INTERNATIONAL COUNCIL FOR
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C.M. 1988/E:21
Marine Environmental
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ORGANOBROMINE IN MARINE AND FRESHWATER FISHERY PRODUCTS

by

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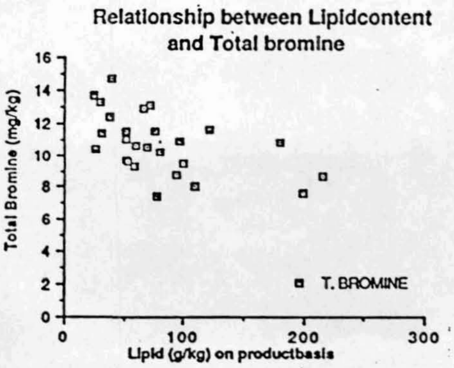
ERRATA: ICES paper C.M. 88/E:21

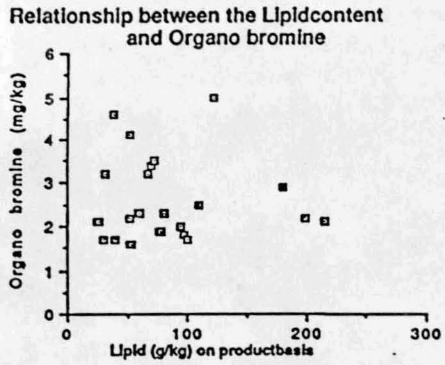
- The lipidcontents in Table II and III have erroneously been expressed in g/kg on a dry weight basis, in stead of on productbasis. The correct figures can be calculated as follows:

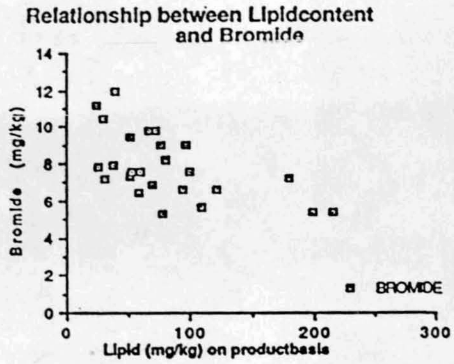
lipidcontent on dry weightbasis X dry weight / 1000 =

lipidcontent on productbasis.

Figures 4 to 6 have been redrawn using the lipidcontent expressed on productbasis, which gave the following pictures:







The conclusion that the bromine level in sprat is inversely related to the lipidcontent remains justified.

RIVO, 26 sept 1988.

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ABSTRACT:

This paper describes some preliminary results of an investigation on the occurrence of organic bromine compounds in fish. In several fish species the contents of total bromine, organobromine and bromide have been analyzed. Total bromine content has been determined by rontgenfluorescence spectrometry. The bromide content could be determined by HPLC after water extraction of the fish tissue and dry-ashing of the residues. The content of organobromine has been calculated as the difference between the total bromine and the bromide concentration.

Great differences exist in the total bromine content of freshwater fish and marine fish: from 1.8 mg/kg bromine in pike-perch (*Stizostedion lucioperca*) to 70 mg/kg bromine on product-basis in shrimps (*Crangon crangon*). Much of the total amount of bromine consists of organically bound bromine, in percentage variing between 15 % and 75 %.

Herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) sampled throughout the North Sea, the English Channel and Irish Sea have been investigated to reveal possible geographical differences in the organobromine content and to elucidate some relationships between bromine levels and fish parameters.

The meaning of the high levels of organobromine compounds in marine fish will be discussed in the context of the increasing industrial use of brominated compounds.

Introduction:

In the last decades several investigations have been published on the occurrence of bromine in fish. Total bromine concentrations show strongly divergent levels for a lot of fish species and products, e.g. fish oils (2.5 - 4.7 mg/kg bromine, ref 1), fish jelly products (10 - 228 mg/kg, ref 2), oils from plankton (100 - 15000 mg/kg, ref 3) and fish fillet (2.4 - 106 mg/kg, ref 4, 5). From experiments of Lunde with fish

oils (3) it is already known that an important part of the bromide found in fish is organically bound.
These natural bromine compounds are found at a higher extent in marine organisms than in terrestrial plants and animals (6). This phenomenon is closely related to the high natural amounts of bromide in sea water of 68 g/m^3 to some hundreds of mg/m^3 in freshwater. Many of these organobromine compounds have been isolated from marine invertebrates (7) serving a lot of applications (e.g. dyes, antibiotics).
However, there is a tendency to substitute chlorinated pesticides and herbicides by bromine compounds. As flame retardants large amounts of bromine compounds are used at the manufacturing of textiles and plastics (8). Eventually, these organobromines may end up in the (marine) environment, where accumulation in organisms, especially fish and invertebrates, can take place. So, in fish one can expect both natural and anthropogenic bromine compounds.
Little information is available about the ecological significance and human toxicity of most organobromine compounds. Therefore some preliminary experiments have been set up to get somewhat more knowledge about the type of bromine compounds in marine organisms. Special emphasis has been paid to the content of bromine in herring and sprat.

