Chapter IX

Study of the pollution in sea fish and shell fish

by

P. HERMAN and N. BOUQUIAUX

I. Mercury content in fish and shrimps caught off the Belgian coast

Introduction

In recent years, the concentration of heavy metals, pesticides and other pollutants in fish has become a cause for concern and has received increased interest. Mercury especially is regarded as a problem since the outbreak of mercury poisonings in Japan was found to originate from the consumption of heavily contaminated fish. Comprehensive reviews on the problems related to mercury in fish were published by Meyer (1972) and Holden (1973).

As in several other countries, a large-scale survey of the levels of contaminants in fish was started in Belgium. This report is the result of a study of mercury concentration in representative fish species, *viz.* plaice *(Pleuronectes platessa L.)*, whiting *(Merlangus merlangus L.)*, cod *(Gadus morhua L.)*, sprat *(Clupea sprattus L.)* and shrimps *(Crangon crangon L.)* caught off the Belgian coast.

This area was surveyed specifically because of the increased risks of pollution in coastal waters. In this connection, the vicinity of the Scheldt estuary should be stressed. Furthermore, Belgian coastal waters are known to be a nursery ground for several important fish species and shrimps. For that reason, special attention was also paid to the pre-recruitment stock (0 , I and for some fish species the II-age groups). Emphasis was laid on the possible relationship between mercury levels and different biological parameters (age, sex, length, weight) of fish. For shrimps, which are sedentary organisms, the fishing ground and the season were taken into account.

1.- Methods and materials

1.1.- Sampling

Experimental fisheries were carried out during a one year's period (October 1971 – October 1972) by the R.V. *Hinders* in an area up to 10 miles off the Belgian coast. Fishing took place during day time with the otter trawl (mesh size 18 mm). Hauling time was 15 min .

Two areas were sampled on a monthly basis, *viz.* five Western stations in the *Westdiep* ground off Nieuport and five Eastern stations in the *Vlakte van de Raan* ground off Zeebruges. Furthermore, an overall survey of 30 stations scattered over the whole area was undertaken in October 1971, May and October 1972. In total, 800 samples were taken.

The whole catch of plaice, whiting and cod was examined and classified in age-groups. The average composition per hour fishing of the catches was :

- plaice : 0-I : 55 % ; II : 16 % ; III + : 29 % ;
- whiting : 0-I : 93 % ; II + : 7 % ;
- cod : 0-I : 67 % ; II + : 33 % .

The samples of these three fish species were analyzed individually and the following parameters were determined :

- length (in cm),
- weight (in g),
- age (otolith readings),
- sex (gonads and testes).

The samples of sprat consisted of 1 to 10 specimens, depending upon their availability.

For shrimps a sample of 250 g was taken. For practical reasons, the shells were not removed.

1.2.- Mercury analysis

Digestion method with H_2SO_4/H_2O_2 ; reduction of Hg with $NaBH_4$. Determination with A.A. (M.A.S. 50) .

1.3.- Statistical analyses

Regression analyses and analyses of variance were carried out as outlined by Snedecor and Cochran (1971).

2.- Results and discussion

The results of the mercury analyses are reported in Table 9.1.

The age-groups of plaice, whiting and cod were considered separately. An estimate of the average mercury content on an individual animal basis of the three populations was also made by taking into account the average composition of the experimental catches in the period under survey.

The population standard deviation was estimated by pooling the age-group variances.

An analysis of variance was carried out to test the differences between males and females per age group but showed no significant differences at the 95 % probability level.

The mercury concentrations tended to increase with the age of the fish.

The increase in mercury levels with age was also reported by several other workers [Johnels *et al.* (1967), Bligh and Armstrong (1971), Thibaud (1971), Forrester *et al.* (1972)].

The nature of this relationship was studied further by calculating the regression between mercury content and length of the fish :

- Whiting, male :

$$y = 8.8 \times 10^{-3} x - 23 \times 10^{-3} \qquad r = 0.566$$

Table 9.1

Mercury analyses of fish and shrimp caught off the Belgian Coast

Species	Age-groups	Number	Hg (ppm)		
			Average	s	v (%)
Plaice	0 - I	66	0.172	0.093	54
	II	91	0.171	0.068	40
	III	73	0.153	0.067	44
	IV	26	0.174	0.070	40
	V-IX	11	0.216	0.094	44
	Total *	-	0.168	0.077	46
Whiting	0 - I	97	0.151	0.059	39
	II	112	0.173	0.087	50
	III	26	0.202	0.090	45
	IV-V	4	0.245	0.072	29
	Total *	-	0.153	0.079	52
Cod	0 - I	37	0.106	0.051	48
	II	37	0.137	0.046	34
	III	5	0.180	0.085	47
	Total *	-	0.116	0.055	47
Sprat	Total	66	0.144	0.064	44
Shrimp	Total	148	0.101	0.039	39

* On an individual animal basis, taking into account the average composition of the catch.

- Whiting, female :

$$y = 7.3 \times 10^{-3}\, x + 26 \times 10^{-3} \qquad r = 0.383$$

- Whiting, male and female :

$$y = 7.0 \times 10^{-3}\, x + 19 \times 10^{-3} \qquad r = 0.410$$

- Cod, male :

$$y = 4.1 \times 10^{-3}\, x - 24 \times 10^{-3} \qquad r = 0.616$$

- Cod, female :

— $r = 0.098$

- Cod, male and female :

$$y = 2.4 \times 10^{-3}\ x + 33 \times 10^{-3} \qquad r = 0.378\ .$$

For the sake of completeness, the equations expressing the relation mercury content to weight are :

- Whiting, male :

$$y = 31.4 \times 10^{-3}\ x^{0.4}$$

- Whiting, female :

$$y = 33.2 \times 10^{-3}\ x^{0.3}$$

- Whiting, male and female :

$$y = 49.6 \times 10^{-3}\ x^{0.3}$$

- Cod, male :

$$y = 18.8 \times 10^{-3}\ x^{0.3}$$

- Cod, male and female :

$$y = 34,4 \times 10^{-3}\ x^{0.2}$$

where r is the correlation coefficient, the significance is 99.9 % . As the relation between weight and length is curvilinear, these equations were of the type $y = ax^b$ where y is the mercury content in ppm and x the body weight in g . For plaice, no significant regressions were found. The regressions for whiting and cod are shown graphically in figures 9.1 to 9.4.

Male and female whiting and male cod showed rather low but very significant (99.9 % probability) regressions. The relationship mercury content to length (or weight) however appeared to be not significant in female cod, indicating different behaviour towards mercury accumulation.

In order to have a better knowledge of the variations in mercury levels, frequency distributions were also calculated. Figures 9.5, 9.6 and 9.7 show the histograms per age-group. The highest age-group of the three species was not taken into consideration owing to the low number of specimens available. The overall frequency distribution of the whole population was also determined by taking the composition of the catches into account.

