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PAPER CHROMATOGRAPHY IN FISH TAXONOMY

By R. Viswanathan and V. Krishna Pillai

(Central Marine Fisheries Research Station, Mandapam Camp)

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Introduction

The correct identification of species is of primary importance to the fishery biologist in his task of assessing the composition of any fishery stock. Conventional taxonomic methods are undoubtedly of great value for this purpose but they have their limitations especially when the specimens under study are too small or when geographical and other factors, unidentified as yet, complicate the picture. Very often the discontinuity in morphological features may not be adequate or the recognition characteristics may be insufficient and in these cases information might well be supplemented by additional methods.

In recent years the technique of paper partition chromatography has been applied with success as an aid in the taxonomy of fishes (Buzzati-Traverso and Rechnitzer, 1953), land snails (Kirk *et al.*, 1954), mosquitoes (Micks, 1954) and fruit flies (Buzzati-Traverso, 1953). The method consists essentially in drying a bit of tissue on filter-paper, dissolving out constituents of the tissue with a chemical solvent travelling along the paper by capillary action, and, after removal of solvent, identifying the linearly separated constituents by the use of suitable indicators or fluorescent light. Buzzati-Traverso and Rechnitzer employed this method in their study of tissue of *Paralabrax clathratus, P. maculatofasciatus* and *Hysterocarpus traski*. They found ninhydrin-positive patterns of a certain tissue taken from various specimens of the same species to be constant, irrespective of the size or age of the fish, while patterns obtained from muscle of different species showed constant but easily recognizable differences. The significance of these results to marine fishery investigations was realised by the Californian Marine Fishery Research Committee (1952–53) who initiated a programme of work on the application of paper partition chromatography to the study of subgroups and migratory habits in sardine populations along the Californian coast. In the present article the possibilities are discussed, on the basis of experiments, of a similar approach to the study of fish populations in the Mandapam area.

Material and Methods

Preliminary Experiments

Fishes were obtained from commercial catches. The fish-muscle samples were spotted on filter paper according to the instructions of Buzzati-Traverso and Rechnitzer. For chromatography, Whatman No. 1 filter-paper was used throughout.

In the first sets of experiments, the apparatus employed was the same as that indicated by Krishna Pillai (1953) for the preparation of ascending chromatograms. The solvent system was made up of *n*-butanol, acetic acid and water, mixed in the ratio of 40 : 10 : 50, v/v. The chromatograms were sprayed with a 0·2% solution of ninhydrin in rectified spirit. The patterns from *Sardinella albella* and *Sardinella gibbosa* were equivocal though differences could be noted in widely different species like *Hemirhamphus georgii* and *Atherina* sp. (Fig. 1).

Final Procedure

Details of subsequent experiments are given in Table I.

Table I

Paper Chromatography of Fish Muscle: Experimental Details

Chromatographic method	Size of filter-paper	Solvent	Paper pre-satd. for (hrs.)	Colour† reagent
Ascending ..	30×13 cm.	*n*-BuOH/AcOH/H_2O (100 : 22 : 50, v/v)	1·5	Ninhydrin
Disk* (Two runs) ..	12·5 cm. diam.	Propanol-ammonia (1%) (4 : 1, v/v)	0·5	Ninhydrin and rectified spirit containing 5% pyridine

* *Cf.* Giri (1953). The solvent was contained in a Petri dish (6·3 cm. diam.) and the chromatograms were developed in between Petri dishes (15 cm. diam.).

† Dip method; patterns traced after keeping chromatograms for a few minutes between 60° and 70° C.

The sardines used ranged from 89 to 126 mm. in total length. Two samples from *S. albella* and two from *S. gibbosa* were spotted on each sheet. The water content of the solvent system was about half that of the solvents used by Buzzati-Traverso and Rechnitzer. This reduction of water content appeared to be essential for a clear differentiation between the patterns from the two species (Figs. 2 and 3).

Chromatograms from Leiognathus *spp.* and Caranx *spp.*

A few chromatograms were prepared from *Leiognathus insidiator* (total length, 58–73 mm.), *Leiognathus daura* (t.l., 72–80 mm.), *Caranx leptolepis* (t.l., 141 mm.) and *Caranx sansun* (t.l., 118 mm.). A double run was employed in the ascending chromatograms similar to the double run in the disk chromatograms. Spots characteristic of the pattern from *L. insidiator* were absent in the case of *L. daura* and likewise a spot typical of *C. leptolepis* chromatogram was missing in the *C. sansun* pattern (Figs. 4 and 5).