Methods and Sampling:

In order to investigate the bromine levels in fishery products samples of several fish species have been collected from the North Sea.
Eel (*Anguilla anguilla*) have been sampled from Lake Grevelingen and Lake IJssel, pike-perch (*Stizostedion lucioperca*) from Lake IJssel, mussels (*Mytilus edulis*) and shrimps (*Crangon crangon*) from the western Wadden Sea.
Both in 1983 and 1984 a lot of samples of herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) have been collected throughout the North Sea, the English Channel and Irish Sea.

Tissues of several fish species were extracted with bidistilled water for isolating the "soluble bromides". To the obtained water-extracts a dry-ashing procedure was applied in order to destruct the organicals. The salt residues were dissolved in buffer and directly used for bromide analysis using HPLC. A standard-addition method with addition of five different concentrations of bromide was used.
The HPLC (high performance liquid chromatography) method is composed of an ionchromatographic analysis of bromide on an amino bonded silica column, using an acidic eluent and UV detection by 202 nm wavelength (4). Bromide contents could be calculated with 95 % confidence limits of

5 - 15 % of the measured values. Recovery percentages of the bromide contents for the overall analysis method amounted to 70 %.
The total bromine content was directly determined in freeze-dried fish samples using rontgenfluorescence spectrometry, carried out by the State Office for Food Quality Control in the Haque (9). Contents of organobromine has been calculated in this experiment as the difference between the total bromine and the bromide concentration.

In the 1983 experiment on herring and sprat samples the bromine contents were measured with rontgenfluorescence spectrometry, according to Klein - Haneveld (9). We have tried to quantify the contents of organobromine by measuring the bromine concentration in the residue remaining the extraction process of the fish tissues. These measured values of the organobromine could be compared with the calculated data. From this comparison it was revealed that deviations of the calculated data remained limited to an averaged value of 14 % (range 0 - 40 %) of the measured data.

In the experiment of 1984 the rontgenfluorescence analysis was replaced by an HPLC analysis. In freeze-dried fish tissues, water extracts and freeze-dried residues of fish tissues (containing the organobromines) the bromine content was measured as described above. A standard-addition method was used with two different additions to each sample. The total bromine contents of the samples could be calculated within 95 % confidence limits of 20 % (averaged value). Recovery percentages for bromide and organobromine were about 90 % and 80 %, respectively.
For the analysis and identification of polybrominated diphenylethers gaschromatography and mass-spectrometry were applied, using a selected ion monitoring procedure.

Results:

Bromine compounds in fishery products:

Figure 1 shows the results of an investigation into the contents of bromide, total bromine and organobromine of fishery products. The results for shrimps and blue mussels are not included in the figure, because the level of bromine was much higher in these benthic organisms. In shrimps the total bromine content varied between 70 and 80 mg/kg bromide on productbasis (see Table I). For mussels a mean concentration level of 46 mg/kg bromide was measured.
On the contrary the levels in fish ranged ten times lower between 1.8 and 6.3 mg/kg, except for sprat, which had a total bromine content of 17.9 mg/kg bromide. The calculated contents of organobromine compounds as percentage of the total bromine content in seafish was about 35 % . In shrimps the organobromine compounds amounted to 75 % of the total

bromine. Therefore, shrimps exhibit the largest values of organobromine in fishery products, exceeding 50 mg/kg bromide on productbasis.
Eel from freshwater lakes (Lake IJssel) has lower bromide levels than eel from a seawater basin (Lake Grevelingen, south-west Holland). It is evident, that the increased bromide content of eel from the Lake Grevelingen is highly related to the bromide concentration of sea water. Furthermore, It appears from the data of figure 1 that sprat did have a much higher bromine level than herring. Differences in behaviour and food pattern might underly this phenomenon.

The contents of bromine in herring and sprat:

The data of figure 2 and 3 gives us a distinct view of the variations in bromide and organobromine contents of sprat and herring from the North Sea and adjacent waters. In the experiment of 1983 total bromine contents ranged from 5 to 14 mg/kg bromide on productbasis. For the bromide content these values were 3 to 12 mg/kg bromine and for organobromine 1.3 to 5 mg/kg bromine. The organobromine compounds fluctuated from 12 to 43 % of the total amount of bromine (see Table II). The measured variations of the bromine content were related to fish parameters rather than to the catch location in the North Sea. There could hardly any geographical effect be observed on the bromine contents of herring and sprat. In fact, the bromine level is related to the fish species, the length of the fish and the lipid content.
The mean total bromine content of eight herring samples amounted to 7.0 (st. dev. 2.1) mg/kg bromide. For sprat a mean bromine content of 10.7 (st. dev. 1.9) mg/kg bromide was calculated with a maximum value of 14.7 and a minimum value of 7.4 mg/kg. Sprat and herring, sampled in 1980, had a total bromine content of respectively 17.9 mg/kg and 4.5 mg/kg bromide. Thus, the bromine level in sprat is significantly higher than in herring.
With increasing length of the fish the bromine content apparently decreased. In Table III the total bromine content and the lipid content in sprat are given. It appeared from these data that sprat with a length beyond 10 cm exhibited a lower bromine content than sprat smaller than 10 cm. The difference between the mean values of the two length classes was significant according to the Student test (< 10 cm: 52.8; > 10 cm: 37.6). Standard deviations were respectively 7.7 mg/kg and 7.5 mg/kg. Simultaneously with the decrease of the bromine content with increasing length of the sprat the lipid content increased significantly. The relation of the lipid content to the bromine content of sprat will be described in the discussion.
The total bromine content in the 1984 experiment varied from 10 to 24 mg/kg bromide on productbasis (Fig. 3). This bromine level is about twice the level measured in 1983, but agreed very well with the bromine content of sprat in 1980 (Table I).