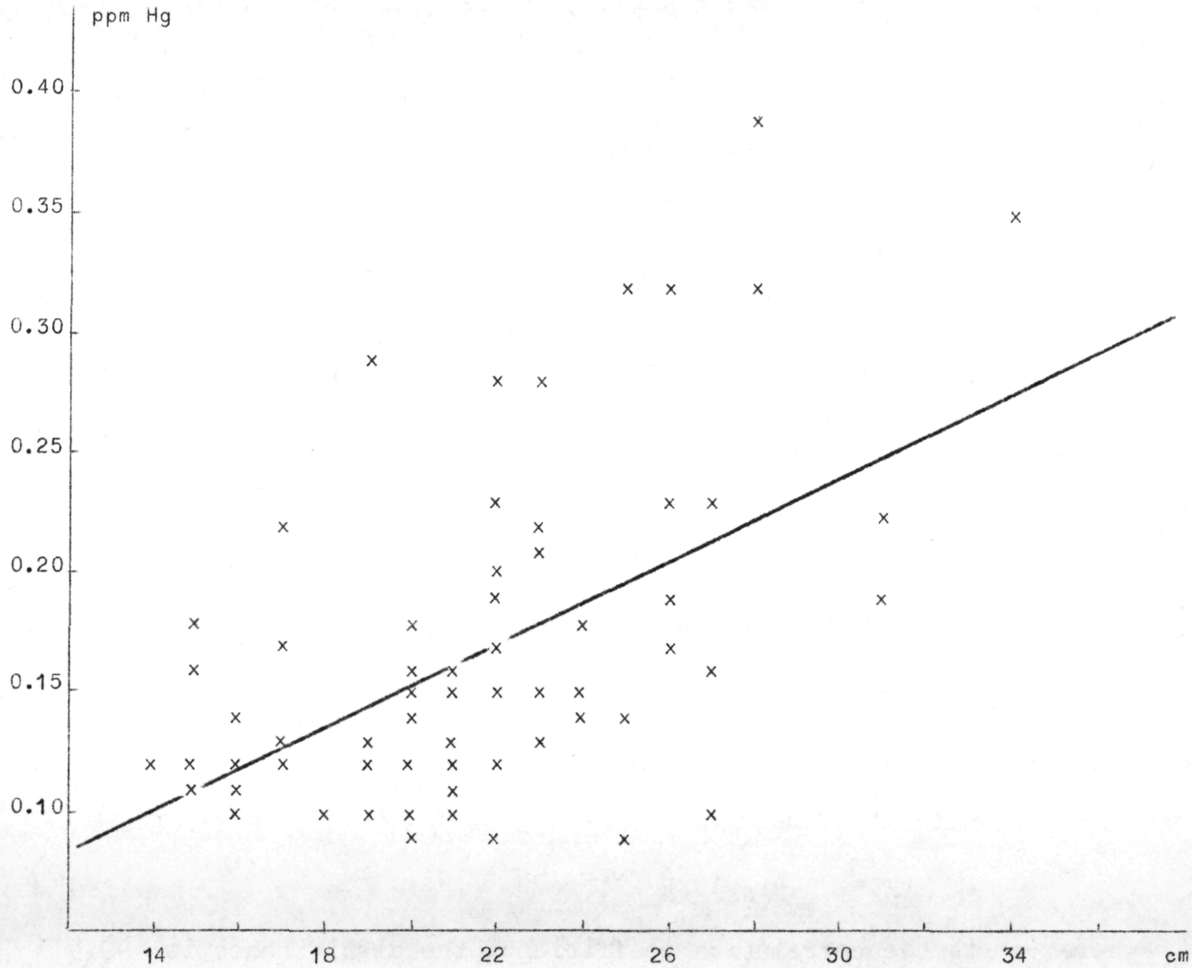


fig. 9.1.- Regression between mercury content and length of male whiting.

The histograms show the Hg concentrations to be fairly well distributed. It should be noted in this respect that the standard deviations of the different groups were very similar (Table 9.1).

The greater portion of values were situated below 0.250 ppm , and were probably within the natural range for the species considered [Holden (1973)]. The highest frequencies occurred between 0.100 and 0.250 ppm .

These data are in general agreement with results obtained in other countries for coastal areas (less than 25 miles from the coast). For plaice, Portmann (1972) reported an average of 0.25 ppm (n = 51) in British coastal waters, Koeman *et al.* (1971) 0.21 ppm (n = 8) in the Dutch Wadden Sea and Antonacopoulos (1973) 0,25 ppm in the Elbe estuary. Greve and Wit (1971) on the other hand mentioned five analyses

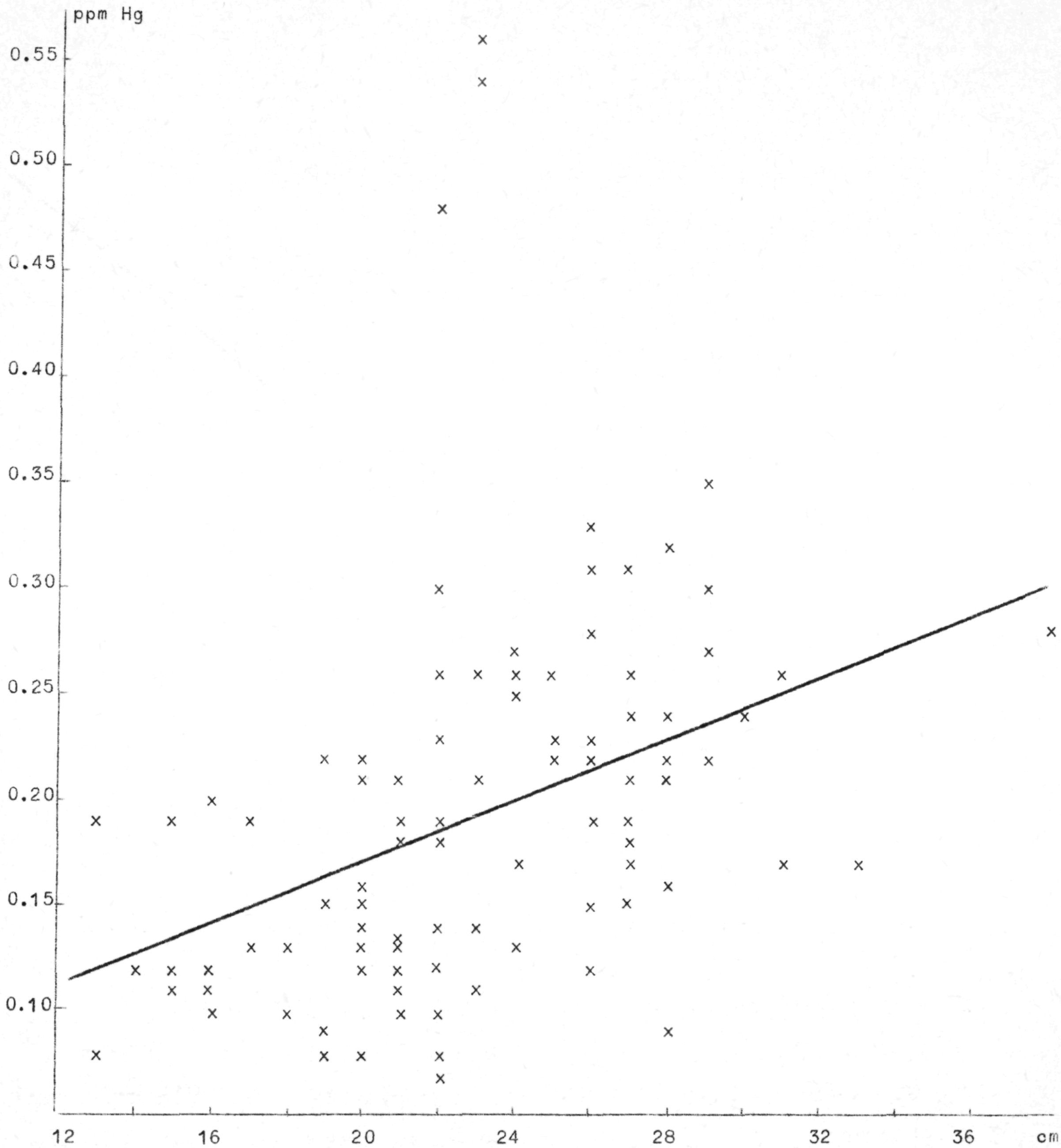


fig. 9.2.- Regression between mercury content and length of female whiting.

of plaice caught off the Southern Dutch coast with a markedly lower range of 0.05 to 0.14 ppm .

For cod, Portmann (1972) found an average value of 0.26 ppm (n = 37) in British coastal waters, Greve and Wit (1971) 0.18 ppm (n = 5) off the Southern Dutch coast and Bligh and Armstrong (1971)

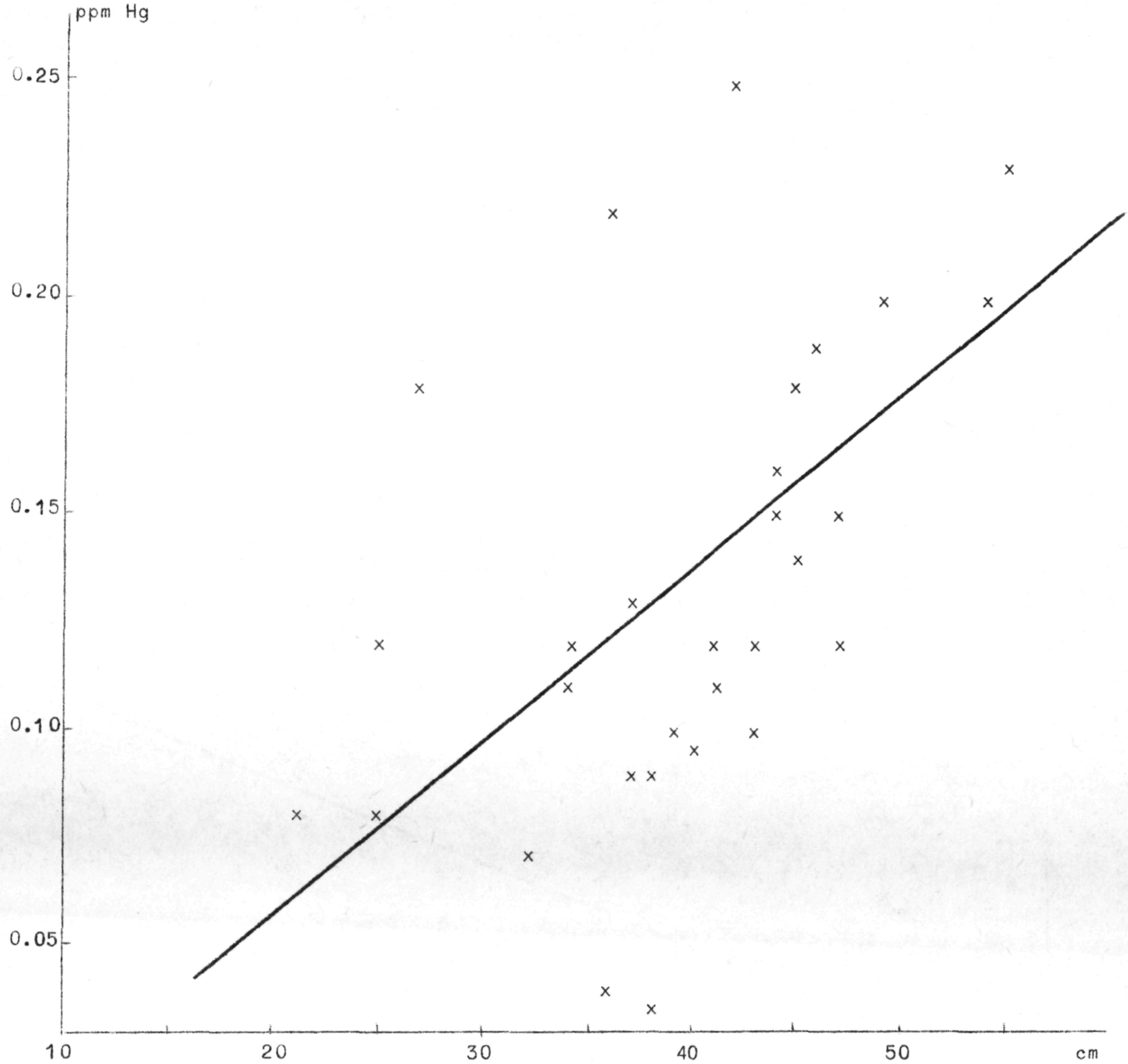


fig. 9.3.- Regression between mercury content and length of male cod.

