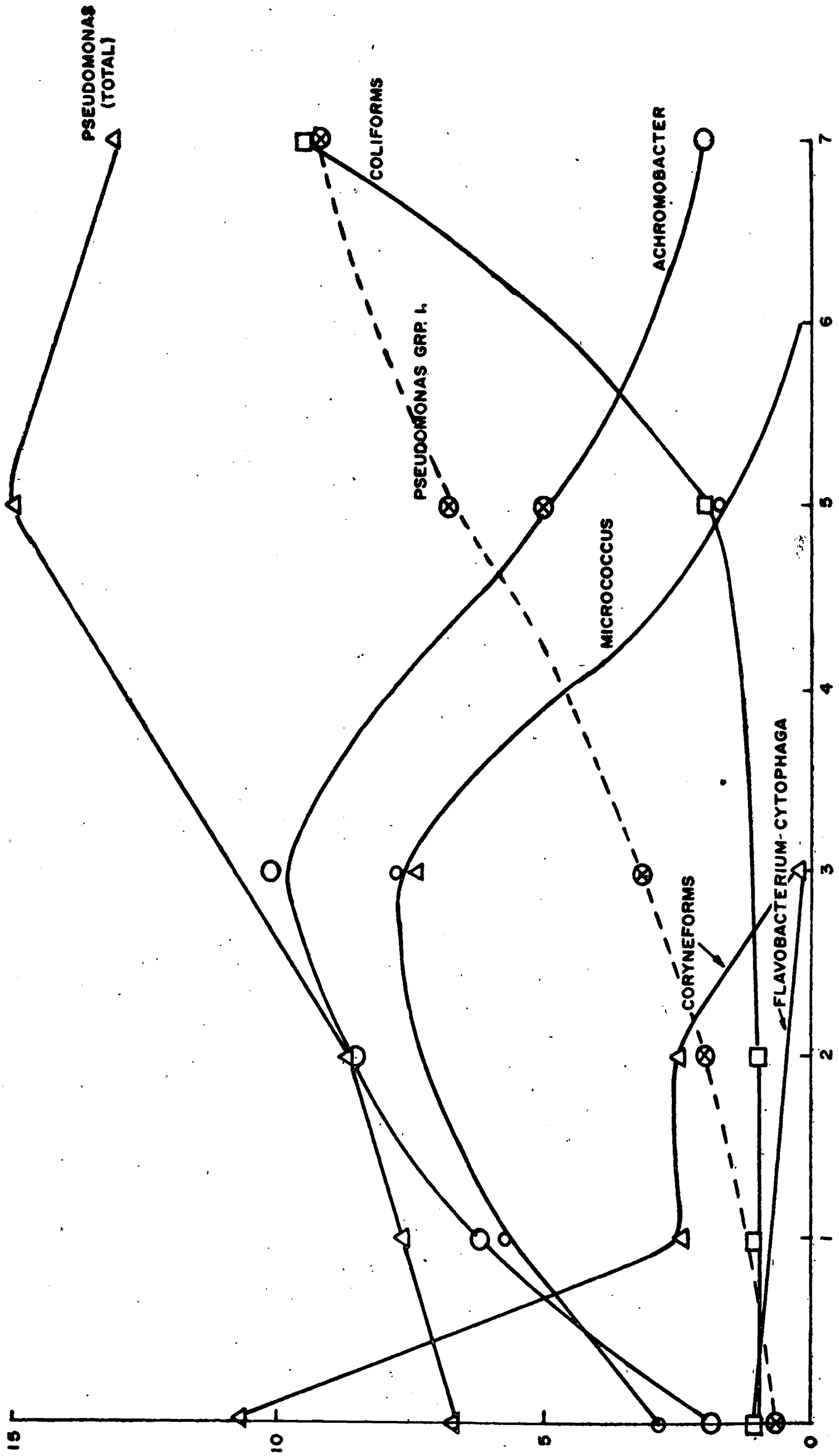


FIG. 2. CHANGES IN THE BACTERIAL FLORA IN CHILLED WATER DURING PRESERVATION OF FISH (TRIAL I).