The same observations were made for the bromide (5 to 18 mg/kg) and organobromine contents (3 to 8 mg/kg), the latter forming 12 % to 54 % of the total bromine content (Table IV), which is comparable with the 1983 data.

Identification of individual bromine compounds in fish: tetrabromodiphenylethers:

Among the many manmade organobromine compounds the flame retardants are an important group of industrial products. Applied in textiles, plastic materials and synthetic fibers, these compounds can easily enter the environment via waste disposals and industrial release (10). Some of these bromine compounds could already be detected in the aquatic environment (11,12).
In our laboratory tetrabromodiphenylether (TBDE) and pentabromodiphenylether (PBDE) have been determined in eel from the river Rhine and some other Dutch surface waters. The content of TBDE ranged in some surface waters from 100 to 200 ug/kg. The level of total organobromine in eel from inland waters was about 1 mg/kg bromide on productbasis. This means that a single component (TBDE) from the group of flame retardants accounts for 10 to 20 % of the organobromines in eels from these waters.
Because of the presence of many other natural bromine compounds in seafish the contribution of bromine containing flame retardants to the total organobromine content will certainly be at quite a lower level. However, in marine algae related polybrominated diphenylethers have been isolated too, being a natural occurring bromine compound (13).

Discussion and Conclusions:

Fish oils contain large quantities of organobromine compounds (4, 6). So, at least part of these compounds are highly lipidsoluble and are extracted from fish together with the lipids. An interesting question therefore is how the organobromine compounds are related to the lipidfraction of the fish itself.
To answer this question the total bromine, bromide and organobromine contents expressed on dry weightbasis have been plotted against the lipidcontent of the fish (fig. 4 - 9).
From these figures it is remarkable clear that the bromine content in sprat is inversely related to the lipid content. At low lipidcontents both bromide and organobromine levels showed relatively higher values, which decreased with increasing lipid content. This inverse relationship of the bromine content and the lipid content is consistent with the decreasing bromine level at increased length of the sprat, because the lipid content increased at increased length.

The lipid contents of the 1984 fish samples were much lower than those of the samples collected in 1983. Concomitantly, some bromine levels of these samples had been increased to a much higher extent (Fig. 7). It might be possible therefore that seasonal changes (e.g., reproduction cycle) can have some influence on the bromide and organobromine levels in sprat.
From our results it is clear, that in marine organisms high amounts of bromine are found, which partly is organically bound. In comparison with terrestrial organisms bromine levels in the marine environment are a hundred times higher. Last decades much research has been done on the biosynthetic origin of the organobromine compounds. Over 600 compounds are identified by now as natural occurring organobromine compounds (14). The majority of these bromo-organicals are produced by benthic invertebrates or plankton species (2). But also xenobiotic bromine compounds occurs in the marine environment, the identity of most of them being just unknown. However, some of these anthropogenic compounds are able to accumulate in marine organisms. Tetrabromodiphenylethers can form an important part (10 to 20 %) of the total organo-bromines of eel from inland surface waters. Much work is needed to elucidate the significance of high amounts of organobromines in marine organisms with respect to the occurrence of anthropogenic bromine compounds in the environment.

In view of the high content of bromine in oils from phytoplankton the bromine level in planktoncells must be very high (3). Also, in shrimps and mussels high values of bromine were found. On the contrary, in sea fish much lower concentrations of bromine are present. Also, lower bromine contents were measured with increasing length and lipidcontent of the fish. Apparently, during the growth of fish dilution of the bromide and organobromine is occurring in the tissues. So, at a low trophic level the bromine contents are the highest, probably being the site at which high production of natural organobromines takes place.