0.02 to 0.23 with an average of 0.12 ppm (n = 163) off the Canadian Atlantic coast.

For shrimps, an average value of 0.12 ppm (n = 50) was reported in the Wadden Sea [Koeman *et al.* (1971)] whilst a range of 0.15 to 0.21 ppm was found in British coastal waters [Ministry of Agriculture, Fisheries and Food (1971)].

It should be noted that all analyses on shrimps in this work were for practical reasons carried out on whole crustaceans. However, a specific investigation was conducted on the mercury distribution in the

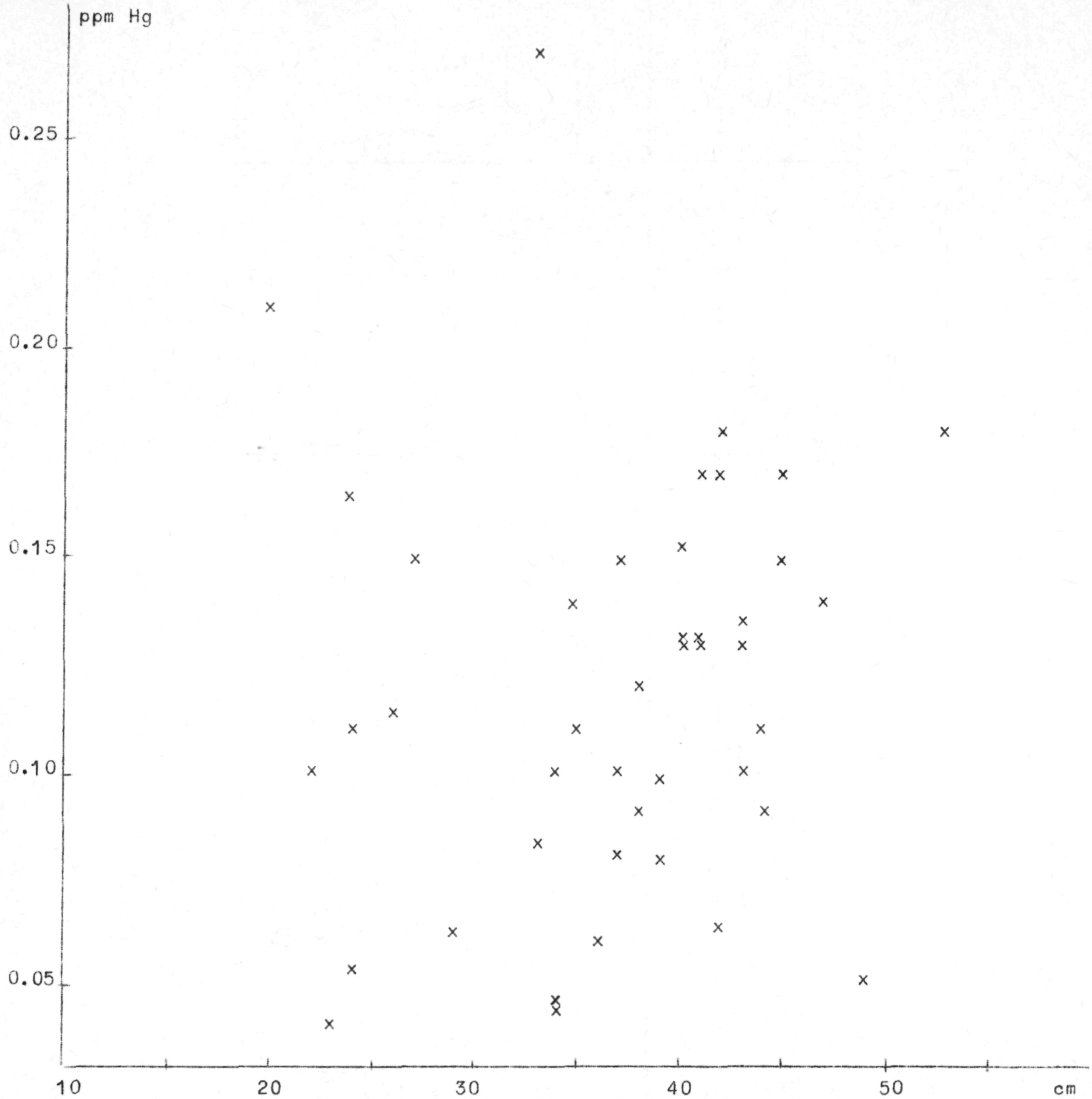


fig. 9.4.- Relationship between mercury content and length of female cod.

body of the shrimps. The results showed a distribution of 56 % in the flesh, 32 % in the cephalothorax, 12 % in the shell and 0 % in the telson. Hence, taking into account an average percentage of 30 % shrimp flesh, a mean content of 0.186 ppm (standard deviation 0.072) was present in the shrimp muscle.

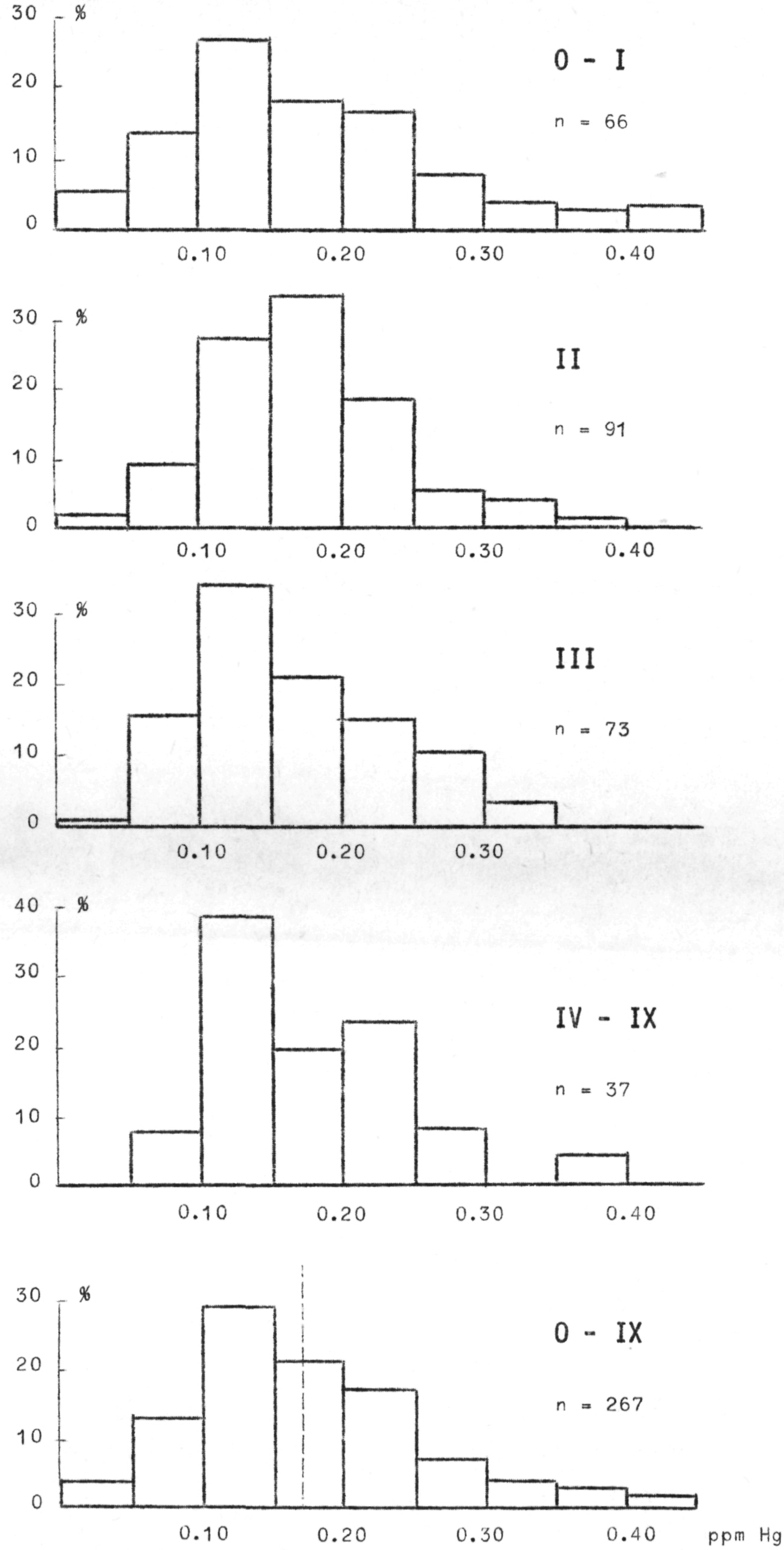


fig. 9.5.- Histogram per age-group of plaice.

