Chapter VIII

Physiological Effects of some Pollutants

by

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Based on experimental work from

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O. MARCQ and J.M. BOUQUEGNEAU (Liège University)

The main contribution of physiologists to the study of pollution of the marine environment lead :

1) to establish toxicity scales;

2) to evaluate the accumulation of pollutants in marine animals and plants either through the food chain or by direct contamination or by both processes.

The experiments are carried out either on whole organisms, organs or isolated tissues, to better understand not only the nature of the observed physiological perturbation, but in fine to explain possible ecological changes and eventual toxic effects on man.

1.- Toxicity scales

1.1.- Effect of heavy metals and organic pesticides on *Euplotes vannus MULLER* (Marine ciliate, Hypotrichida)

G. PERSOONE and G. UYTTERSPROT (1973a)

Euplotes is an ubiquitous marine benthic ciliate living in sand-mud sediments, of importance as nutrient regenerator and as food for other organisms. Significant increase or decrease in its population density should be reflected at other trophic levels.

The test organisms are cultured on a yeast-sea water suspension (Fleischmann's dry yeast and artificial sea water 35 ‰). Heavy metals (Pb, Cu, Cd, Zn, Hg) are added as chlorides; organic pesticides and PCB are first dissolved in acetone (1 cc for 1 ℓ sea water). After 48 h incubation at 28° C , the number of cell divisions is calculated from cell counts after fixation, and the inhibition is evaluated from :

$$\% \text{ inhibition} = 100 - \left(100 \times \frac{\text{average number generations + pollutant}}{\text{average number generations control}}\right)$$

Figure 8.1 summarizes the results. The toxicity scale for heavy metals is Hg > Cu > Pb > Cd > Zn . It is however obvious that the growth of *Euplotes* is affected only at very high concentrations compared to those normally found in North Sea coastal waters and even in the Scheldt estuary as indicated in Table 8.1 :

Table 8.1

	North Sea coastal water (ppb)	Scheldt water (ppb)	North Sea sediments (ppm)	Scheldt sediments (ppm)
Zn	100	430	271	926
Cd	5	15	?	?
Pb	58	35	280	185
Cu	59	130	58	221
Hg	0.8	?	1.24	?

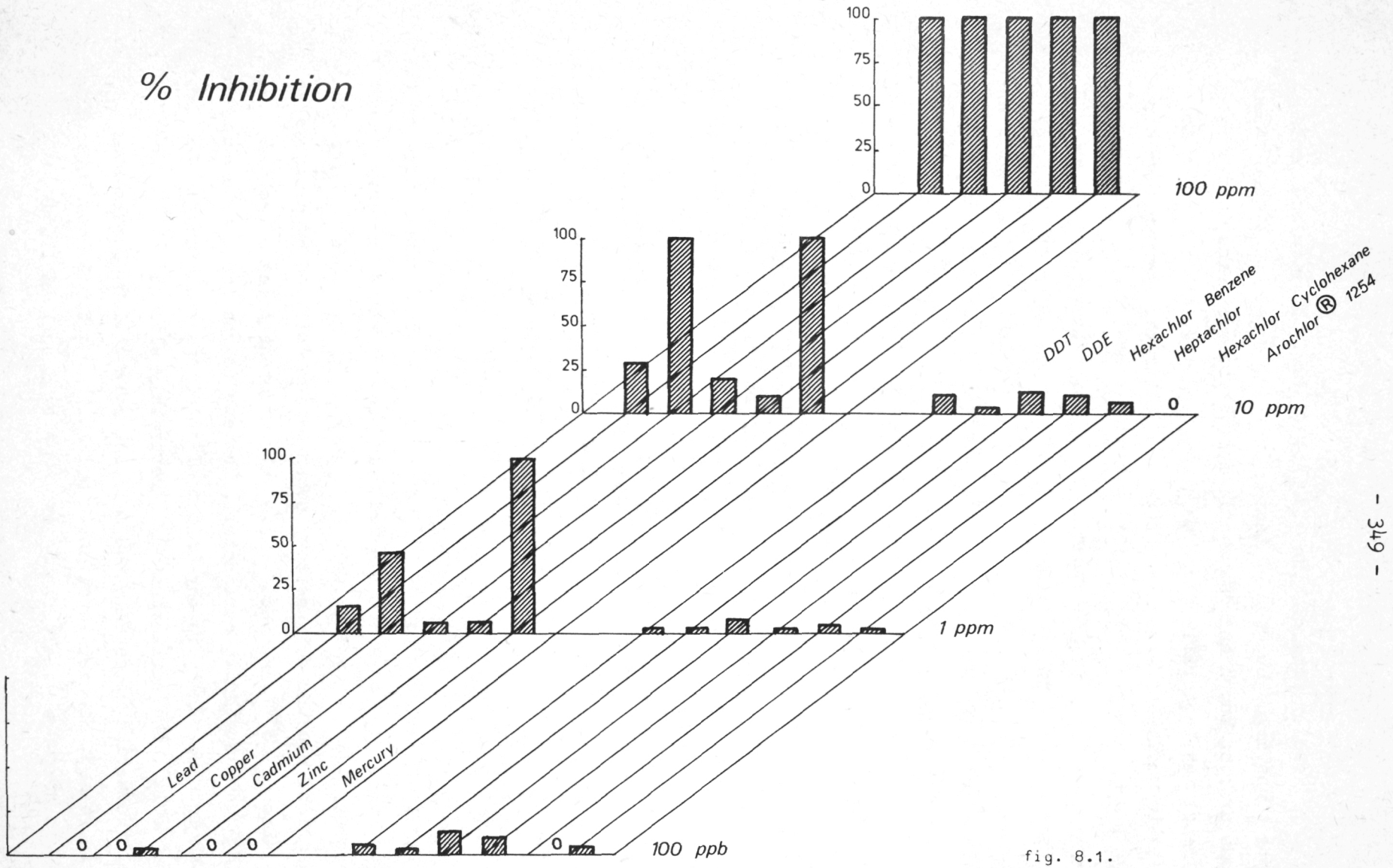

fig. 8.1.