Briefly, the following conclusions can be made from the results described in this paper:

1. in comparison with seafish, shrimps and mussels have relatively high bromine contents.
2. with increasing length the bromine content of sprat decreases.
3. the bromine level in sprat is higher than in herring.
4. the bromine level in sprat is inversely related to the lipidcontent
5. predator fish has lower bromine levels than prey fish. (Table I)

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Table I : Bromine in several fish species

	A	B	C	D	E
1	Fish species	Total bromine	Bromide	Organobromine	Dry weight
2		mg/kg on productbasis	mg/kg on productbasis	mg/kg on productbasis	
3	Mussels-1	56.4			191
4	Mussels-2	43.5			157
5	Mussels-3	38.9			185
6	Shrimps-1	74.5	16.0	58.5	272
7	Shrimps-2	69.5	17.3	52.3	250
8	Shrimps-3	78.9	21.2	57.8	275
9	Sprat	17.9	7.1	10.8	296
10	Whiting	6.3	4.8	1.6	190
11	Cod	5	2.8	2.1	192
12	Plaice	4.9	3.2	1.7	215
13	Sole	6.6	4.3	2.2	206
14	Mackerel	4.6	3.0	1.7	220
15	Herring	4.5	2.9	1.6	271
16	Eel-Lake IJssel	2.8	1.7	1.1	475
17	Eel-Grevelingen	7.1	4.2	2.9	268
18	Pike-perch	1.8	1.1	0.7	210

Table II : Bromine content of herring and sprat in 1983

Sampling Area: North Sea

	A	B	C	D	E	F	G	H	I	J	K
1	SAMPLE NR		FISH	LOCATION	Length (cm)	TOTAL BROMINE	ORGANOBROMINE	% ORGANOBROMINE	BROMIDE	DRY WEIGHT	LIPIDCONTENT
2						mg/kg on productbasis	mg/kg on productbasis		mg/kg on productbasis	G/KG	G/KG
3	1		SPRAT	ENGLISH COAST	> 10	8.7	2.1	24.1	5.4	410	524
4	2		SPRAT	ENGLISH COAST		7.6	2.2	28.9	5.4	387	514
5	3		SPRAT	SOUTH-ENGLAND		10.8	2.9	26.9	7.2	377	477
6	4		SPRAT	SOUTH-IRELAND	< 10	11.6	5.0	43.1	6.6	331	369
7	5		SPRAT	CORNWALL	> 10	8.1	2.5	30.9	5.6	324	336
8	6		HERRING	NORTH-IRELAND		4.8	1.7	35.4	3.0	306	304
9	7		HERRING	NORTH-IRELAND		6.2	2.0	32.3	4.4	272	243
10	8		HERRING	NORTH-IRELAND		7.4	1.5	20.3	7.2	262	122
11	9		SPRAT	SOUTH-IRELAND	> 10	11.5	4.1	35.7	7.3	245	212
12	10		HERRING	SOUTH-IRELAND	> 20	8.6	1.9	22.1	7.3	227	111
13	11		SPRAT	GERMAN BIGHT		10.2	2.3	22.5	8.2	285	281
14	12		SPRAT	NETH. COAST	> 10	8.8	2.0	22.7	6.6	281	335
15	13		SPRAT	NETH. COAST	< 10	12.4	4.6	37.1	7.9	234	162
16	14		SPRAT	CENTRAL NORTH SEA	> 10	11.0	1.6	14.5	9.4	251	207
17	15		SPRAT	CENTRAL NORTH SEA	< 10	13.7	2.1	15.3	11.2	222	113
18	16		SPRAT	CENTRAL NORTH SEA	> 10	9.3	2.3	24.7	6.4	249	233
19	17		SPRAT	CENTRAL NORTH SEA	< 10	13.3	1.7	12.8	10.5	229	131
20	18		HERRING	CENTRAL NORTH SEA	> 20	4.8	1.4	29.2	3.4	278	270
21	19		HERRING	NETH. COAST	< 20	6.6	2.3	34.8	4.3	230	126
22	20		SPRAT	CENTRAL NORTH SEA	> 10	9.6	1.6	16.7	7.6	247	215
23	21		SPRAT	NETH. COAST	> 10	7.4	1.9	25.7	5.3	267	292
24	22		SPRAT	NETH. COAST	< 10	10.4	2.1	20.2	7.8	225	116
25	23		SPRAT	GERMAN BIGHT	< 10	10.5	3.4	32.4	6.9	260	269
26	24		SPRAT	GERMAN BIGHT	> 10	9.5	1.7	17.9	7.6	295	336
27	25		SPRAT	NETH. COAST	> 10	10.6	2.3	21.7	7.6	253	237
28	26		SPRAT	NETH. COAST	< 10	11.4	3.2	28.1	7.2	223	143
29	27		SPRAT	GERMAN BIGHT		11.5	1.9	16.5	9.0	260	298
30	28		SPRAT	CENTRAL NORTH SEA	> 10	12.9	3.2	24.8	9.8	268	250
31	29		SPRAT	CENTRAL NORTH SEA	< 10	14.7	1.7	11.6	12.0	248	161
32	30		SPRAT	CENTRAL NORTH SEA	> 10	13.1	3.5	26.7	9.8	282	255
33	31		HERRING	GERMAN BIGHT	< 20	11.2	4.1	36.6	7.1	213	89
34	32		SPRAT	CENTRAL NORTH SEA		9.7	2.2	22.7	7.4	240	217
35	33		HERRING	CENTRAL NORTH SEA		6.7	1.3	19.4	5.7	240	150
36	34		SPRAT			10.9	1.8	16.5	9.0	303	320

Table III: Total bromine and lipid content in sprat of two different length classes