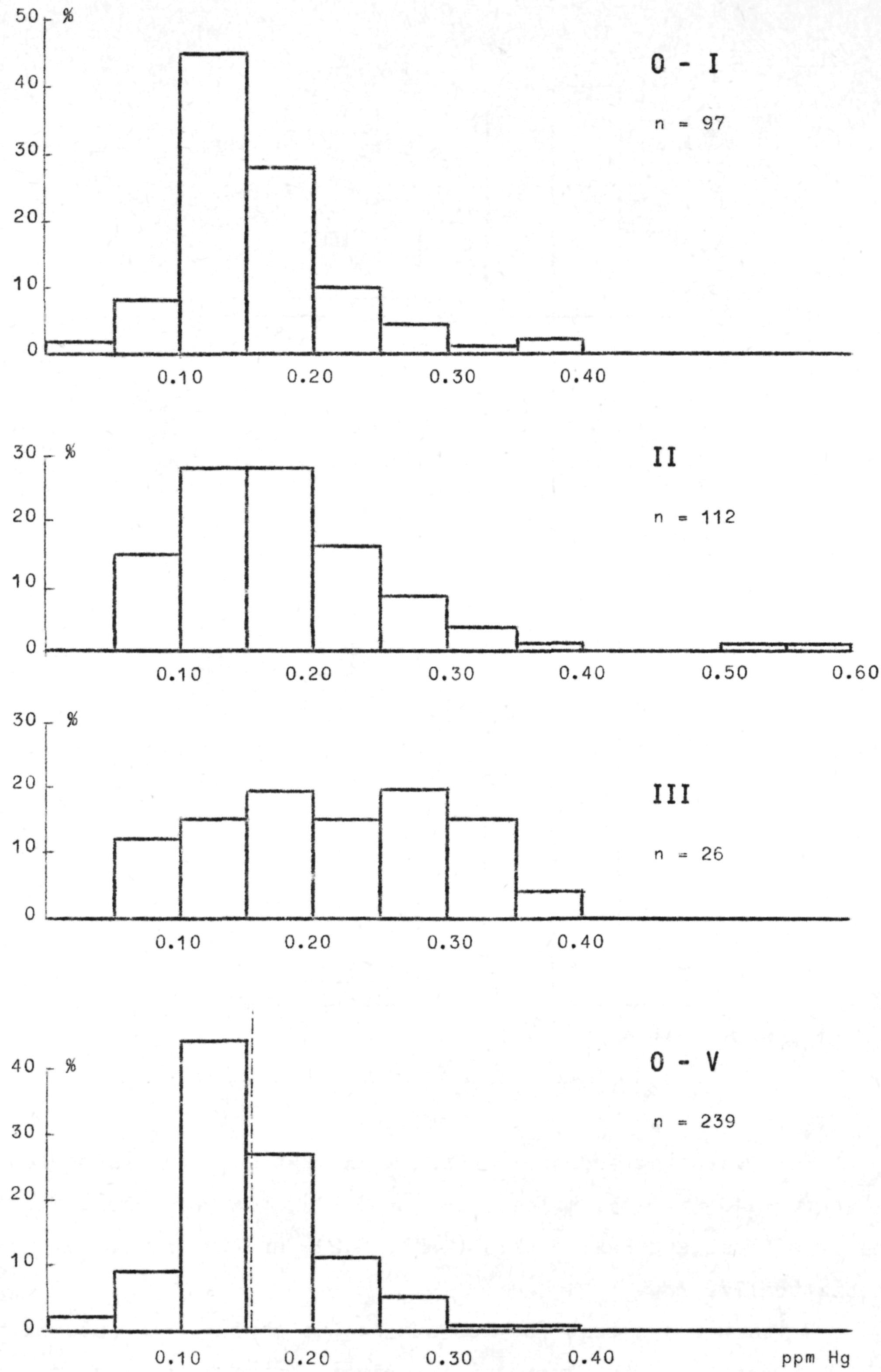


fig. 9.6.- Histogram per age-group of whiting.

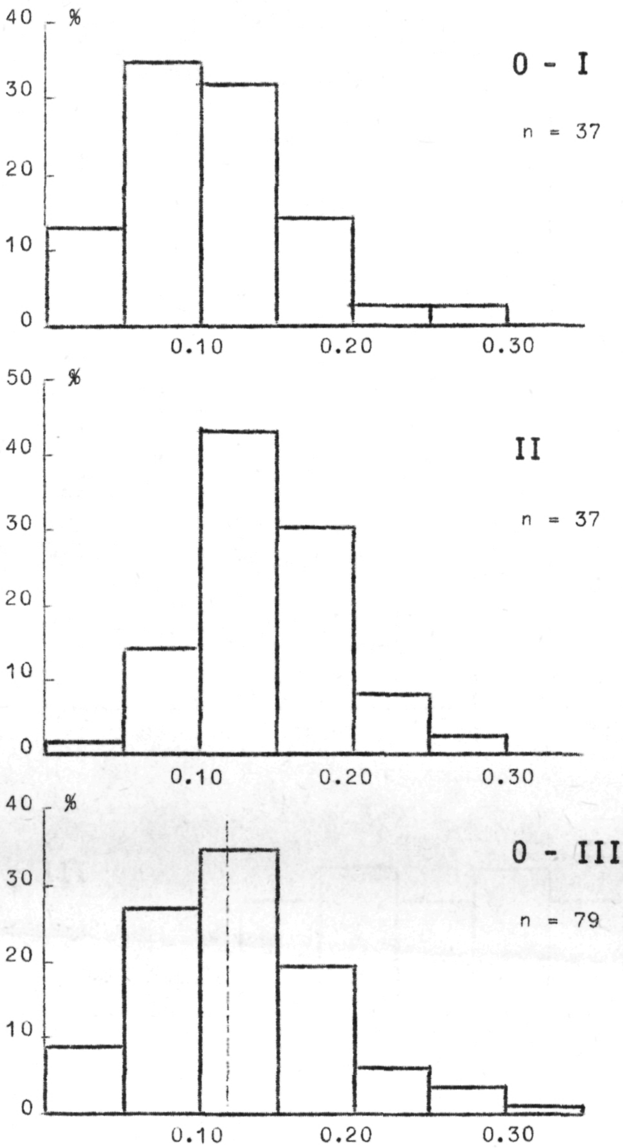


fig. 9.7.- Histogram per age-group of cod.

Shrimps being sedentary animals, migrating over distances not exceeding 10 to 15 miles [Tiews (1963)], the possible influence of the area (West and East coast) (Table 9.2), and the season was evaluated statistically.

Neither the t-test carried out on the mercury contents of the West and East coast nor the regression analysis between mercury and time of catching appeared to be significant, stressing again the fairly uniform distribution of mercury levels over the whole area.

Table 9.2

Mercury content (ppm) of shrimps from the East and West coast

Area	Number	Average	s	v (%)
Eastern	65	0.099	0.040	40
Western	83	0.103	0.039	38

The mercury concentration in sea water off the Belgian coast averages 0.15 ppb [C.I.P.S. (1972)] which seems to be normal for surface waters [Sillen (1963), Beasley (1971)]. Sediments on the other hand contained about twice as much mercury as in more distant waters (0.27 versus 0.15 ppm) [C.I.P.S. (1972)]. Hence, it is not surprising that mercury concentrations in Belgian inshore fish appeared to be higher than in fish caught in distant waters where reported levels are almost exclusively below 0.100 ppm [Westöö (1967), Bligh and Armstrong (1971), Thibaud (1971), Antonacopoulos (1973), Portmann (1972), Icelandic Fisheries Laboratories (1973)].

On the other hand, the reported values contrast with the high concentrations recorded in heavily contaminated fishing grounds. Ackefors (1968) reported values up to 3.1 ppm for plaice and 2.7 ppm in cod caught in the Oresund. Also in cod, Boëtius (1971) found an average of 0.667 ppm (n = 15) with maximum concentrations of 1.29 ppm in the Sund (Denmark). In a survey covering 13 Norwegian fjords, Berge and Palmork (1971) recorded maximum levels of 4.70 (n = 215) and 1.88 ppm (n = 18) in cod and whiting respectively.

References

ACKEFORS, H., (1968). I.C.E.S. Fish Improv. Com., *Report C.M. 1968/E3.*

ANTONACOPOULOS, N., (1973). *Inf. G. Fischwirtschaft, 20,* (1), 17.