Measurements of Pattern Spread

The quantity of muscle spotted varied from sample to sample and, for comparing the systematic differences found, the ratios of the spreads of chromatographic patterns were calculated in each experiment in preference to the absolute values. In nine experiments in which the solvent front advanced a distance of 19–21 cm., the lengthwise spread of the patterns in the ascending chromatograms had the following chracteristics: Spread (gibbosa)/spread (albella): range, 1·05 to 1·26; mean, 1·12; s.d., 0·07; and coeff. of variation, 6·3%. In the patterns obtained by the disk method, the ratio, R, of the distance from base to lower edge of bottom spot to the distance from base to upper edge of topmost spot was higher in *S. gibbosa* than in *S. albella*, the values of *R.* (*gibbosa*)/*R.* (*albella*) in three experiments being 1·16, 1·14 and 1·12.

Chromatography of Preserved Muscle

A study was also made of the related question of the chromatography of preserved samples. Preservation of muscle in rectified spirit resulted in loss of some of the ninhydrin-positive spots. Treatment with acetone rendered the muscle too fibrous to be conveniently spotted on the filter-paper. Prolonged drying at room temperature caused a loss of the intensity of the patterns and drying the muscle at 100° C. (*cf.* Ceriotti, 1955) resulted in a distortion of the patterns. The best results with minimum or no distortion were obtained when the muscle was pre-treated with ether overnight. The ether-treated muscle could be pressed on to a filter-paper by applying pressure for a few minutes with a glass rod.

DISCUSSION

The chromatographic patterns described by Buzzati-Traverso and Rechnitzer were obtained from fresh fish-tissue; but the experiments reported here indicate that even when the fish are not quite fresh, similar species-specific patterns can be obtained by suitably modifying the experimental conditions, especially the water content of the irrigating solvent-system. The test samples of muscle can be preserved, if necessary, in

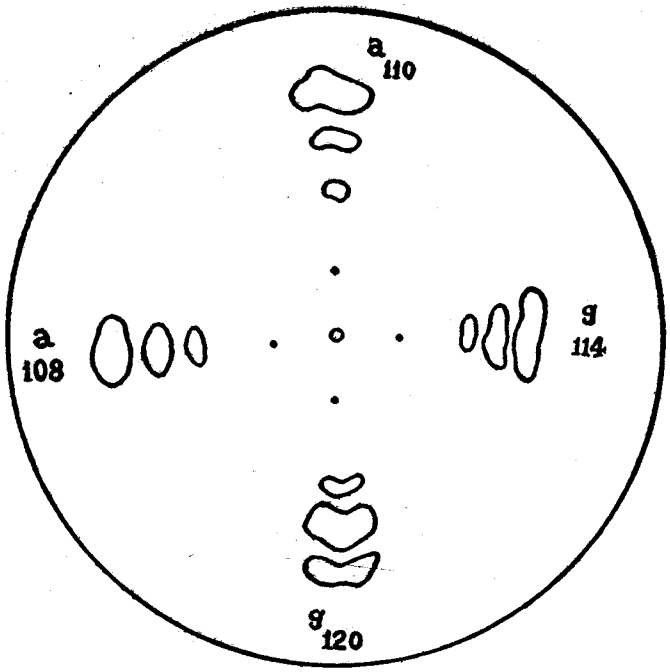

FIG. 3. Ninhydrin-positive patterns of *Sardinella albella* (a) and *Sardinella gibbosa* (g) (Disk chromatography).