		< 10 cm:		> 10 cm:	
	Catch location:	Total bromine: (mg/kg dry weightbasis)	Lipid: (g/kg)	Total bromine: (mg/kg dry weightbasis)	Lipid: (g/kg)
1.	Central North Sea	61.9	113	43.9	207
2.	Central North Sea	58.1	131	37.5	233
3.	Central North Sea	59.3	161	48.3	250
4.	Dutch Coast	53.1	143	31.3	237
5.	Dutch Coast	46.3	116	27.7	292
6.	Dutch Coast	50.9	162	42	335
7.	German Bight	40.4	269	32.3	336
	Mean value:	52.8	156	37.6	270
	Standard deviation:	7.7	53	7.5	51

Table IV : Bromine content of herring and sprat in 1984

Sampling Area: North Sea

	A	B	C	D	E	F	G	H	I
1	MONSTER	FISH	LOCATION	Total bromine	Organobromine	% Organobromine	Bromide	Dry weight	Lipidcontent
2				mg/kg on productbasis	mg/kg on productbasis		mg/kg on productbasis	g/kg	g/kg
3	1	HERRING	S-NORTH SEA	15.6	5.6	36	14.9	374	65
4	2	HERRING	S-NORTH SEA	18.1	5	28	13.4	310	42
5	3	HERRING	HEBRIDEN	10.1	5.5	54	8.4	366	
6	4	HERRING	S-NORTH SEA	19.7	7.8	40	14.6	340	71
7	9	SPRAT	GERMAN BIGHT-ELBE	12.7	4.7	37	9.5	266	91
8	10	SPRAT	GERMAN BIGHT-ELBE	18.3	5.3	29	13	266	53
9	11	SPRAT	GERMAN BIGHT-ELBE	11.8	3.5	30	7.2	258	90
10	12	SPRAT	GERMAN BIGHT	20.1	4	20	16.9	228	48
11	13	SPRAT	CENTRAL NORTH SEA	23.3	4.2	18	8.2	424	142
12	14	SPRAT	SKAGERAK	14.8	5.4	36	13.3	226	76
13	15	SPRAT	S-NORTH SEA	14.2	3.7	26	13.8	224	71
14	16	HERRING	S-NORTH SEA	14.3	4	28	7	288	25
15	17	SPRAT	CENTRAL NORTH SEA	13.8	4.8	35	8.9	240	50
16	18	SPRAT	NETH. COAST	11.4	2.6	23	11.6	196	55
17	21	SPRAT	ENGLISH CHANNEL-WEST	21.6	5.4	25	18.3	212	16
18	25	HERRING	S-NORTH SEA	13.4			5.1	330	42
19	26	SPRAT	CENTRAL NORTH SEA	42.6	5	12	14.2	388	40
20	27	HERRING	CENTRAL NORTH SEA	17.6	7	40	12.3	382	
21	30	SPRAT	NETH. COAST	24	5.2	22	16.9	288	59
22	31	SPRAT	ENGLISH CHANNEL-WEST	11	3	27	11.1	458	216
23	32	SPRAT	GERMAN BIGHT-ELBE	11.8	4.4	37	11	316	152
24	33	SPRAT	CENTRAL NORTH SEA	10.4	4.5	43	10.7	284	131
25	34	SPRAT	NETH. COAST	20.1	3.9	19	13.2	334	89
26	35	HERRING	NETH. COAST	12.6	2.7	21	11.9	436	108