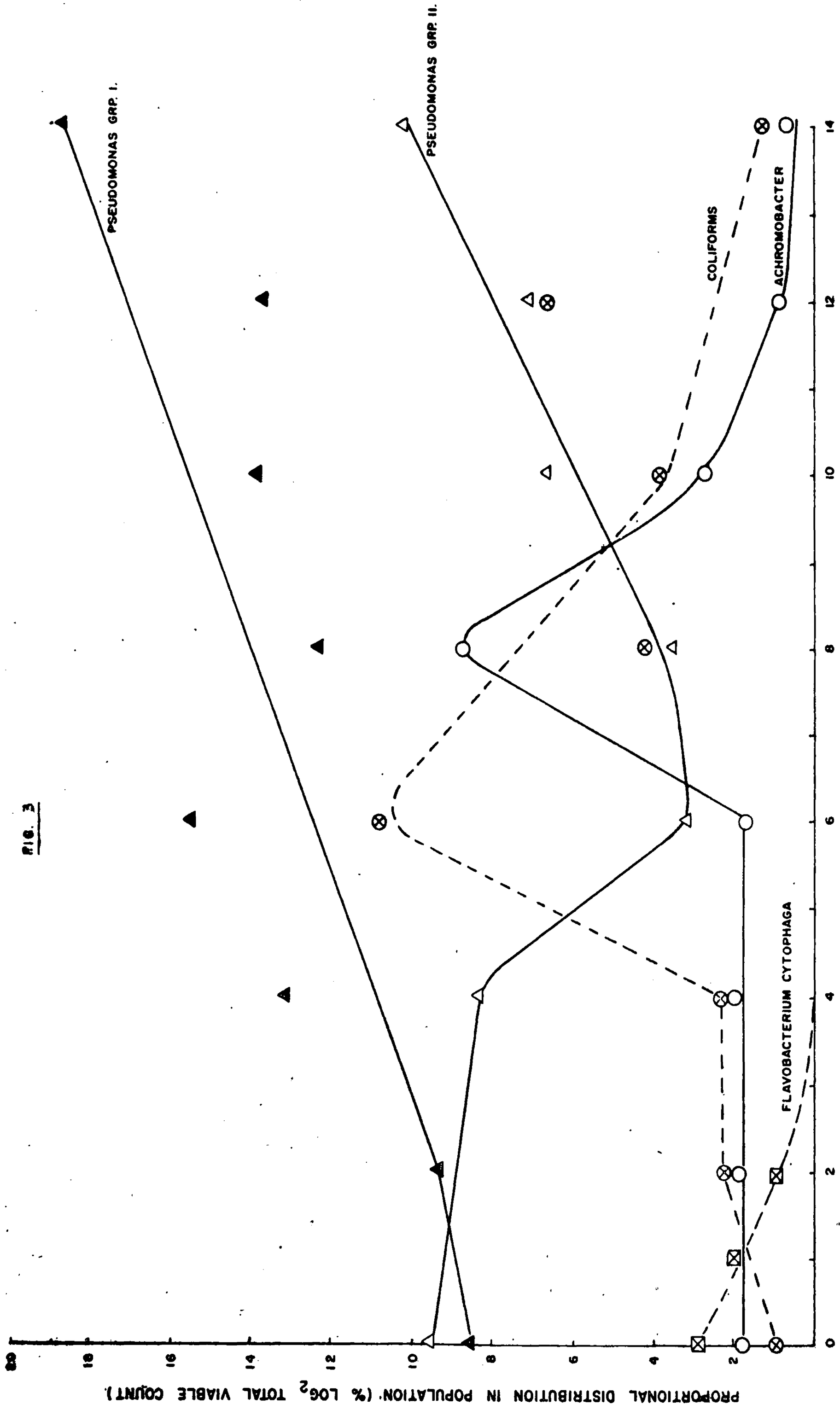