Effect of heavy metals, pesticides and PCB on the growth of Euplotes Vannus Muller (benthic ciliate);
% inhibition =

$$100 - \left(100 \times \frac{\text{average number generations + pollutant}}{\text{average number generations control}}\right).$$

It is however possible that in interstitial water in sediments, concentrations might be reached that affect *Euplotes*, but these concentrations are not known and one should further bear in mind that the heavy metals are generally bound to organic particulate matter or to minerals in sediments and only partially free in the surrounding solutions.

Euplotes's growth is extremely unsensitive to pesticides and PCB. The actual concentrations in sea water are of the order of 0.01 ppb , exceptionally up to 1.5 ppb ; in sediments one finds 1 ppb up to a few ppb , far below the lowest concentration used by Persoone and Uyttersprot in their experiments.

On the whole *Euplotes* appears as very resistant which is perhaps not surprising since it is commonly found among fouling organisms in harbours.

It would be interesting to find out whether this ciliate, because of its high resistance, does accumulate or not pollutants, for example DDT known to rapidly penetrate living cells as algae, being very soluble in lipids. If accumulation does occur *Euplotes* might turn out to be a dangerous input path to higher trophic levels for substances potentially toxic.

1.2.- Effect of methyl-Hg and organic pesticides on the activity of the isolated heart atrium of eels (*Anguilla anguilla*)

O. MARCQ (1973a,b)

Previous work [Marcq (1972)] has shown that the contractions of the isolated atrium of sea water adapted eels are decreased by heavy metals in the order Hg > Cd > Pb > Cu > Zn > Mn > Ni ; Hg , Zn , Mn , Cu and Ni ions produce a transitory potentiation followed by progressive inactivation; Co^{++} and UO_2^{++} have only a potentiating effect.

Hg was studied in more detail. The atrium was found to accumulate large amounts of Hg^{++} in physiological solutions containing 2-3 ppm $HgCl_2$. The atrium beat is irreversibly altered; Hg^{++} decreases the action potential, the membrane permeability to all ions being increased. However intoxicated eels with Hg-blood contents of the same order show no alteration of the atrium beat amplitude.

The experiments described below have been carried out to learn more about the protective effect of plasma and to investigate the effect of CH_3HgCl and organic pesticides.

1) CH_3HgCl : the atria after 5 min intoxication in physiological solutions containing 2 ppm $HgCl_2$ or CH_3HgCl are washed during 25 min in mercury free solutions; an irreversible decrease corresponding to 60-70 % of the normal heat amplitude is observed for both $HgCl_2$ and CH_3HgCl but the effect of the methylated compound appears to be faster, especially during the initial potentiating phase (fig. 8.2). The accumulation in the tissue reaches 39.5 ppm and 20.8 ppm for $HgCl_2$ and CH_3HgCl respectively.

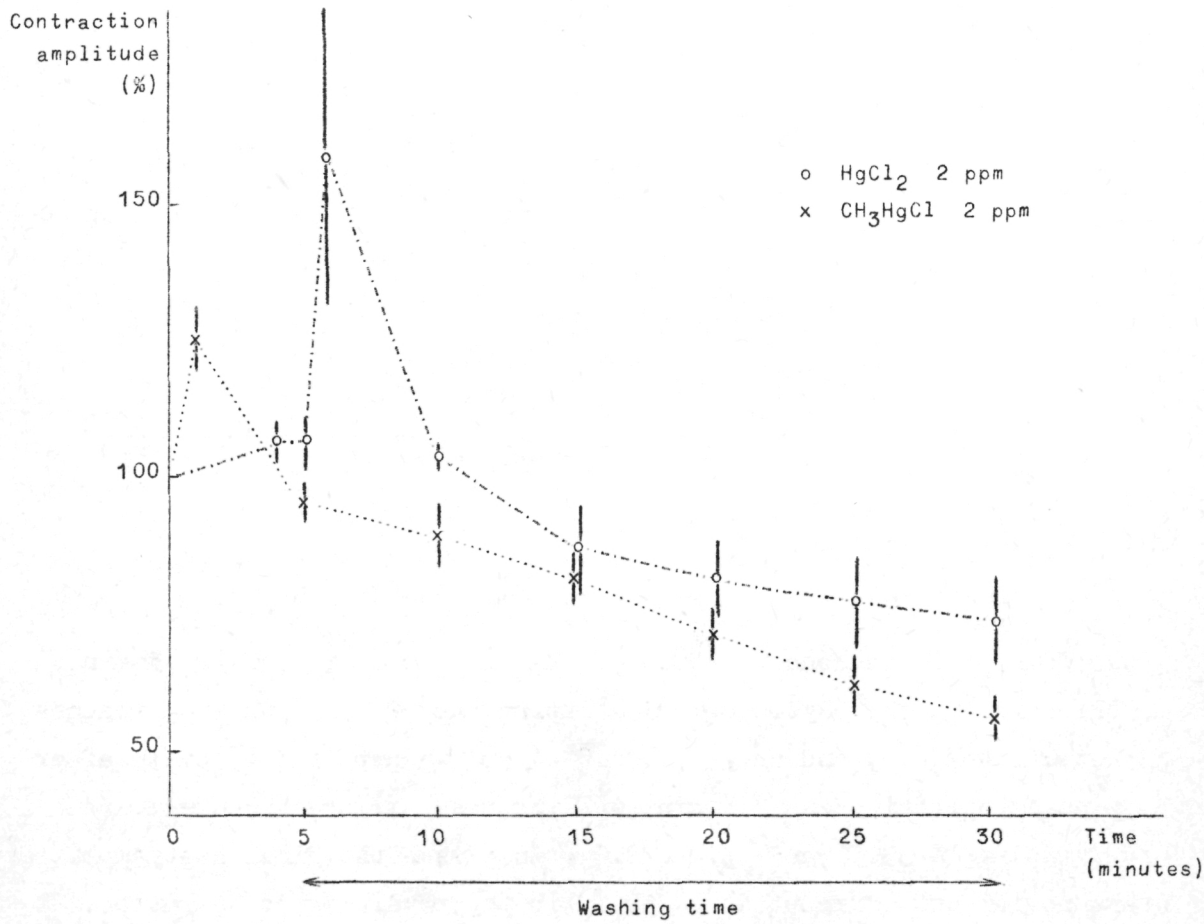

fig. 8.2.