Fig. 1 **Total Bromine in fish**

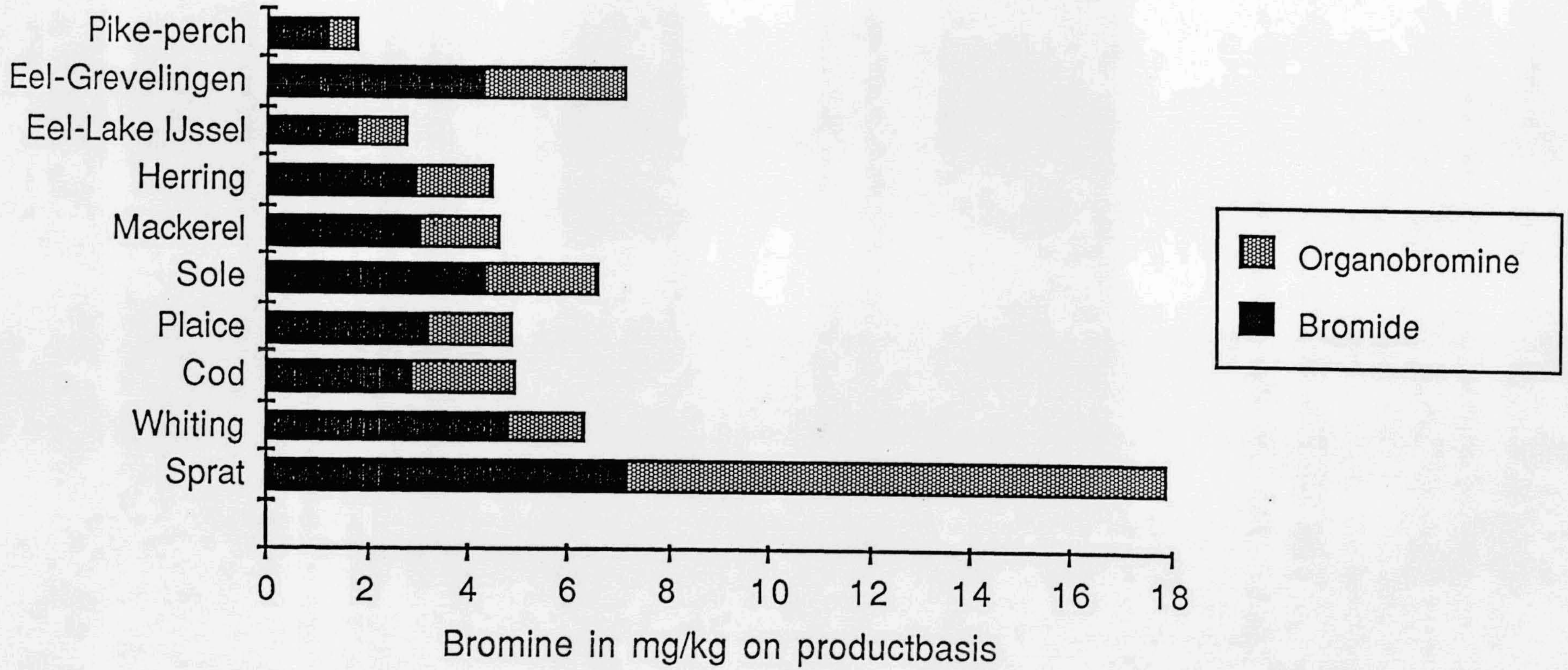

Fig. 2 Bromine species of sprat and herring sampled in 1982/83

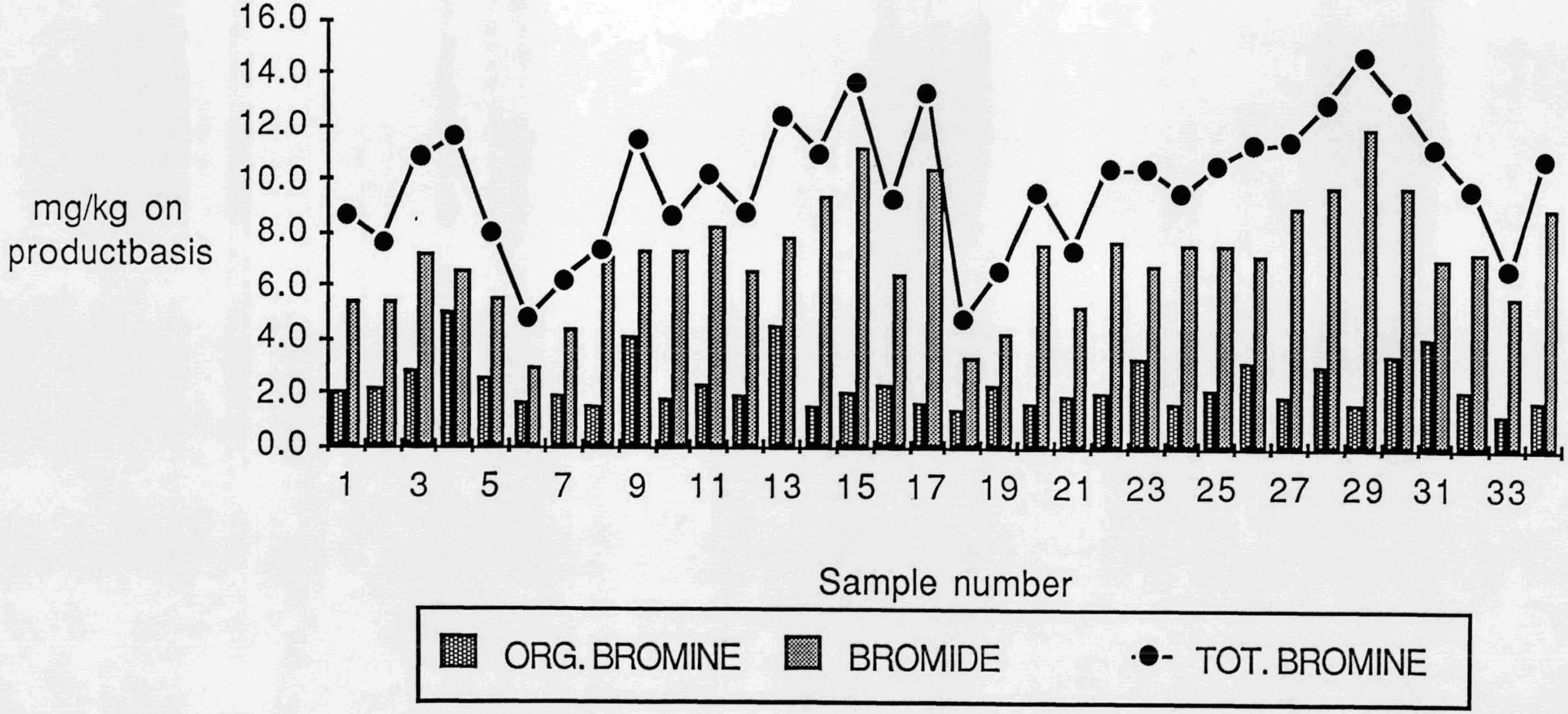

FIG. 3 Bromine species of sprat and herring sampled in 1984