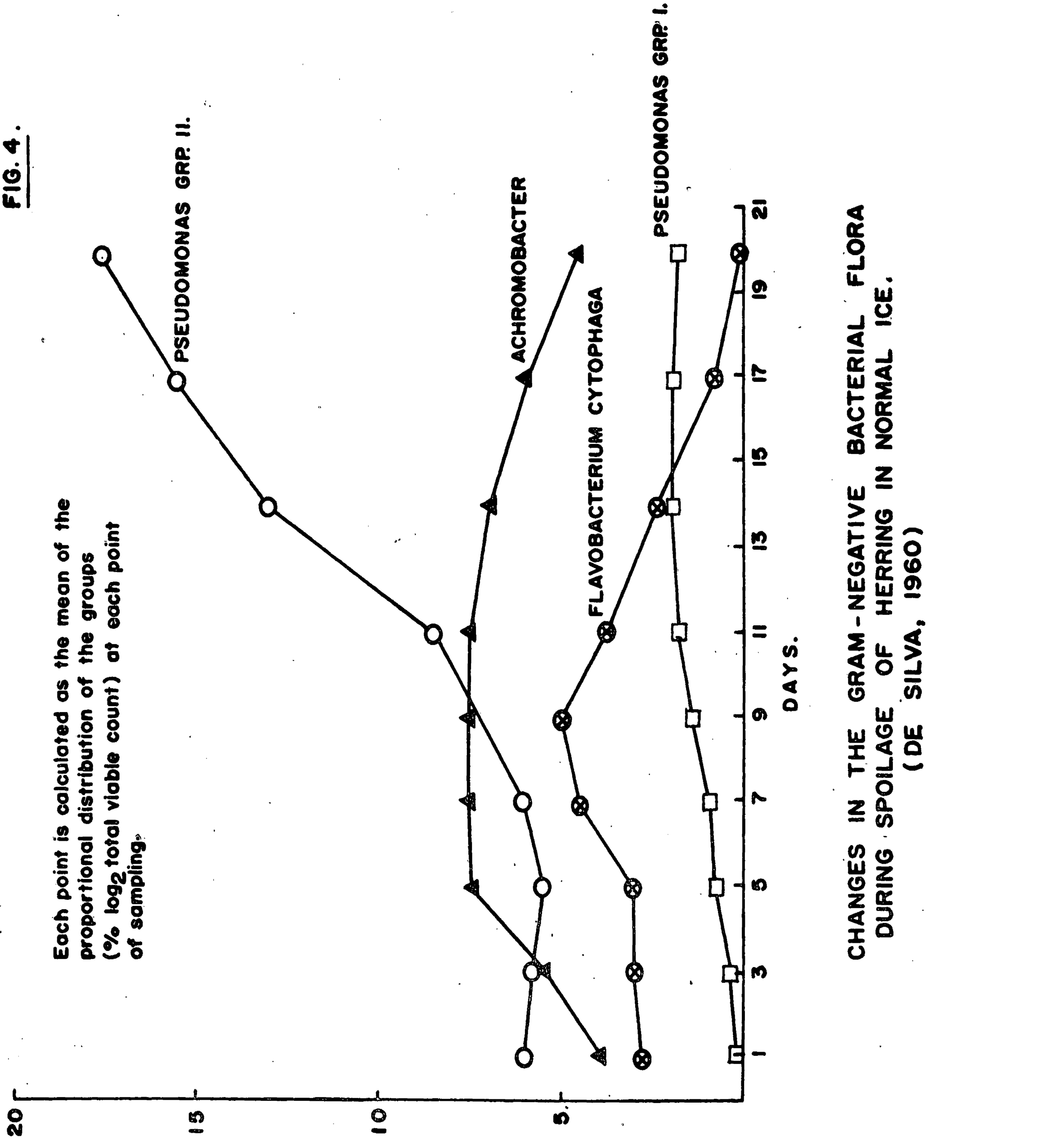