Effect of $HgCl_2$ (2 ppm) and CH_3HgCl (2 ppm) on the contraction amplitude of the isolated eel atrium; 5 minutes intoxication followed by 25 minutes recovery in mercury free physiological solution.

2) Protective effect of plasma : Figure 8.3 shows the protective effect of plasma compared to the physiological solution, both containing

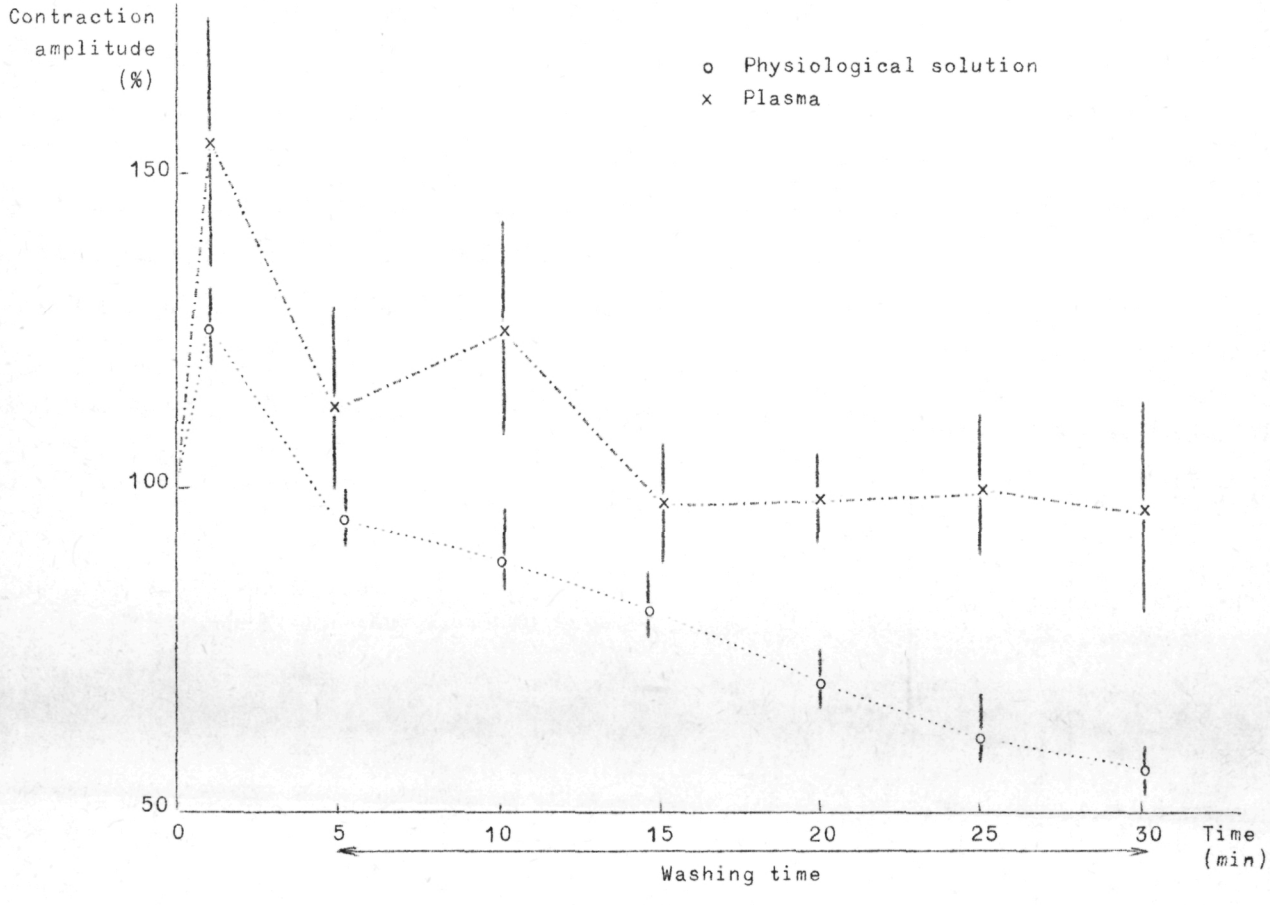

fig. 8.3

Protective action of plasma on the contraction amplitude of isolated eel atrium; intoxication in presence of 2 ppm CH_3HgCl lasts 5 minutes either in plasma or in physiological solution; mercury free fluids are used for washing.

2 ppm CH_3HgCl . The concentration of Hg in the atrium after 5 min intoxication in the physiological solution and 25 min washing reaches 21.0 ± 2.0 ppm Hg , and only 1.6 ± 0.4 ppm when plasma is used; after 30 min intoxication and 15 min washing these figures become respectively 166.0 ± 33.0 and 3.6 ± 2.0 . In plasma the final beat amplitude stays normal but is reduced to 50 % in the physiological solution. It is well known [Hugues (1946)] that serum albumin (SA) reacts with Hg which binds to SH groups, to finally form AS-Hg-SA . If the affinity of this albumin for Hg is greater than that of the reactive sites on

the atrium proteins, it might explain the protective action of plasma compared to physiological solutions devoided of SH bearing substances.

To test this hypothesis cystein has been added to the physiological fluid used to wash the preparation first intoxicated in the normal solution. The Hg concentration in the atrium in a series of experiments of this type falls from 24.0 ± 3.0 ppm when no cystein is used to 18.0 ± 4.0 ppm , 3.0 ± 1.0 ppm and 4.0 ± 1.0 ppm in presence of respectively 2.5 and 8 mM cystein in the washing solution.

Cystein thus binds the Hg first attached to the atrium proteins; however no improvement in the amplitude of the contractions is observed. On the contrary, they decrease and arythmicity is observed. This effect can be observed in absence of Hg but in presence of cystein and is probably due to the formation of Ca and Mg undissociated cystein complexes, upsetting the normal ionic conditions required for normal activity of the contractile tissue.

3) Toxicity of pesticides : the following pesticides have been tested : DDE , DDD , heptachlor epoxide, aldrin, dieldrin, endrin, heptachlor, lindane, polychlorobiphenol (PCB), pyrethrin I and II. The atrium proved to be exceedingly resistant and the effects of the toxic substances cannot be distinguished from that of the solvent used : acetone or cyclohexane; for example, 210 ppm DDT in a physiological solution containing 2.5 % acetone produces the same decrease in the beat amplitude as the acetonic solution alone.