BEASLEY, T., (1971). *Environ. Sci. Technol., 5,* 634.

BERGE, G. and PALMORK, K., (1971). I.C.E.S. Fish Improv. Com., *Report C.M. 1971/E33.*

BLIGH, E. and ARMSTRONG, K., (1971). I.C.E.S. Fish. Improv. Com., *Report C.M. 1971/E34.*

BOËTIUS, J., (1971). I.C.E.S. Fish. Improv. Com., *Report C.M. 1971/E36.*

C.I.P.S. (Comité Interministériel de la Politique Scientifique), Brussels (1972). *Report M22.*

FORRESTER, C., KETCHEN, K. and WONG, C., (1972). *J. Fish. Res. Board Canada, 29,* 1487.

GREVE, P. and WIT, S., (1971). *TNO-Nieuws, 26,* 395.

HOLDEN, A., (1973). *J. Fd Technol., 8,* 1.

ICELANDIC FISHERIES LABORATORIES, (1973). *Annual Report 1971,* p. 22.

JOHNELS, A., WESTERMARK, T., BERG, W., PERSSON, P. and SJOSTRAND, B., (1967). *Oikos, 18,* 323.

KOEMAN, J., CANTON, J., WOUDSTRA, A., PEETERS, W., DE GOEY, J., ZEGERS, C. and VAN HAAFTEN, J., (1971). *TNO-Nieuws, 26,* 402.

MEYER, V., (1972). *Arch. Fischereiwiss., 23,* (Suppl. 1), 1.

PORTMANN, J., (1972). *Aquaculture, 1,* 91.

SNEDECOR, G. and COCHRAN, W., (1971). *Statistical Methods,* 6th Ed., Iowa State Univ. Press, Ames Iowa, USA.

SILLEN, L., (1963). *Sverisk kem. Tidskr., 75,* 161.

THIBAUD, Y., (1971). *Science et Pêche, 209,* 1.

TIEWS, K., (1963). I.C.E.S., *Report N° 7.*

WESTÖÖ, G., (1967). *Acta Chem. Scand., 21,* 1790.

II. Mercury and other pollutants in sea fish and shell fish of Belgian fishing

1.- Mercury and other metallic pollutants

1.1.- Generalities

This study refers to fish caught off the Belgian coast as well as in other areas.

The results are given in function of some parameters (age, geographical localization of the fishing, season) and relate to specimens caught from autumn 1971 till the end of autumn 1972.

The fish studied in I.R.C. were all caught or brought by the Fisheries Research Station of Ostend which carries out the biometric measurements.

The purchases done in the "fish auction" relate to fish caught by Belgian fishers in the North Sea, the Irish Sea, the English Channel and the Bristol Channel.

The fish caught off the Belgian coast by the Fisheries Research Station of Ostend originate from areas for which the coordinates have already been given before.

The determination of total Hg was made on all the specimens; the determinations of Cu , Zn and Pb commenced in April 1972 in function of the development of our determination technics.

The total results are given in Tables 9.3 to 9.7 hereafter.

The determination of cadmium in the same specimens is in progress.

1.2.- Seasonal variation of the average amounts of Hg

According to the results obtained for different fishing periods, we observe an increase of the Hg amount in the fish we have examined

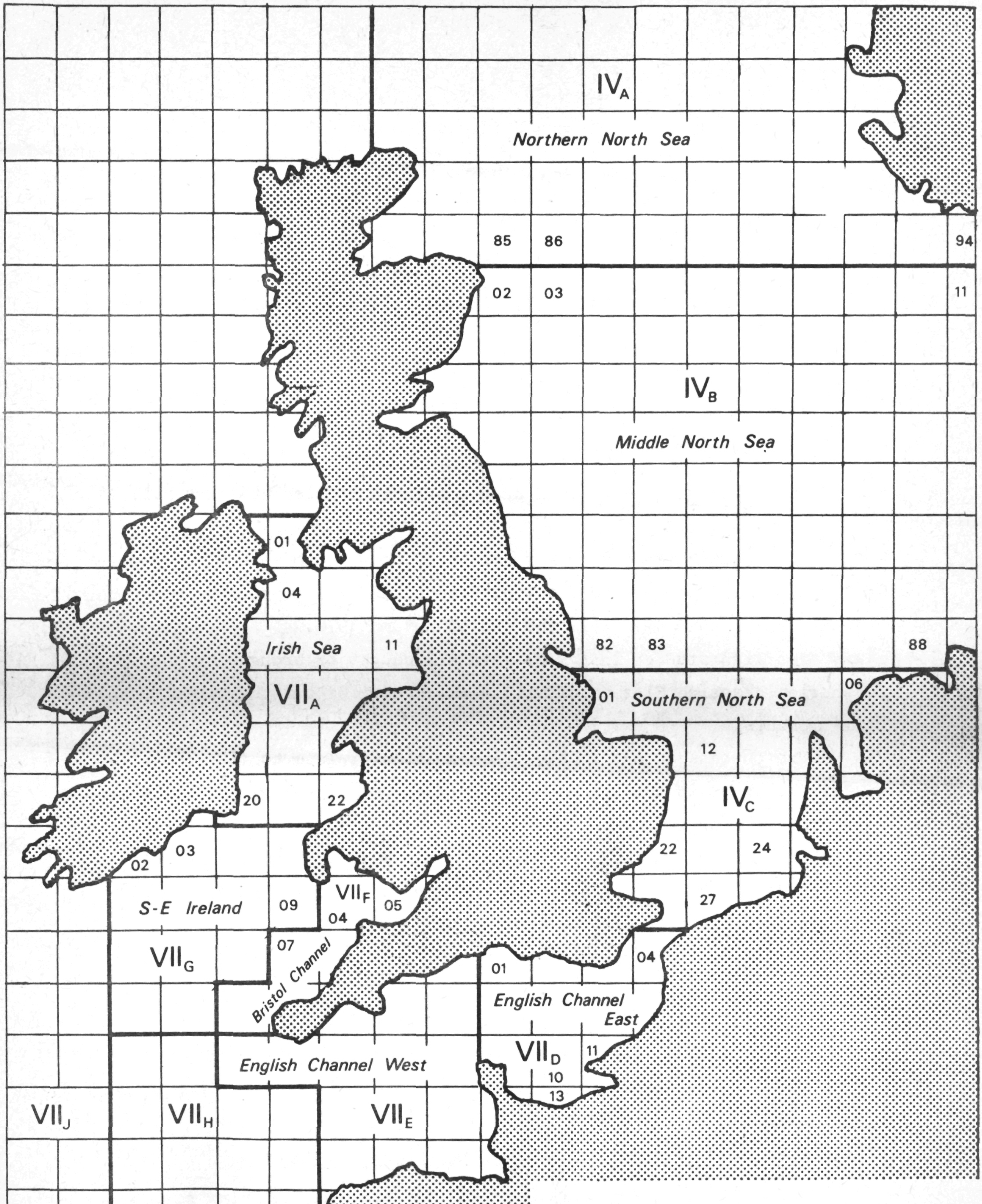


fig. 9.8.- Chart for locating fishing grounds.

Table 9.3

Mercury in ppm - Collection 1972

Species	Area	Square n°	Year class.	N	X_{max}	X_{min}	$\overline{X}$	σ
Plaice	IV_C	27	1967	5	0.36	0.18	0.24	0.09
	IV_C	27	1968	23	0.39	0.06	0.17	0.08
	IV_C	27	1969	76	0.32	0.04	0.16	0.06
	IV_C	27	1970	60	0.39	0.04	0.13	0.08
	IV_C	27	1971	37	0.39	0.03	0.22	0.09
	IV_C IV_C	12 17	1968	5	0.37	0.13	0.26	0.10
	VII_G VII_F VII_F	09 04 07	1966	7	0.37	0.08	0.17	0.1
	VII_G VII_F	09 04	1968	8	0.42	0.13	0.23	0.09
	VII_F	04	1969	6	0.34	0.06	0.19	0.11
	VII_A	11	1964	6	0.84	0.09	0.39	0.30
	VII_A VII_A	10 11	1965	4	0.60	0.19	0.38	0.17
	VII_A	11	1966	5	0.46	0.10	0.34	0.15
	VII_A	11	1967	7	0.71	0.10	0.34	0.19
	VII_A	11	1968	7	0.37	0.13	0.27	0.09
	VII_A	11	1969	5	0.46	0.07	0.33	0.15
Whiting	IV_C	27	1969	18	0.37	0.08	0.29	0.08
	IV_C	27	1970	93	0.56	0.07	0.19	0.09
	IV_C	27	1971	40	0.36	0.11	0.17	0.06
Sprats	IV_C	27	-	47	0.29	0.04	0.15	0.06
Sole	IV_C IV_C IV_C	02 26 27	1968 to 1971	13	0.48	0.036	0.21	0.12
	VII_F VII_F	04 09	1963 1966 1969	10	0.38	0.04	0.16	0.10
Cod	IV_C	27	1969	4	0.20	0.03	0.15	0.06
	IV_C	27	1970	52	0.27	0.04	0.13	0.05
	IV_C	27	1971	8	0.21	0.04	0.11	0.02
Shrimps	IV_C	27	?	99	0.25	0.04	0.11	0.04

from the first sending of autumn 1971 till the summer 1972, for almost all fishing places. It appears that the average amount of Hg in the fish caught in the summer of 1972 is distinctly highest and consequently