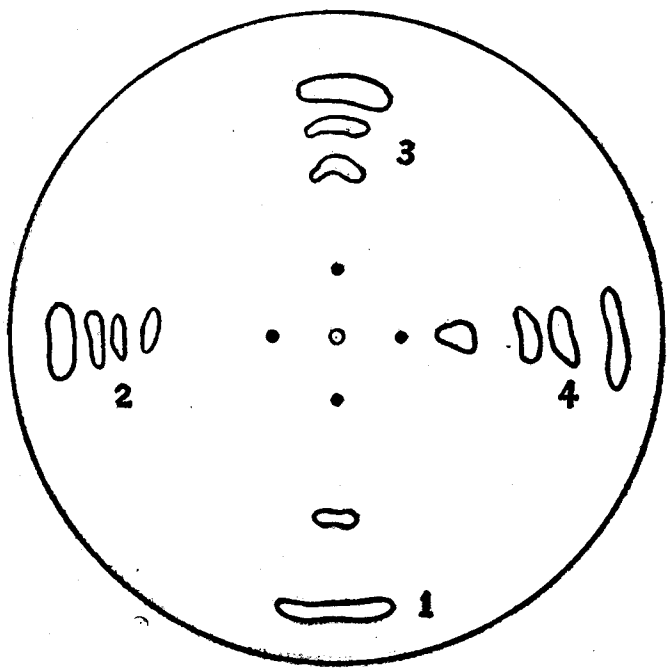

FIG. 5. Ninhydrin-positive patterns of (1) *Leiognathus daura*, (2) *Leiognathus insidiator*, (3) *Caranx sansun*, and (4) *Caranx leptolepis*.

suitable solvents like ether which do not significantly alter the patterns and they can be later chromatographed against standard or authentic specimens and compared. Further, the amount of muscle required in each experiment is only of the order of a few c.mm., and difficulties due to smallness of size of fish are minimised. These features of the method are of obvious advantage in dealing with a large and representative number of samples from commercial catches which are usually of varying degrees of freshness.

The chemical identity of the substances responsible for the specificity of the chromatographic patterns* is not known with definiteness (Buzzati-Traverso and Rechnitzer). This accounts to a large extent for the necessity for standardising *ab initio* the experimental conditions of solvent system, equipment, types and sizes of filter-paper, etc., best suited for the species under comparison. This notwithstanding, the data available suggest the scope for an extensive use of the chromatographic method in standard fishery biology problems of the type enumerated by Kesteven and Deacon (1955). In the sardine fishery near Mandapam, for instance, the bulk of the catches belong to two closely related species (Sekharan, 1955) and basic taxonomic issues are involved in the analysis of fish stocks with reference to their composition, fluctuations in composition from year to year and from area to area, effects of geographical isolation on the stocks, etc. It is in the study of these that paper chromatography with its high degree of specificity is likely to be helpful and even necessary.

SUMMARY

Paper partition chromatography by the ascending and disk methods has been applied to the study of muscle from *Sardinella* spp., *Leiognathus* spp., *Caranx* spp., *Hemirhamphus georgii* and *Atherina* sp.

Experimental conditions have been standardised under which differences among species of fish could be made out qualitatively and on the basis of quantitative measurements, using fish tissue of varying degrees of freshness. Methods have been studied for the preservation of fish muscle for chromatographic work.

The applications of paper chromatography to problems in fishery biology have been indicated.

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* Very recently Fox (*Science*, 1956, **123**, 143) has been able to detect sex-specific differences (*Drosophila melanogaster*) also by substituting two-dimensional chromatography for the one-dimensional procedure of Buzzati-Traverso and Rechnitzer. His results are of particular interest since they substantiate evidence presented in the present article in regard to the scope of the method in modern taxonomy and to the necessity for a quantitative approach to the problem.