Only pyrethrin was found to be active at low concentrations (0.6 ppm) in presence of 0.06 % cyclohexane : it accelerates the beat frequency by 50 % , but the amplitude falls to 20 % of the normal value in 0.06 % cyclohexane with or without pyrethrin.

The toxicity of the solvents, the precipitation of the pesticides in the aqueous solution alone, the extreme resistance of the tissue, carries the inevitable conclusion that the eel isolated atrium is useless even to evaluate the relative toxicity of the tested organic pesticides.

Since the preparation is also relatively immune to large doses of heavy metals its use as bioassay has to be abandonned.

The resistance to toxics shown by many marine organisms emphasizes the danger of possible accumulation at sublethal doses, leading to toxic effects at higher trophic levels, including man.

1.3.- Synergic effects of pollutants extracted from North Sea sediments on the growth of *Dunaliella viridis Teodoresco* (flagellate alga)

G. PERSOONE and G. UYTTERSPROT (1973b)

The method used by Persoone and Uyttersprot has been developped to try to test the potential quality or toxicity of sediment material by measuring the growth of pure algae inocula in sediment extracts.

The sediment samples (Van Veen grab samples) are taken at 25 grid points located as shown in figure 8.4 in the test region where their content in organic matter (difference between dry weight and ash weight) is indicated.

Extracts are prepared using the amount of sediment required to provide 10 g of organic matter, suspended in 1 ℓ of artificial sea water, followed by filtration on 0.45 Millipore filter. Extract samples (100 cm^3) are inoculated with 100.000 algae cells [either with addition of Vlasblom culture medium ($FeSO_4$, NaH_2PO_4 , $NaNO_3$, $MnCl_2$, glycine) or not]. Controls are run on artificial sea water either or not enriched with the Vlasblom substrate. To compare the growth of the different cultures the surface of the S-shaped growth curve is used or the number of cells counted after 5 days .

Relative enhancement or decrease of the growth compared to control samples is expressed by

$$\% \frac{\text{S-growth curve sample}}{\text{S-growth curve control}} \quad \text{and} \quad \% \frac{\text{N cells sample}}{\text{N cells control}} \quad \text{after 5 days.}$$

Figures 8.5 and 8.6 show the results of these experiments. Fig. 8.5 indicates that in presence of the culture substrate, the difference between the growth in sediment extracts and the control is small, the mean for all points being practically zero. The only exception is point 5 where 30 % inhibition is observed.

Figure 8.6 shows what happens in sediment extracts in absence of culture substrate. On the average there is growth stimulation (65 %) .

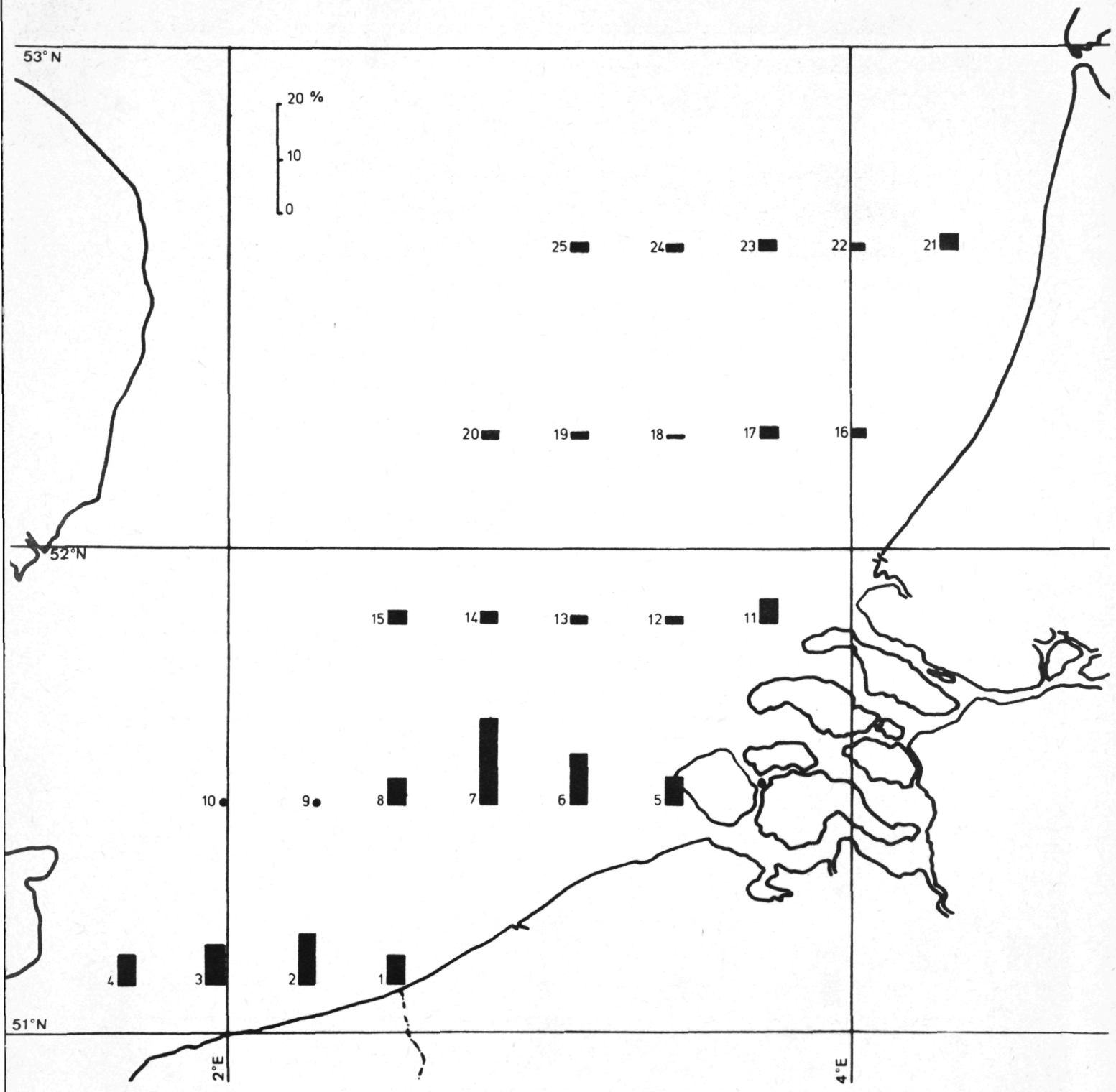

fig. 8.4.- % organic matter of sediment.