Table 9.4

Copper in ppm - Collection 1972

Species	Area	Square n°	Year class.	N	X_{max}	X_{min}	$\bar{X}$	σ
Plaice	IV_C	27	1967	5	1.33	0.56	0.74	0.33
	IV_C	27	1968	14	1.30	0.51	0.74	0.25
	IV_C	27	1969	51	1.67	0.26	0.63	0.24
	IV_C	27	1970	39	1.63	0.42	0.66	0.29
	IV_C	27	1971	12	1.20	0.41	0.77	0.32
	IV_C IV_C	12 17	1968	5	0.81	0.34	0.55	0.20
	VII_G VII_F VII_F	09 04 07	1966	5	0.77	0.35	0.58	0.16
	VII_G VII_F	09 04	1968	7	1.66	0.45	0.91	0.47
	VII_F	04	1969	6	0.88	0.26	0.62	0.25
	VII_A	11	1964	6	0.83	0.31	0.58	0.20
	VII_A VII_A	10 11	1965	4	0.92	0.32	0.61	0.32
	VII_A	11	1966	5	0.82	0.39	0.50	0.18
	VII_A	11	1967	7	1.17	0.25	0.57	0.31
	VII_A	11	1968	7	0.75	0.31	0.48	0.15
	VII_A	11	1969	5	1.10	0.39	0.76	0.27
Whiting	IV_C	27	1969	16	1.39	0.44	0.88	0.28
	IV_C	27	1970	78	1.84	0.34	0.98	0.38
	IV_C	27	1971	26	3.36	0.27	1.26	0.90
Sprats	IV_C	27	-	47	2.41	0.74	1.31	0.43
Sole	IV_C IV_C IV_C	02 26 27	1968 to 1971	8	1.36	0.53	0.74	0.31
	VII_F VII_F	04 09	1963 1966 1969	10	0.86	0.31	0.47	0.19
Cod	IV_C	27	1970	35	1.24	0.30	0.67	0.29
Shrimps	IV_C	27	?	89	22	6.6	12.8	3.2

positively affects the total year average for each considered species (except shrimps).

In table 9.8 we noted the average amount in Hg for a number n of fish caught off the Belgian coast, relative to each of the fishing seasons.

Table 9.5

Zinc in ppm - Collection 1972

Species	Area	Square n°	Year class.	N	X_{max}	X_{min}	$\bar{X}$	σ
Plaice	VII_G VII_F VII_F	09 04 07	1966	4	5.97	3.70	4.69	0.94
	VII_F	07	1968 1969	4	4.79	3.00	3.97	0.82
	VII_A VII_A	10 11	1964 1965	5	6.46	3.66	5.44	1.12
	VII_A	11	1967	5	5.94	3.83	5.10	0.85
	VII_A	11	1968 1969	6	5.49	2.52	4.47	1.10
Whiting	IV_C	27	1969 to 1971	16	8.2	4.9	5.9	1.1
Sprats	IV_C	27	-	23	29.3	16.7	23.8	3
Cod	IV_C	27	1970	14	5.07	3.26	4.16	0.51
Shrimps	IV_C	27	?	24	44.44	17.23	26.1	7.28

Table 9.6

Lead in ppm - Collection 1972

Species	Area	Square n°	Year class.	N	X_{max}	X_{min}	$\bar{X}$	σ
Plaice	VII_G VII_F VII_F	09 04 07	1966	4	0.22	0.15	0.20	0.03
	VII_F	07	1968 1969	4	0.20	0.11	0.15	0.04
	VII_A VII_A	10 11	1964 1965	5	2.81	0.13	0.68	1.18
	VII_A	11	1967	5	1.69	0.18	0.65	0.67
	VII_A	11	1968 1969	6	0.80	0.17	0.29	0.25
Whiting	IV_C	27	1969 to 1971	16	4	0.2	0.83	1.06
Sprats	IV_C	27	-	23	5.3	0.1	1.98	1.5
Cod	IV_C	27	1970	14	0.83	0.21	0.30	0.16
Shrimps	IV_C	27	?	22	16.8	0.40	4.32	4.4

Table 9.7

Mercury, Copper, Zinc, Lead in ppm
Fishing period : January-May 1972

Species	Area	N	Hg			Cu		
			$\bar{X}$	X_{max}	X_{min}	$\bar{X}$	X_{max}	X_{min}
Plaice	Belgian Coast	57	0.14	0.36	0.04	0.67	1.33	0.32
	Middle North Sea	10	0.15	0.43	0.05	0.55	0.83	0.31
	Southern North Sea	8	0.17	0.50	0.05	0.6	0.95	0.40
	Eng. Channel	9	0.17	0.32	0.10	0.54	0.91	0.26
	Bristol Channel	12	0.16	0.34	0.06	0.73	1.80	0.26
	Irish Sea	19	0.27	0.84	0.07	0.66	1.17	0.25
Whiting	Belgian Coast	25	0.17	0.33	0.04	0.84	1.98	0.27
Cod	Belgian Coast	43	0.14	0.27	0.04	0.7	1.3	0.27
Sprats	Belgian Coast	23	0.15	0.24	0.06	1.2	1.42	0.74
Shrimps	Belgian Coast	57	0.10	0.22	0.04	12.2	21.9	6.6

Species	Area	N	Zn			Pb		
			$\bar{X}$	X_{max}	X_{min}	$\bar{X}$	X_{max}	X_{min}
Plaice	Belgian Coast	53	6.48	11	3.10	0.30	0.51	0.20
	Middle North Sea	9	4.3	5.52	2.8	0.17	0.22	0.14
	Southern North Sea	8	4.86	6.88	3.63	0.21	0.32	0.14
	Eng. Channel	9	5.10	6.70	2.52	0.23	0.29	0.15
	Bristol Channel	11	5.33	13.8	3	0.19	0.25	0.11
	Irish Sea	19	4.93	6.46	2.42	0.57	2.88	0.13
Whiting	Belgian Coast	16	5.9	8	4.75	0.84	0.97	0.19
Cod	Belgian Coast	43	4.8	8.01	3.04	0.32	0.83	0.21
Sprats	Belgian Coast	23	23.9	29.3	16.7	2.22	5.3	0.10
Shrimps	Belgian Coast	23	26.3	45.2	19.2	4.7	16.8	0.3

The values quoted in the table 9.8 seem to confirm that the Hg amounts are effectively rising for the fish caught during the summer campaign.

As the causes which may involve an increase of the Hg amount in the fish during the summer they are perhaps relative to the increase of primary productivity in the North Sea : 100 mg/m^2.day in winter,

Table 9.8

Average contents
(total Hg in ppm)

Fishing campaigns	Plaice		Whiting		Sprats		Cod	
	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$	n
Autumn 1971	0.14	46	0.14	67	0.14	12	0.10	34
Winter 1972	0.14	57	0.17	25	0.15	23	0.14	43
Spring 1972	0.16	41	0.17	59	0.11	9	-	-
Summer 1972	0.22	86	0.23	59	0.17	16	-	-
Autumn 1972	0.19	73	0.13	34	0.12	6	-	-
$\bar{X}$: 1 - 3	0.15	144	0.16	151	0.14	44	0.12	77
$\bar{X}$: 1 - 4	0.18	230	0.18	210	0.15	60	0.12	77

1500 mg/m^2.day in summer (*cf. Modèle Mathématique, Rapport de Synthèse II*). If we take the absorbtion capacity of phytoplankton into account, in respect to heavy metals, it seems logic to record a conjugated increase of the amounts of those elements in the fish.

The increase of the total Hg amount is again more characteristic if we compare fish of the same age (see Table 9.9).

Table 9.9

Species	Age	Fishing ground	Fishing period			
			January-15 June		15 June-September	
			$\bar{X}$	n	$\bar{X}$	n
Plaice	1969	Southern North Sea	0.14	53	0.20	23
"	1970	"	0.14	30	0.21	30
"	1971	"	0.13	7	0.25	30
Whiting	1970	"	0.16	61	0.24	32

As far as the respective distribution of the amounts in one species of the same age is concerned, it appears that the greatest frequency is located between the same limits, whatever the fishing period. On the other hand, the distribution curve is more spread out towards the high values for the summer fishing (see Table 9.10).

Table 9.10

Plaice; year class : 1970; fishing period : 1972

Southern North Sea n = 30	Frequency	
Hg total (ppm)	January-June	June-September
0.17 - 0.21	33 %	33 %
0.04 - 0.09	25 %	0 %
0.26 - 0.39	0 %	25 %

Recall, average summer : 0.21 ; winter : 0.14 .

Further work will permit us to know whether it is really a periodic increase as desirable.

In this case, it would be necessary to study the correlation which might exist between the quoted phenomenon and the subsequent elimination of mercury by sea organisms.

