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International Council for
the Exploration of the Sea

C.M. 1975/K:18
Shellfish and Benthos Committee

Vibrio cholerae El Tor in shellfish beds
of the South Coast of Portugal



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INTRODUCTION

When in 25/4/74 appeared the information about the occurrence of the first human case of cholera in Tavira, we were worried immediately with the possibility of the pollution by the choleric vibrio to attain the local shellfishes beds.

From this moment, was mounted a net of sampling stations for water and shellfish, firstly limited to the area under the influence of Tavira sewage system, later widespread to all the lagoon that forms the portuguese coast at this zone.

This scheme included in the final phase 23 sampling stations where the samples were taken every three days during the first month.

The purpose of this note is to report the results obtained in a year of work, in spite of keeping the monitoring scheme.

From April 1975 the monitoring scheme was modified, including now 25 sampling stations located in all the Algarve coast, but sampling only water. The most of the sampling stations of this scheme was located near the main urban seawages effluents or at places where the pollution probability by fluency will be conspicuous. This scheme is an alarm system from which we can detect the vibrio and then to start to the organization of an intensive programm with a more closed sampling net and with a shorter periodicity.

The analyses were accomplished in collaboration with the Laboratory of the Regional Health Service.

METHODS

The methods used in the search of the choleric vibrio were proposed by W.H.O.

The samplings of water have a volume of 1 liter which is inoculated in two flasks with 50 ml of alkaline peptone water. These flasks are incubated at 37°C during 18 to 24 hours. Starting from this flasks are inoculated plates of T.C.B.S. - Agar (Thiosulphate Citrate Bile Salts Sucrose-Agar) and of Blood-Agar and a tube with alkaline peptone water. After incubation in the same conditions agglutinations tests are made with suspect colonies. The sera used are the polyvalent cholera serum the Inaba serum and the Ogawa serum. From the second enrichment tube we do new inoculations in plates with the same media. In the case of positive agglutination test, we make a new culture in tubes with T.S.I. Agar (Triple Sugar Iron-Agar) and plates of Blood-Agar for the sensibility test to polymyxin B. If these tests are positive, cultures are sent to the National Health Institute Dr Ricardo Jorge at Lisbon for complete identification of the isolated organisms. The same procedure is used with the cases of anomalous results.

To the search of vibrio in shellfishes, the samples have ten mollusks each in the case of clams, cockles and mussels. The samples of oysters have only 6 mollusks. The mollusks are aseptically opened and the flesh is mechanically macerated and incubated in an Erlenmeyer flask with 150 ml of alkaline peptone water. From this stage the method is the same used to the water sample. We don't use the membrane filter technic, because the water of our samples contains a big quantity of suspended matter.

RESULTS

During 1974 are made 165 water analyses and 166 of mollusks. We obtained 40 positive samples (25,6%). Concerning the

shellfish we obtained 64 positive results (38,6%). All the positive results were identified as *Vibrio cholerae* El Tor Inaba serotype.

The samples distribution by the different stations, as well as the respective results are presented in the fig. 1, 2, 3 and 4.

We don't obtain till the present (17 Jun. 75), any positive results.

CONCLUSIONS

Shellfishes have been reported in the past to cause outbreaks of cholera (Malaysia and Italy). In 1973 in Italy mussels were shown to be vehicle of transmission. In spite of only one of many samples of shellfish tested and yielded *V. cholerae*. Our results show that both shellfish and sea water samples from the shellfish grounding areas were heavily contaminated with *V. cholerae*.

Epidemiologic studies, demonstrated that mollusks caused cases of cholera, supporting the established conclusion of P. C. Wood in his report on a mission to Portugal (1974).

The conclusions of Cachola and Nunes (1974) that all the shellfish growing areas of Algarve were heavily polluted by the urban sewage system, are confirmed by these results.

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APPENDIX ICULTURE MEDIA

Enrichment media

Alkaline peptone water (shellfish)

Sodium chloride	10 gr.
Peptone	20 gr.
Distilled water	1000 c.c.
Final pH	8,8

Alkaline peptone water (waters and sewage)

Sodium chloride	50 gr.
Peptone	100 gr.
Distilled water	1000 c.c.
Final pH	8,8-9

Alkaline peptone water (2nd enrichment)

Sodium chloride	10 gr.
Peptone	10 gr.
Distilled water	1000 c.c.
Final pH	8,6

Selective media

Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS)

Yeast Extract	5 gr.
Bacteriological peptone	10 gr.
Sodium thiosulphate	10 gr.
Sodium citrate	10 gr.
Ox bile	8 gr.
Sucrose	20 gr.
Sodium chloride	10 gr.
Ferric citrate	1 gr.
Brom-thymol blue	0.04 gr.
Thymol blue	0.04 gr.
Agar	14 gr.
Distilled water	1000 c.c.