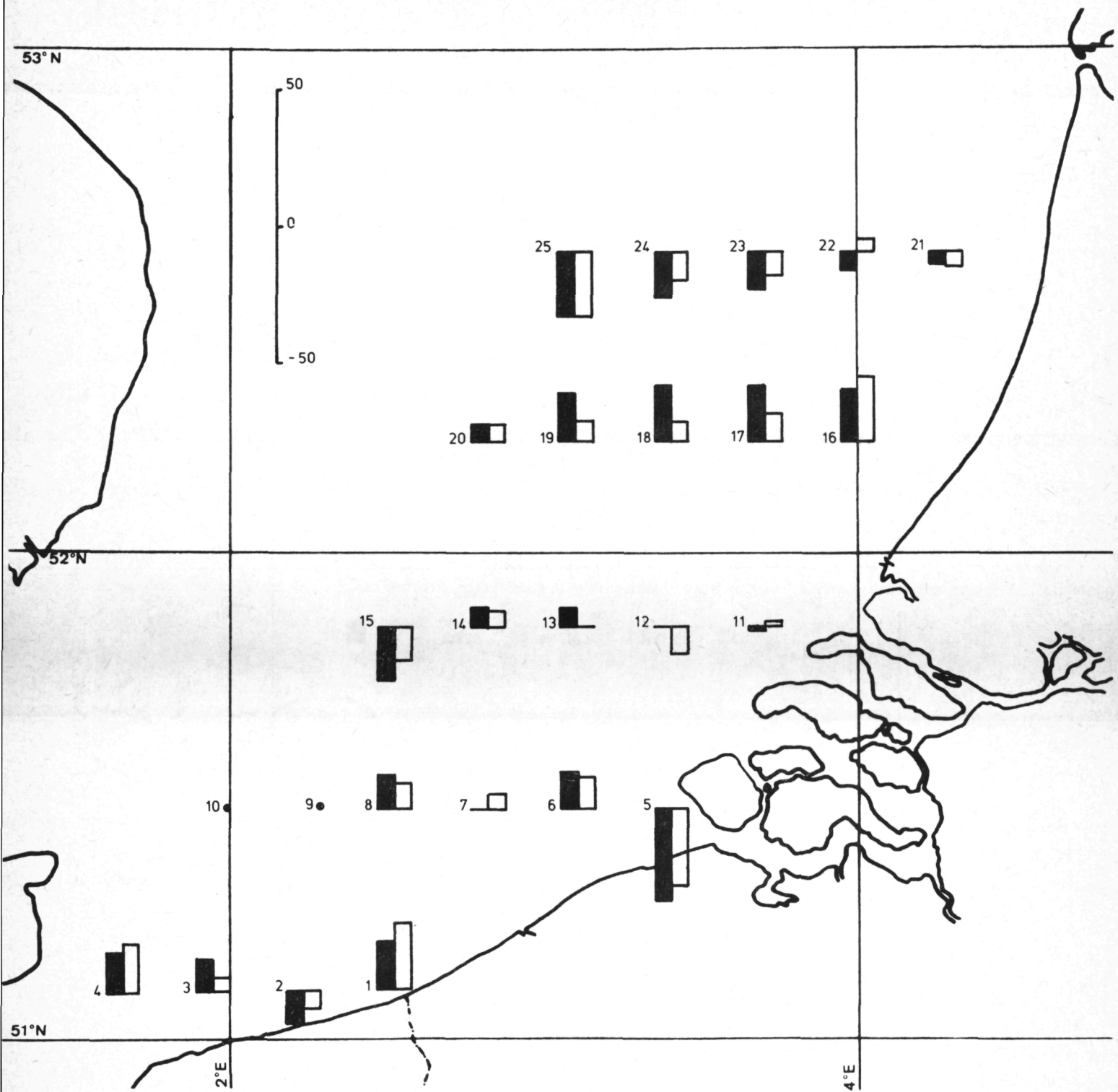

fig. 8.5.- Growth of Dunaliella viridis in usual culture medium with extract of sediment.

■ % $\frac{\int \text{growth curve sample}}{\int \text{growth curve control}}$ □ % $\frac{N_{sample}}{N_{control}}$ after 5 days