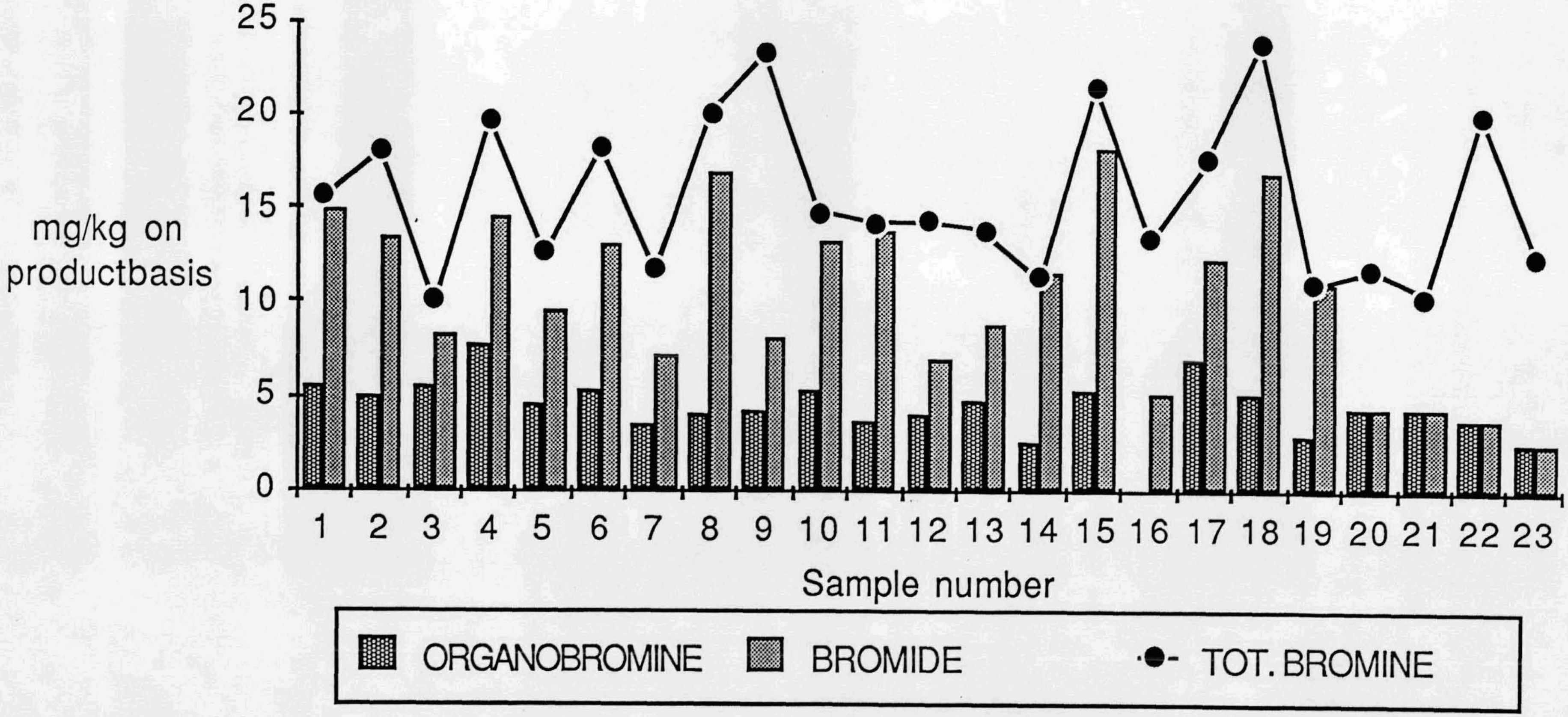

Fig. 4 : Total bromine as a function of the lipidcontent in sprat sampled in 1983.

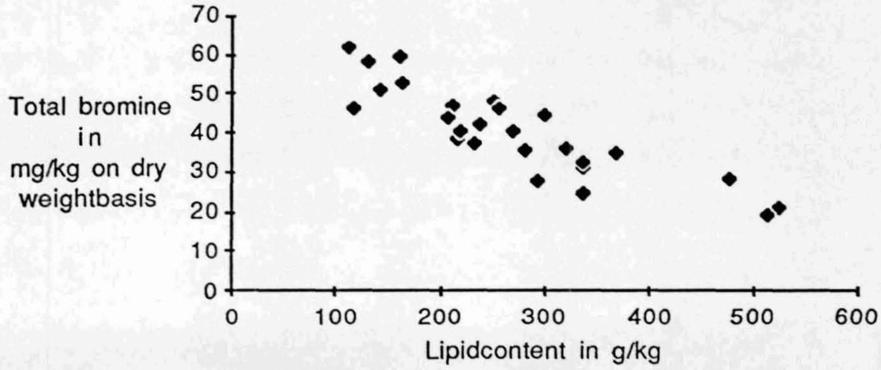

Fig. 5 : Organobromine as a function of the lipidcontent in sprat sampled in 1983.