1.3.- Supervision of pollution

The discussion of the numerous chemical results already obtained permits to bring out the bases of a methodology useful for the supervision of the pollution of fish destinated for consumption, from a chemical point of view. First of all, the number of specimens of the same species must be large enough to be representative for the fishing place in regard to the unavoidable large distribution of the amounts. According to the foregoing, and for all security, it is necessary to include the parameter "fishing period". On the other hand, it is not evident that the age should be taken into consideration as an essential parameter, although there is a correlation between age and amount of Hg .

About the systematic supervision to be ensured after the period of investigation, which has to be spread over a period of at least two years, we expect that a minimum of thirty specimens of the same fish species, caught during the same season, will be necessary in order to characterize the pollution of the fish originating from one fishing area. On the one hand the solution may be to hold inspections within sufficiently spaced periods. On the other hand, we could effectuate more frequent studies of one species from one fishing area immediately when anomalies are observed. As to the frequencies to be observed they have to be determined for each species separately in function of the actual average amounts.

The dispositions mentioned above should permit the competent authorities to decide in time what measures have to be taken, for a defined species of fish or fishing area, with respect to the protection of the consumers.

1.4.- Other heavy metals

As for the other heavy elements, we observed important average amounts in Zn and Pb for sprats; in Cu , Zn and Pb for shrimps ($\overline{X}$ = 12.8 ppm Cu ; more than 60 % of the results are situated between 10 and 15.5 ppm Cu) (see Tables 9.4 to 9.7).

If we have to take care of the normal amounts for these species, it is not less true that the toxic character of the mentioned elements must be taken into consideration.

The histograms amounts-frequencies (reason = $\sqrt{1.5}$ particularly appropriate for the majority of the studied cases), reveal a quasi log-normal distribution for Hg and Cu . For plaice the distribution is rather normal for Zn and presents some discontinuities for Pb .

In Table 9.7 we notice the following increasing order of average amounts for plaice and cod : Hg (about 0.2 ppm) ; Pb (about 0.3 ppm) ; Cu (about 0.6 ppm) ; Zn (about 5.5 ppm) .

For whiting the same order is maintained, but the amounts are slightly higher (with Pb = Cu = 0.89 ppm).

On the other hand, for sprats, the order is : Hg , Cu , Pb , Zn . The amounts in Zn of the sprats and shrimps are distinctly higher. In the shrimps the Cu content reaches 12.2 ppm and the Pb content 4.7 ppm . Here we must also take into consideration the normal amount for the species.

The search after possible correlations between amounts in pollutants (Hg , Cu , Pb , Zn) for the different fish species studied did not yield any positive results. As a matter of fact the calculated correlation coefficients generally had non significant values and even reverse signs for the same fish species.

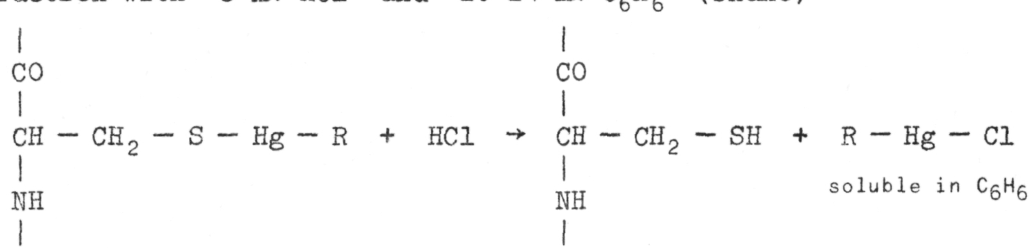
Nevertheless for cod we may reckon on a significant correlation between zinc and lead. Indeed, from 43 cods of the Belgian coast, the 23 of the Westdiep had an $r = 0.32$; $Zn = 2.98\ Pb + 4$ (significant at ± 95 %) and the 15 cods originating from Sierra had an $r = 0.67$, $Zn = 4.87\ Pb + 2.96$ (significance higher than 99 %).

2.- Total organic mercury

The method for the determination of the total organic mercury was perfected and then systematically applied to a first series of samples; the results were compared with those of the total Hg .

2.1.- Method

- 10 g fish + 10 mℓ water are homogenized in a mixer and brought in an obturable centrifugation tube and rinced with 5 mℓ water.
- add pepsine and 1 mℓ of concentrate HCl ; incubate at 37 °C during 2 days. In doing so the protein chains are partially destroyed.
- extraction with 8 mℓ HCl and 20-24 mℓ C_6H_6 (shake)



- centrifugation of the suspension
- 5 mℓ of the benzene solution + 5 mℓ CCl_4

+ { 1 mℓ $Ca(NO_3)_2$ 10 % solution
  { 10 mℓ Na_2S 10 % solution

The organic mercury passes into the waterphase.

$$R - Hg - Cl + Na_2S \rightarrow R - Hg - S - Na + NaCl$$

soluble in water

- oxydation of the waterphase with Cl_2 (dissolved in CCl_4)

$$R - Hg - SNa \xrightarrow{Cl_2} HgCl_2 \rightarrow \text{MAS 50}$$

Blank

1) The solution in water of $CH_3 - Hg - Cl$ is treated with concentrated HCl and C_6H_6 and the above mentioned method applied.

Results : theoretical : 1.19 µg Hg ; 1.19 µg Hg ; 1.16 µg Hg ;

found : 1.12 µg Hg ; 1.04 µg Hg ; 1.28 µg Hg .

2) Addition of $HgCl_2$ or $CH_3 - Hg - Cl$ after incubation;

- application of the method.

2.2.- Results

Table 9.11

Tests on fishes

Species	Addition of Hg to fish (ppm)	Total organic Hg			Total Hg (ppm Hg)
		Theoretical (ppm Hg)	Found (ppm Hg)	Found %	
		(1)	(2)		
Plaice (890)	-	-	0.17	-	0.39
"	0.046 ($HgCl_2$)	0.17 (2)	0.16	-	0.44
"	0.074 (CH_3-Hg-Cl)	0.239	0.22	92	0.46
Cod (897)	-	-	0.28	-	0.51
"	0.2 (CH_3-Hg-Cl)	0.48	0.45	94	0.71
" (bis)	0.2 (CH_3-Hg-Cl)	0.48	0.40	84	0.71
Cod (730)	-	-	0.32	-	0.30
"	0.08 (CH_3-Hg-Cl)	0.40	0.39	97	0.38
" (bis)	0.10 (CH_3-Hg-Cl)	0.42	0.39	89	0.40

Conclusions

1) The addition of $HgCl_2$ has no effect on the determination of the total organic mercury $R - Hg - Cl$, because of the clear separation between the water phase

$$HgCl_2 + 2\ HCl \rightarrow H_2(HgCl_4)$$

and the organic phase ($R - Hg - Cl$ dissolves in C_6H_6).

2) Addition of $CH_3-Hg-Cl$ increases the amount of organic Hg . Allthough there is some less.

Table 9.12

Analysis of fish and shrimps

Hg in ppm. First figures total organic Hg; second figures total Hg.

Cod	Belgian coast	0.37/0.51	0.38/0.30		
Plaices	Belgian coast	0.17/0.39	0.22/0.20		
	Irish Sea	0.07/0.77 (1962)	0.28/0.42 (1965)	0.06/0.18 (1961)	
		0.14/0.43 (1960)	0.10/0.46 (1964)	0.29/0.43 (1963)	
	Bristol Channel	0.15/0.18 (1964)	0.19/0.14 (1963)	0.11/0.37 (1966)	
		0.08/0.15 (1966)	0.07/0.11 (1968)	0.10/0.21 (1963)	
Sprats	Belgian coast	0. /0.06			
Shrimps	Belgian coast	0.01/0.08	0.02/0.10	0.06/0.07	0.08/0.07
		0.02/0.11	0.13/0.14	0.09/0.16	0.09/0.07
		0.09/0.08	0.05/0.15		
Sole	North Sea	0.15/0.19	0.38/0.32		
Pike	?	0.03/0.21			
Calamary	?	0.17/0.30			

2.3.- Comments

It appears from the examination of our first results, some of which are given above, that the total organic mercury is most frequently situated between $\frac{1}{3}$ and $\frac{2}{3}$ of the total Hg amount.

Note also that we find sometimes higher values for total organic Hg than for total Hg (sampling, precision !). This can be explained by the sampling procedure and the lack of precision in the determinations. However, we have undoubtedly to do with specimens in which the proportion of organic mercury is very high.

Those results represent the first elements of the practical application of the method. A method for the determination of $CH_3 - Hg - Cl$ was also perfected; the first applications are in progress. This determination will be effectuated systematically and will permit us to evaluate the proportions of total organic Hg and $Me - Hg$ in relation to different parameters retained for total Hg .

3.- Pesticides and PCB

3.1.- Analytical method for determining pesticides and PCB in fish

3.1.1.- Extraction

Sample weight : 10 g of fish fillet mixed with Na_2SO_4 anhydrous.

Solvent : petroleum ether (Boiling range : 65 to 70 °C).

Apparatus : non-siphoning (plain tube) extractor.

Extraction time : at least 6 hours.

Extract concentrated in Rotavapor apparatus and then to 1 mℓ .

3.1.2.- Clean-up

- on alumina micro-column de-activated with 5 % water [adapted from Law and Goerlitz, JAOAC 53 (6) 1276 (1970)].
- elution with 7 mℓ petroleum ether. Extract concentrated to 1 mℓ .