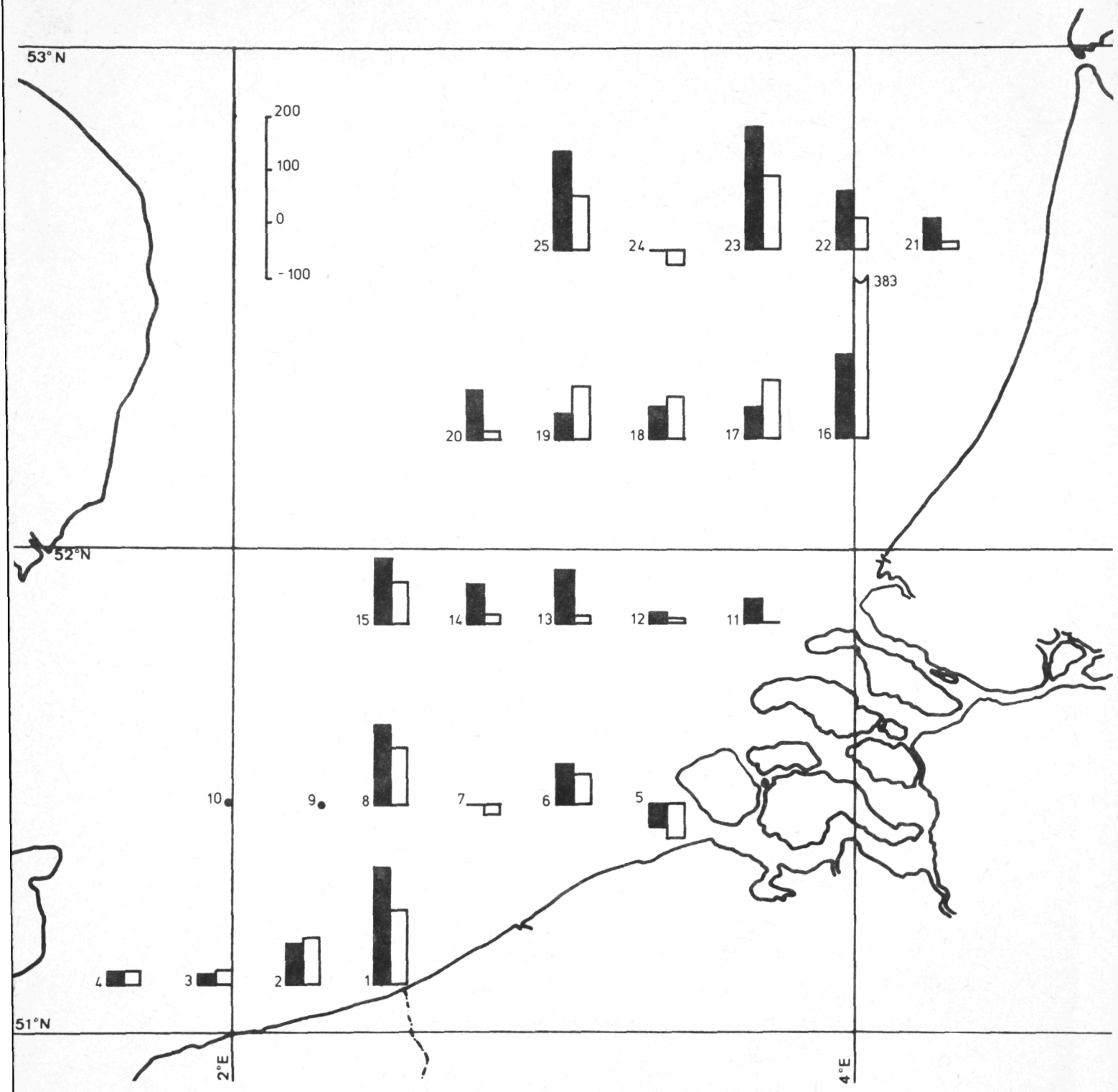

fig. 8.6.- Growth of Dunaliella viridis on extract of sediment solely.

■ % $\frac{\int \text{growth curve sample}}{\int \text{growth curve control}}$ □ % $\frac{N_{sample}}{N_{control}}$ after 5 days

Point 5 reveals 50 % inhibition. The growth rate is smallest around the estuarine region but it increases with distance from the coast; on the contrary growth is highest near the coast in transects 1-4 and 16-20 but a different and more complicated situation is found in 20-25.

It is obvious that when culture medium is added, there is no substrate limitation, which might explain that the algae grow at the same rate in control and sample; in absence of culture medium, although general stimulation is observed, it seems clear from what happens at point 5 that the influence of toxic substances increases when the environmental conditions become worse, the organism being submitted to multiple stress.

Point 5 is known to be in the vicinity of dumping sites for industrial waste. Evaluation of benthic biomass and fish population around points 5 and 6 shows however a relative increase, in contradiction with the laboratory results of Persoone and Uyttersprot.

Although figure 8.6 probably shows how growth of *Dunaliella* is activated by sediment extracts and modulated by synergic effects of toxic or inhibiting substances localised in the organic matter of these sediments, it remains doubtful whether this picture can be taken to reveal the global pollution situation in the test region. It should be remembered that Persoone and Uyttersprot (1972) obtained similar results when growing *Dunaliella* in natural sea water sampled at the 25 grid-points but almost an opposite picture when using the flagellate *Monochrysis lutheri*.

It seems obvious for the author of this report that the use of a single organism for water or sediment quality tests is very much questionable, the more that the nature of the pollutants remains totally unknown as well as the nature of nutrients and eventual natural growth inhibitors or potentiators.

It is clear that *in situ* primary production measurements, zooplankton evaluation and inventories of the biomass of benthic and pelagic populations, including diversity indexes, is by far much more reliable (see reports by Polk and De Coninck).

The methodology developped by Persoone and Uyttersprot for mass culture of algae leads however to important future possibilities to

determine incorporation rates of given pollutants either heavy metals or pesticides, and to initiate experimental food chains (see § 2.2.2 below).

2.- Accumulation processes

2.1.- Accumulation of heavy metals in mussels (*Mytilus edulis*)

Ch. PERPEET and M. VLOEBERGH (1973)

2.1.1.- *In situ* accumulation at different localities between Morgat Bay (Finistère, France) and the Scheldt Estuary

Mussels samples (70-90) collected at Morgat, Knokke, Hoofdplaat, Terneuzen and Perkpolder have been divided in groups of ten according to size and the tissues have been analyzed for Hg , Cu , Zn , Pb , Cd , Fe , Cr , using atomic absorption methods. The results shown in figures 8.7 and 8.8 are given in ppm dry weight except for mercury where one refers to wet weights (ppb).