Final pH 8,6 (approx.)

Triple Sugar Iron Agar (TSI)

Beef Extract	3 gr.
Yeast Extract	3 gr.
Peptone	20 gr.
Sodium chloride	5 gr.
Lactose	10 gr.
Sucrose	10 gr.
Dextrose	1 gr.
Ferric citrate	0,3 gr.
Sodium thiosulphate	0,3 gr.
Phenol red	q.s.
Agar	12 gr.
Distilled water	1000 c.c.

Final pH 7,4 (approx.)

APPENDIX IILIST OF SAMPLING STATIONS1 - VILA REAL DE SANTO ANTÓNIO

1A - Lota

1B - Santo António

2 - TAVIRA

2A - Cacela - Fábricas

2B - Cabanas - Lota

2C - Ponte Rodoviária

2D - Lota

2E - Quatro Águas

2F - Santa Luzia

2G - Torre d'Aires

3 - FUZETA

3A - Livramento - Moinho Santos

3B - Lota

4 - OLHAO

4A - Cavacos

4B - Marim

4C - Doca de Pesca

4D - Coroa dos Mouros

4E - Pernada da Cruz

4F - Alcorão

4G - Culatra

4H - Machiles

4I - Barra Nova

5 - FARO

5A - Praia dos Estudantes

5B - Horta da Areia

5C - S. Francisco

5D - Marchil

5E - Quatro Águas

5F - Ramalhete

5G - Carga Palha

5H - Ancao

5I - Cascalheira

6 - ALBUFEIRA

6A - Praia dos Pescadores

7 - PORTIMAO

7A - Ferragudo

7B - Lota

7C - Mexilhoeira