PSEUDOMONAS GRP. I.
PSEUDOMONAS GRP. II.
COLIFORMS
ACHROMOBACTER
FLAVOBACTERIUM CYTOPHAGA
CHANGES IN THE GRAM-NEGATIVE BACTERIAL FLORA IN CHILLED WATER DURING PRESERVATION OF FISH (TRIAL II.)
TIME OF STORAGE IN DAYS.
PROPORTIONAL DISTRIBUTION IN POPULATION. (% LOG2 TOTAL VIABLE COUNT)

FIG. 3

FIG. 4.



CHANGES IN THE GRAM-NEGATIVE BACTERIAL FLORA DURING SPOILAGE OF HERRING IN NORMAL ICE. (DE SILVA, 1960)

Each point is calculated as the mean of the proportional distribution of the groups (% log_2 total viable count) at each point of sampling.

Two points of interest may be mentioned. Firstly, there was little change in the proportion of coliforms till about the 5th day of storage after which they began to increase in importance, especially due to the increase within the population of those lactose fermenters which do not grow at 44°C. Secondly, the establishment of the climax spoilage flora by about the 10th day of storage.

Discussion

The increased use of chilled water for storage of fish has focussed attention on the bacteriology of this form of storage particularly in view of the findings of the Scottish workers that the micro-aerobic conditions encountered in this form of storage could cause a change of the spoilage pattern. Such a change could be of special significance under tropical conditions of temperature and handling which could favour the establishment of coliform organisms of enteric origin which are tolerant of microaerobic conditions.

The pattern of change indicated by the present investigation is clearly different from the pattern of spoilage encountered when fish is preserved in ice (Fig. 4). To some extent at least the changes of the bacterial flora are temperature dependent as indicated by the observation that the flora at 37° remains unchanged or increases slightly while that at 20°C. increases markedly. It would thus seem that on tropical fish at least there are two components of the flora : one that is tolerant of low temperatures and the other tolerant of higher temperatures. The temperature of storage (average air temperature=27° to 30°C.) would determine which of these components would dominate during spoilage.

But upon these temperature dependent factors are superimposed those primarily due to the micro-aerobic conditions that are without doubt established during chilled water storage. The rapid establishment of micro-aerobic conditions could cause the rapid selection of the climax flora when compared to fish stored in ice. It was also observed that the flavobacterium-cytophaga group which increase relatively till about the 9th day in iced fish, does not increase proportionately at any time during chilled water storage. This is consistent with the observation that this group is obligately aerobic. The selection of the pigmented pseudomonads and the coliforms could be related to their being facultative aerobes. Even among the Gram positive groups the results of Trial I indicated that the decrease in coryneforms and the slight, though significant, increase of micrococci may be related to the aerobic requirements of these two groups.

The increase in relative importance of the " fluorescens-putida " group and the coliforms during chilled water storage was significant. While it is no doubt explicable on the assumption that chilled water storage favours the selection of groups of bacteria tolerant of micro-aerobic conditions, it is interesting to note that even among the coliforms " 44° " coliforms were replaced by the ones that grew at 37°C. A similar replacement of one group of coliforms by the other has also been reported by Burman (1960) in a study of the bacteriology of effluence of sewage into water. However the increase of coliforms during a part of the spoilage succession involves many problems of sanitary importance and a more realistic assessment of the use and technology of chilled water storage. It is certainly true that during all stages of spoilage the numbers of typical " spoilage " bacteria swamp the coliforms. This is indicated by the quantitative results (Fig. 1) where the coliforms are insufficient to show significant changes in the 37°C. counts. But there is no doubt that at least to some extent a certain epidemiological risk is involved during the storage of fish in chilled water particularly under conditions where chance contamination by pathogens of enteric origin cannot be totally eliminated.

The increase in the proportions of pseudomonads during chilled water storage has been reported from Torry (Ann. Rept. T. R. S., 1959). But their results indicated that the selected group was the non-pigmented variety. Torry workers do not mention the coliforms. While it could be due to their total absence in the chilled water during their experiments, it is more likely that the plates were incubated at 20°C for 48 hours, a temperature at which many of the coliforms will not grow during the period of incubation.

It may also be noted that the present investigation was done with chilled freshwater. To what extent the inclusion of salt alters this pattern of spoilage has yet to be investigated. It may be possible that some prolongation of storage life may be obtained by the use of chilled brine as compared to chilled freshwater ; but it is doubtful if there would be major differences in the bacterial succession during spoilage.

CONCLUSIONS

The present study of the population dynamics of the bacterial flora of chilled water during storage of fish indicated that there was a marked difference in the spoilage pattern between fish held at +1°C in ice and that held at the same temperature in water. The differences in the pattern of change of flora were explicable on the assumption that on the temperature dependent factors which mainly determined the nature of the bacterial flora at any given time, there were super-imposed micro-aerobic factors during chilled water storage. Micro-aerobic conditions were no doubt caused by the low temperature when the solubility of oxygen is lowered in relation to carbo-di-oxide, poor circulation of the water when the tank was loaded with fish and the high content of decomposing organic matter in the water.

The flavobacterium-cytophaga group were suppressed quite early during spoilage. After an initial rise the micrococci decreased in importance. The achromobacters and the coliforms also showed relative increases especially during the second stage of spoilage but were finally replaced by the pigmented and the non-pigmented pseudomonads, which formed the dominant climax flora during spoilage.

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