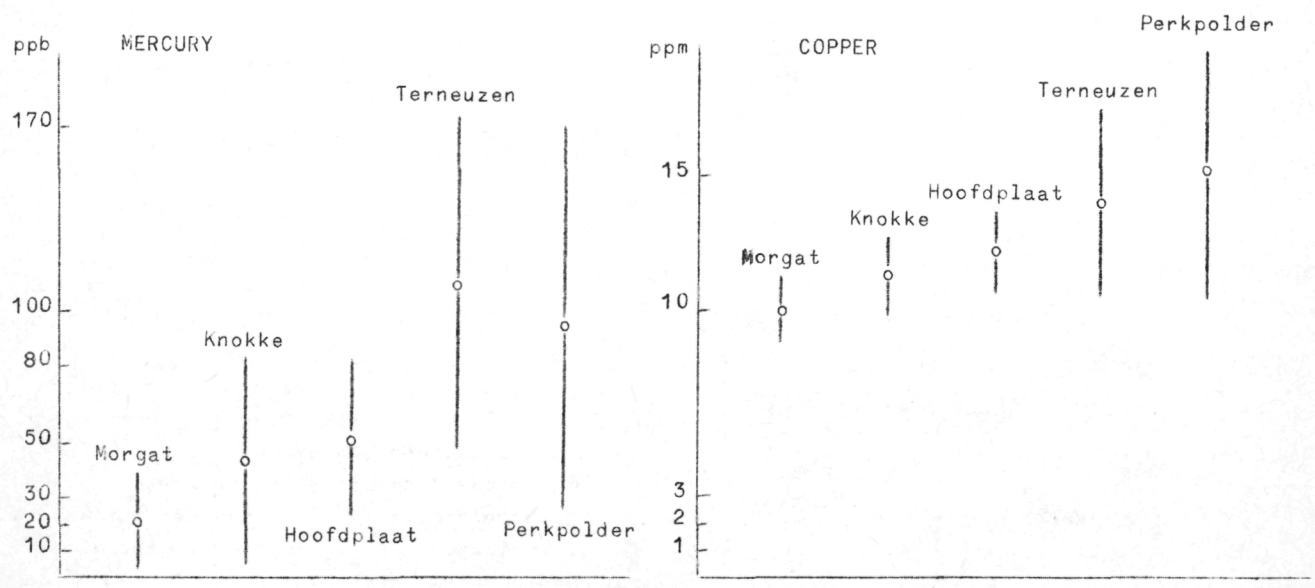

fig. 8.7.

Heavy metal content of mussels collected at various localities in the North Sea and the Scheldt estuary (Hg ppb wet weight; other metals ppm dry weight; height of vertical bars corresponds to the dispersion of the results).

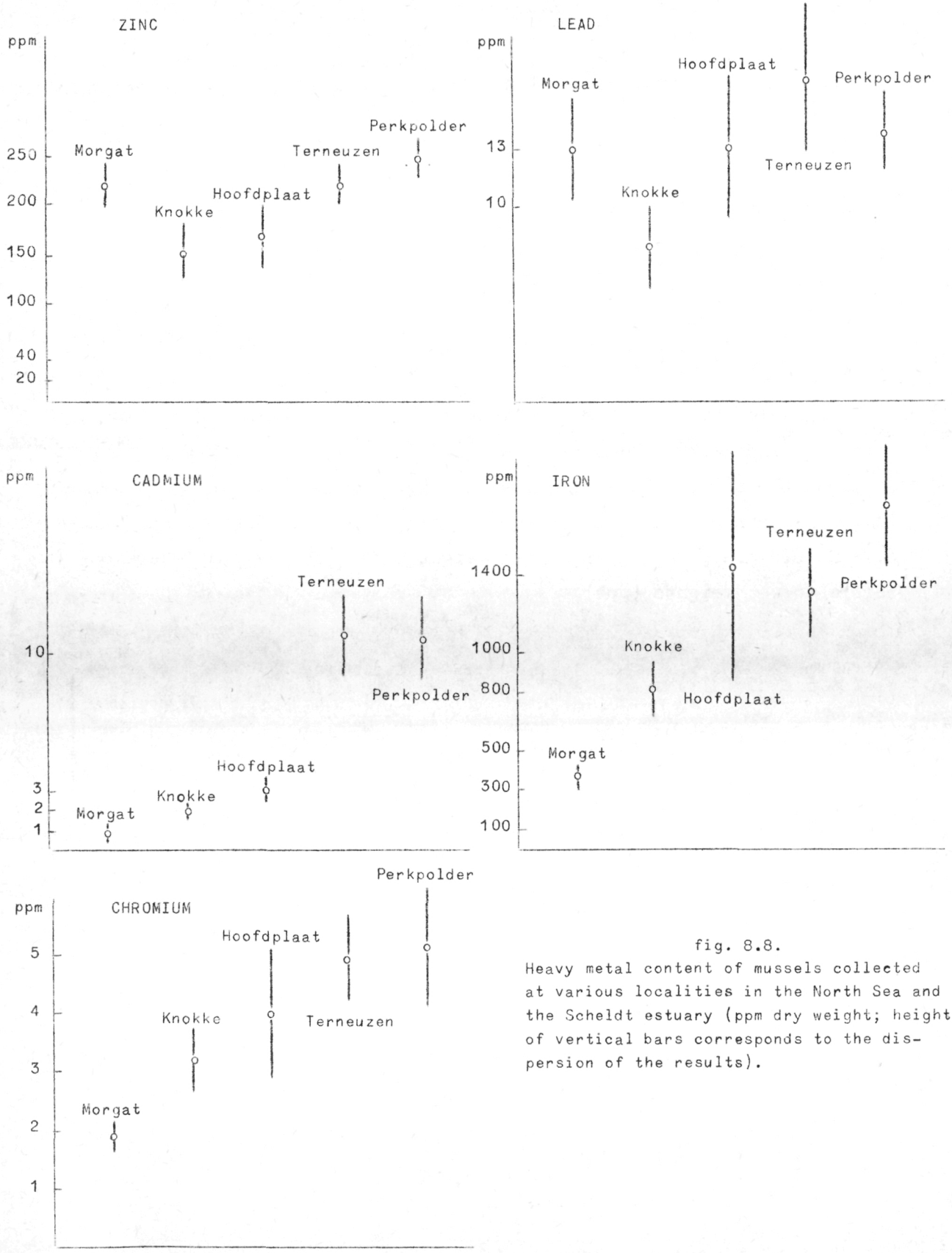

fig. 8.8.
Heavy metal content of mussels collected at various localities in the North Sea and the Scheldt estuary (ppm dry weight; height of vertical bars corresponds to the dispersion of the results).

No correlation is found with the size of the mussels and the results vary greatly at the same locality as is indicated by the size of the vertical bars centered on the mean value in the different graphs. It is however obvious that generally the heavy metal load increases going from Morgat towards the Scheldt. Exceptions are found for Pb and Zn , rather high at Morgat probably because of local industry.

Comparison with data of Bertine and Goldberg (1972) on mussel tissues sampled at Nieuwpoort in 1971 shows reasonable agreement : these authors give values ranging between 400-1400 ppm for Fe , 0.5-2.0 ppm for Cr , 0.5-1.6 ppm (dry weight) for Hg , but their values for Zn lie between 30 and 60 ppm , that is about 4 times less than those reported here.