3.1.3.- Pre-GLC separation

a) Silicic acid – celite microcolumn

Adapted from Armour and Burke [JAOAC 53 (4) 761 (1970)]

- mixture silicic acid + 3 % water (4 parts) and Celite 545 (1 part)
- first elution with 6 mℓ petroleum ether. This eluate contains : PCB's, aldrin, the most part of DDE and of heptachlor. Eluate concentrated to 1 mℓ → GLC .
- second elution with 4 mℓ of eluting mixture : acetonitrile (1 part) petroleum ether (19 parts), dichloromethane (80 parts). Eluate concentrated to 1 mℓ and then separated on b).

b) Alumina microcolumn

Alumina de-activated with 5 % water.

- first elution with 2 mℓ petroleum ether. This eluate contains pp'DDT, DDD, the most part of lindane and the remainder, if any, of DDE and heptachlor. Eluate concentrated to 1 mℓ → GLC .
- second elution with 5 mℓ petroleum ether. This eluate contains dieldrin, endrin, heptachlor epoxide and the remainder, if any, of lindane. Eluate concentrated to 1 mℓ → GLC .

3.1.4.- GLC determination

Apparatus : Varian 1200.

Detector : ECD, tritium source.

Column : glass, 1.80 m × 3 mm o.d.

Column packing : 4 % OV.1 / 6 % QF.1 on Chromosorb W. 80/100 mesh.

Carrier gas : N_2 flow rate : 30 mℓ/min.

Temperature : Column 170 °C , Injector 220 °C , Detector 280 °C .

Injection : 8 μℓ .

3.1.5.- Peak measurements

- for pesticides, area or height reported on a calibration curve, checked up from time to time with reference solutions.
- for PCB's , profile method with 5 peaks (Reference : Arochlor 1254).

3.1.6.- Identification of peaks (not always as routine method, but as checking up where necessary)

a) first eluate after silicic acid — celite separation
   - alcoholic NaOH hydrolysis : PCB and DDE are stable;
   - CrO_3 — HAc : DDE → dichlorobenzophenone.

b) first eluate after alumina separation
   - alcoholic NaOH hydrolysis : pp'DDT → DDE
     DDD → 2.2-bis (p-chlorophenyl)-1-chloroethylene
     lindane → disappears.
   - as supplementary checking up where necessary CrO_3 — HAc : DDE → dichlorobenzophenone.

c) second eluate after alumina separation
   - H_2SO_4 concentration : dieldrin and endrin disappear.

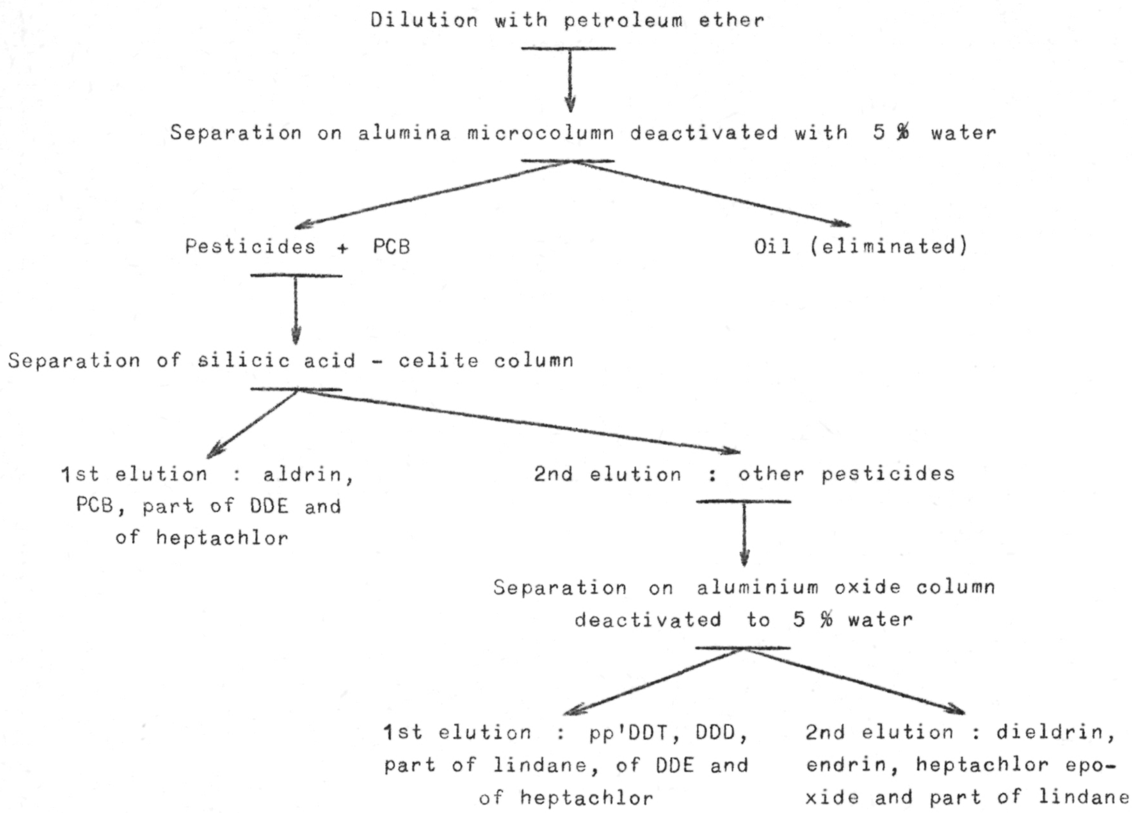
3.1.7.- Alternative methods

According to the difficulties encountered in the analysis for some samples of fish, the usual method (as described here above) is sometimes modified as follows, only for the clean-up procedure :

- a liquid-liquid partition clean-up with DMF (adapted from Richardson and Coll, *Pestic. Monit. Journ.*, *4* (4) 169, March 1971).

- Florisil microcolumn clean-up. Elution with petroleum ether-diethyl ether.

Scheme of the method used for determining pesticides and PCB in the fish oil samples



3.2.- <u>Comments</u>

From the comparison of the average results obtained for pesticides and PCB in the species caught during the two periods mentioned in Table 9.13, it appears that :

- the contents are higher in the autumn 1971;
- for one and the same species, we notice an influence of the fishing area, especially for PCB ;
- a differentiation according to the species may also exist, particularly on the basis of the PCB contents.

Table 9.13

Average amounts of pesticides and P.C.B.
(results in ppm)

Fishing campaigns Autumn 1971									
Species	Number	pp' DDT	DDE	DDD	Dieldrine	Endrine	Lindane	PCB	
Belgian Coast - Westdiep									
Cod	8	0.023	0.005	0.002	0.006	< 0.001	0.003	0.074	
Plaice	5	0.027	0.007	0.003	0.010	< 0.001	0.008	0.210	
Whiting	6	0.022	0.004	0.002	0.008	< 0.001	0.007	0.117	
Shrimps	5	0.021	0.003	0.001	0.006	< 0.001	0.006	0.082	
Belgian Coast - Sierra									
Cod	5	0.038	0.008	0.005	0.010	< 0.001	0.006	0.173	
Plaice	5	0.025	0.004	0.004	0.010	< 0.001	0.006	0.252	
Whiting	4	0.014	0.004	0.001	0.007	< 0.001	0.005	0.084	
Shrimps	5	0.018	0.002	0.001	0.007	< 0.001	0.007	0.078	
Belgian Coast									
Plaice	7	0.023	0.004	0.008	0.010	< 0.001	0.007	0.189	
Belgian fisheries outside the Belgian Coast									
Plaice	8	0.025	0.010	0.002	0.006	< 0.001	0.007	0.126	
Sole	7	0.034	0.010	0.003	0.007	< 0.001	0.005	0.207	
Fishing campaigns January-May 1972									
Species	Number	pp' DDT	DDE	DDD	Dieldrine	Endrine	Lindane	PCB	Hept. epoxide
Belgian Coast									
Cod	23	0.004	0.002	0.001	0.001	-	0.002	0.059	< 0.001
Sprats	5	0.037	0.013	0.053	0.022	0.002	0.007	0.518	0.002
Shrimps	9	0.011	0.003	0.002	0.002	-	0.003	0.108	< 0.001
Plaice	18	0.005	0.002	0.002	0.002	-	0.002	0.058	-
	16	0.004	0.002	0.003	0.003	-	0.001	0.068	-

These are relatively more abundant in the plaice and the sole and distinctly higher in the sprats.

For this last species the amounts of the different kinds of pesticides and PCB are clearly higher than in all the other investigated species. Besides we have to remind that sprats have also a high amount of heavy metals.

In the I.C.E.S. reports relative high amounts of pesticides and PCB in fish of the Southern North Sea are mentioned. If however we take into account the low toxicity of these products we can conclude that in both cases they do not present any danger for men. However, care must be taken with dieldrine, highly toxic for mammals.

According to Egon (1967) it is recommended that dieldrine in food should not exceed 0.1 ppm (on fresh material).

The amounts we have found and those given by I.C.E.S. are much lower than this value.