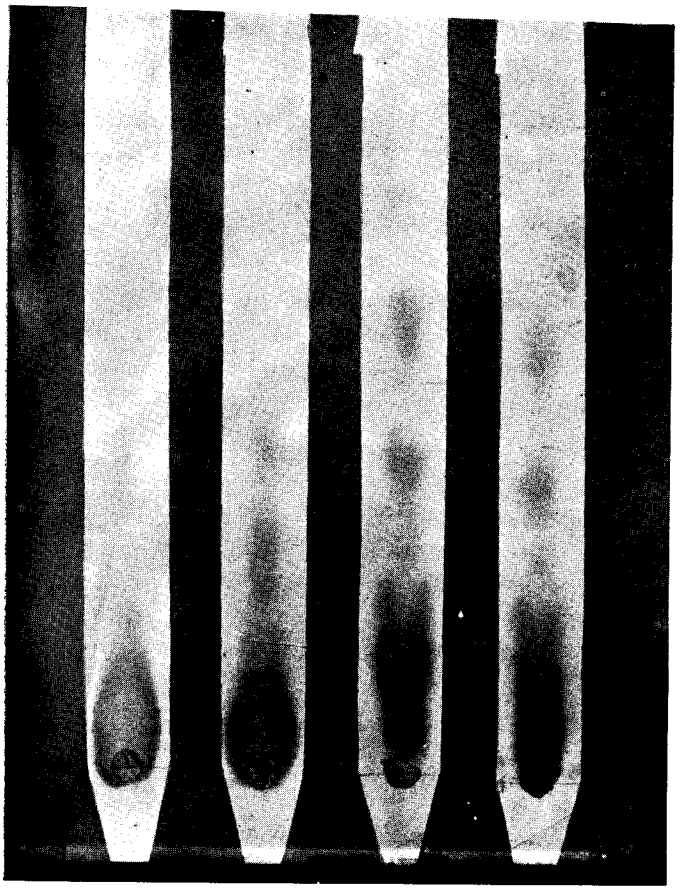

FIG. 1

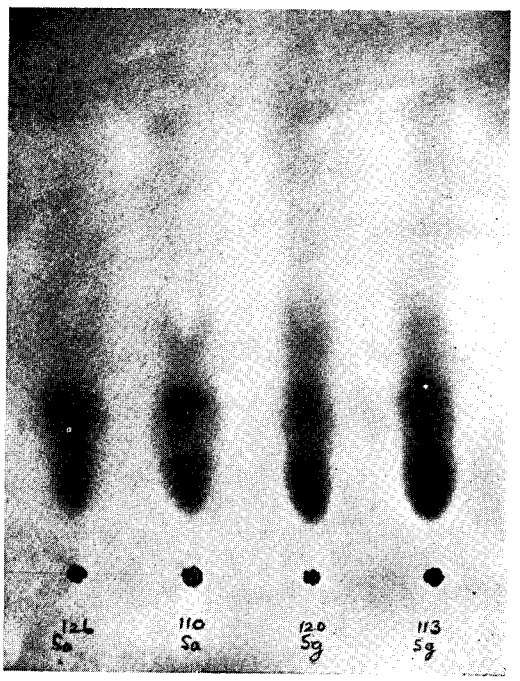

FIG. 2

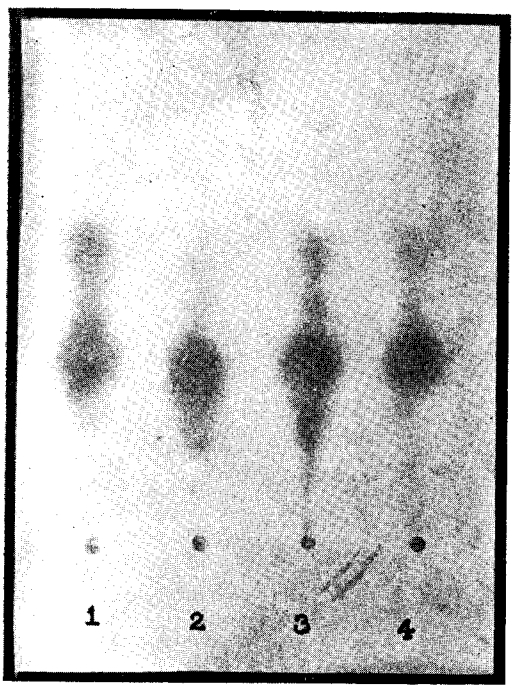

FIG. 4

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EXPLANATION OF PLATE

FIG. 1. Ninhydrin-positive patterns of (left to right) *Hemirhamphus georgii* (1 and 2) and *Atherina* sp. (3 and 4).

FIG. 2. Ninhydrin-positive patterns of (left to right) *Sardinella albella* (1 and 2) and *Sardinella gibbosa* (3 and 4).

FIG. 4. Ninhydrin-positive patterns of (1) *Leiognathus insidiator*, (2) *Leiognathus daura*, (3) *Caranx leptolepis*, and (4) *Caranx sanson* (Ascending chromatography).