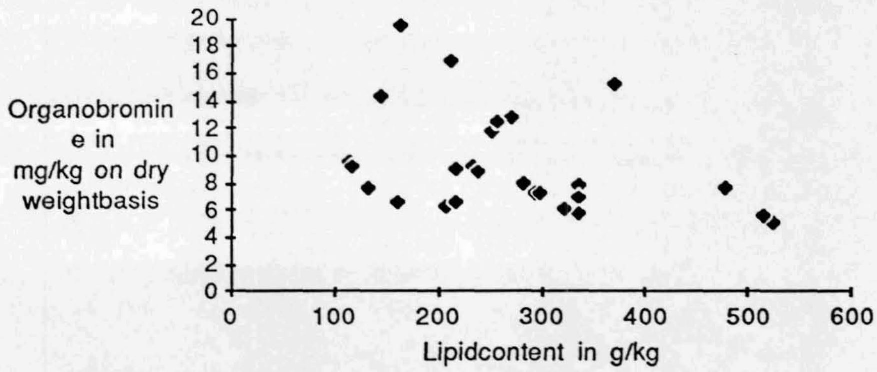

Fig. 6 : Bromide as a function of the lipidcontent in sprat sampled in 1983

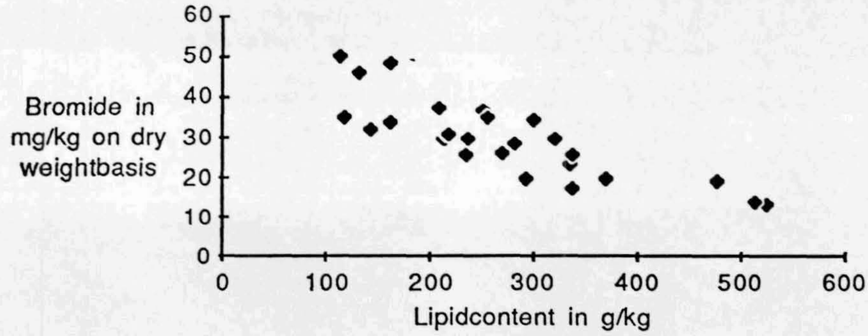

Fig. 7 : Total bromine as a function of the lipidcontent of sprat sampled in 1984

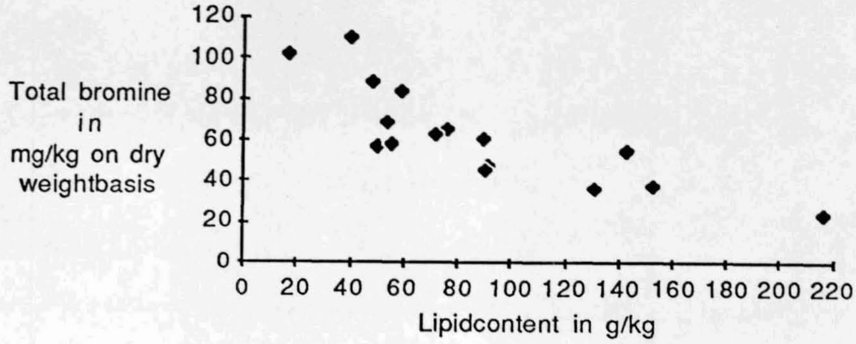

Fig. 8 : Organobromine as a function of the lipidcontent in sprat sampled in 1984.

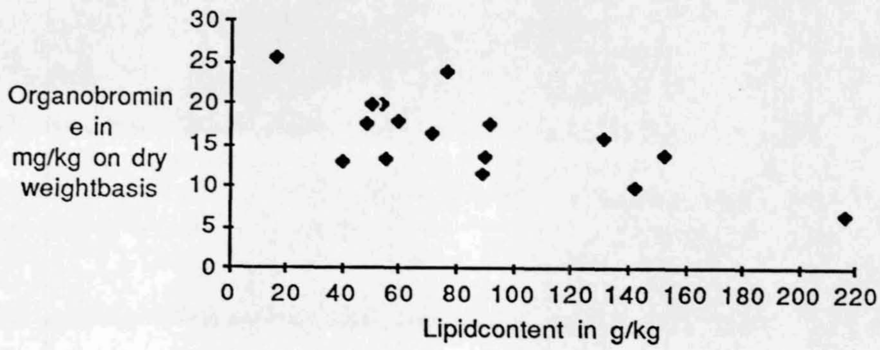

Fig. 9 : Bromide as a function of the lipidcontent of sprat sampled in 1984.