7D - Alvor - Lota

8 - LAGOS

8A - Estaleiro

FIG. 1
 WATER ANALYSIS
 1974

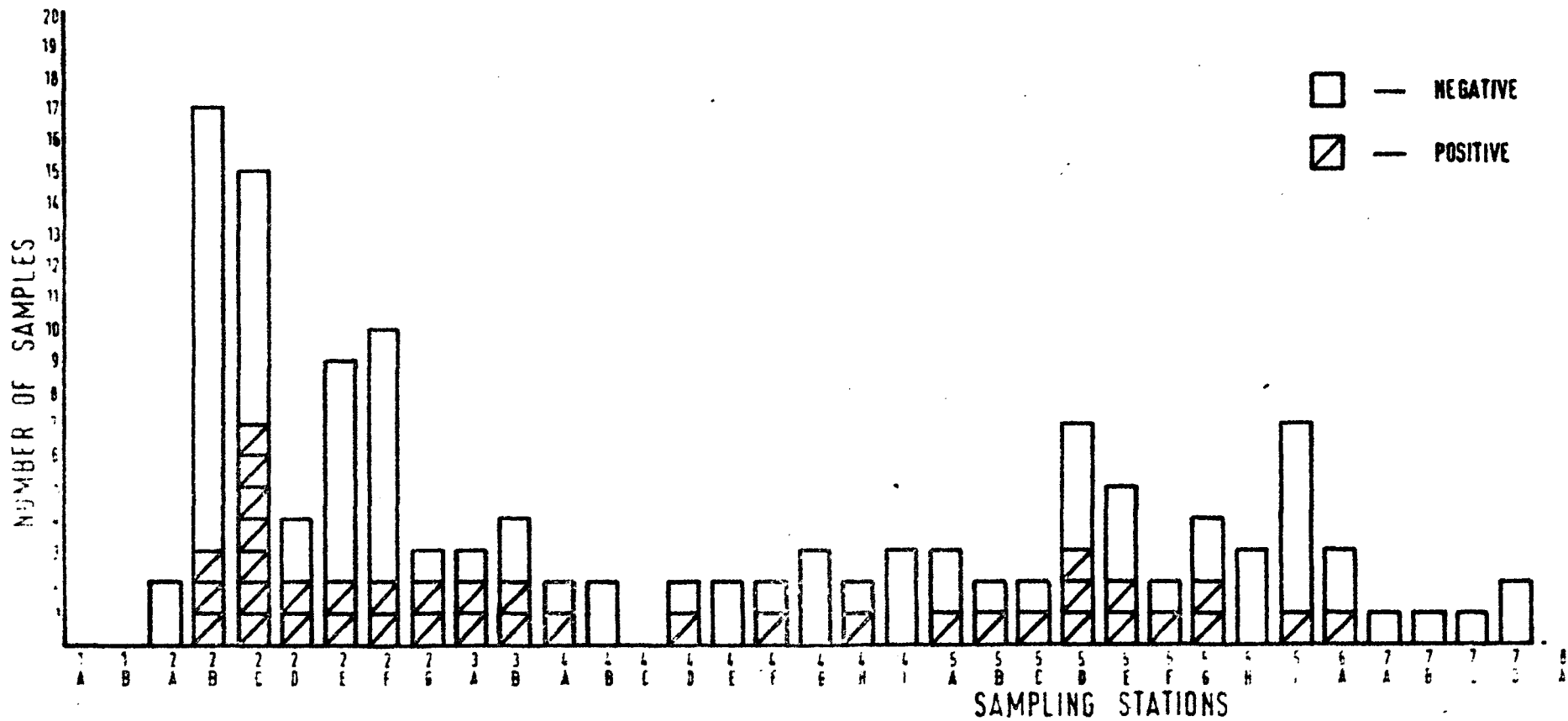


FIG. 2
WATER ANALYSIS
1975

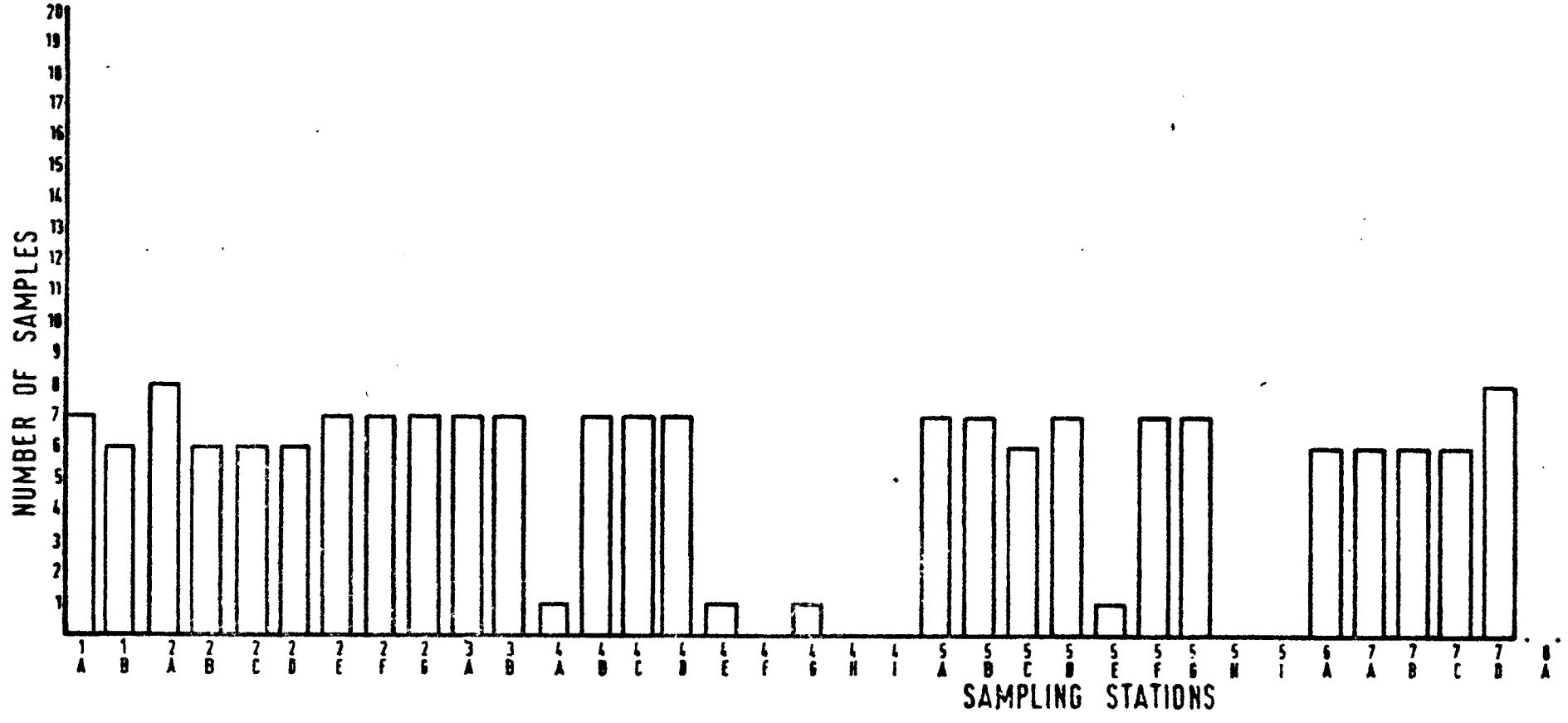


FIG. 3
MOLLUSC ANALYSIS
1974

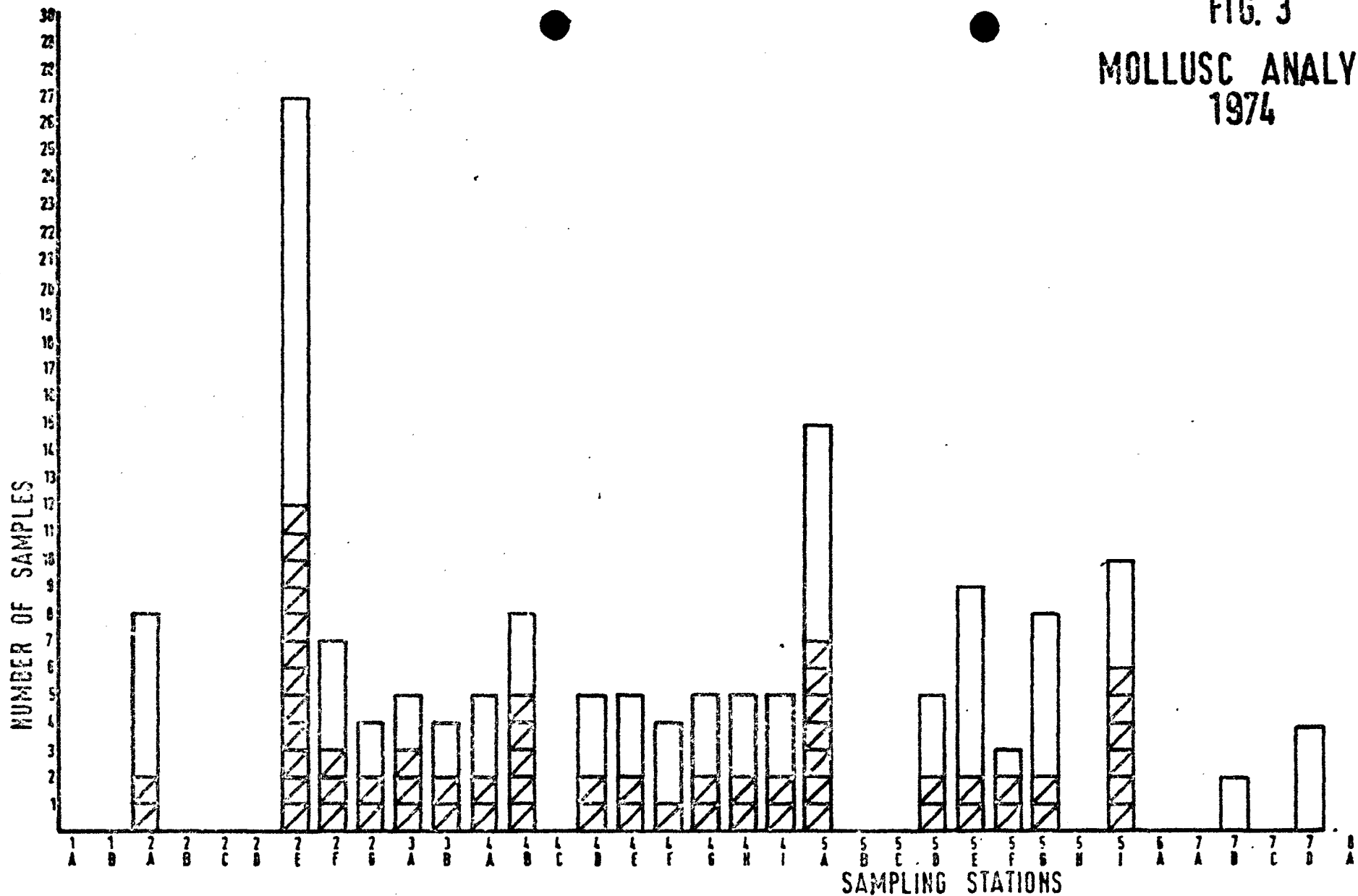


FIG. 4
MOLLUSC ANALYSIS
1975

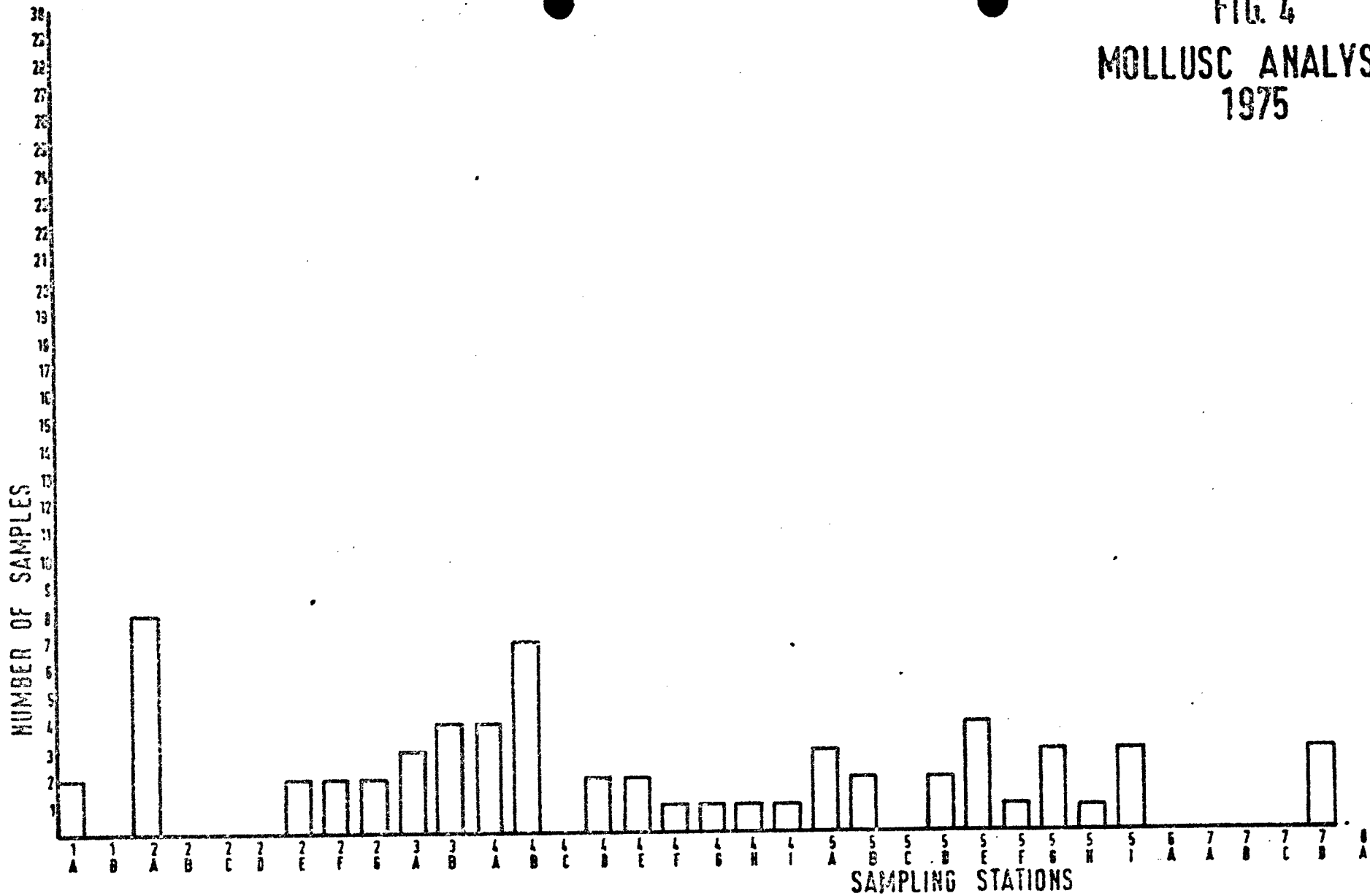


FIG. 5
ALGARVE COAST