Although these results tend to suggest that mussel might prove a good indicator for pollution, they do not necessarily mean that the heavy metals are really accumulated in the tissues. A large amount of the pollutants is probably simply located in the digestive tract and it should be interesting to repeat these analysis on mussel tissues separated from the digestive tract. It is known (see Bertine and Goldberg) that in the case of museum specimens a great part of the metals, Fe for instance, in the tract slowly pass into solution and contaminate the tissues.

If this is true then whole mussels should be regarded more or less as receptacles for suspended matter having undergone the effect of total or partial digestion, removing organic material. This would add to the eventual accumulation in other tissues either from food or due to the water flow and perhaps explain the scattering of results and the absence of correlation with size.

2.1.2.- Accumulation of ^{203}Hg in mussels kept in aquaria

Figure 8.9 shows preliminary results on the accumulation in mussels of ^{203}Hg added to artificial sea water. Concentration reaches the highest levels in the gills and the digestive tract after respectively 72 and 130 hours , then declines. The authors believe this to be due to changes in the ^{203}Hg concentration in the water because of precipitation or adsorption on mucus, organic particles, or on the aquarium

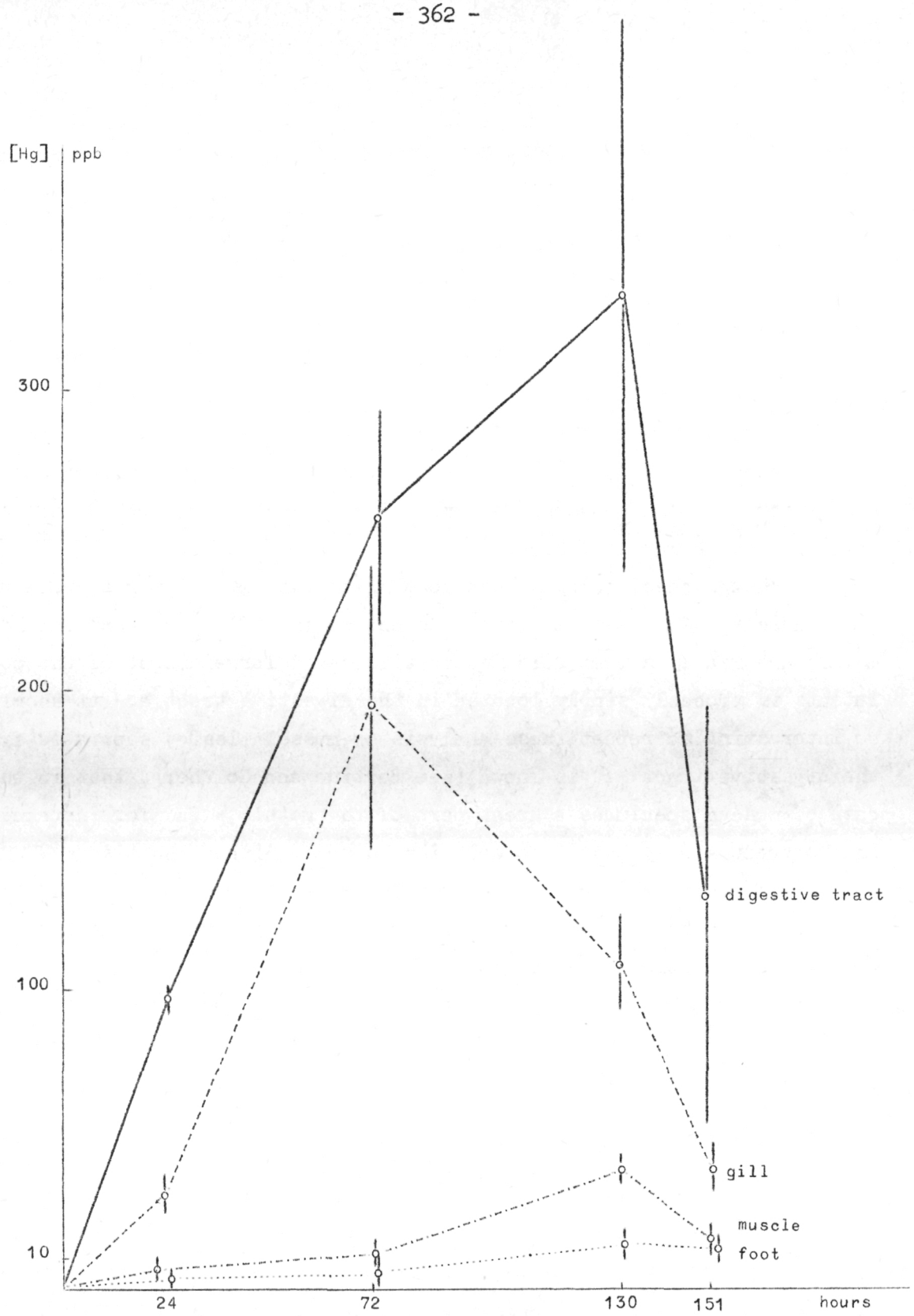

fig. 8.9.

Accumulation of ^{203}Hg in the tissues of mussels exposed to an initial concentration of 5 ppb ^{203}Hg in artificial sea water.

walls. If it is true, then obviously fixation and release of Hg in the gills and the digestive tract are fast compared to what happens in the other tissues.

At 1 ppm Hg , 100 % mortality is observed within a few days, and heavy accumulation is noticed in the gills.

Although the experiment must be repeated, sea water and the mussels being analyzed simultaneously and the Hg level being kept constant, it shows that accumulation can result from direct contact with contaminated water containing only 5 ppb Hg . This is still about 10 times more than what is found in the coastal region of the North Sea, but is a realistic figure and shows how promising the use of radio-isotopes is.

One will of course have to bear in mind the possibility of adsorption on organic particulate matter or bacteria in suspension in the sea water, which as food might contribute to the intoxication of the test animals; redistribution between different organs is also not to be neglected.

2.2.- Accumulation of heavy metals in the food chain

2.2.1.- Mussel-Starfish (*Mytilus edulis – Asterias rubens*)

Ch. PERPEET and M. VLOEBERGH (1973)

Figure 8.10 shows the accumulation of the radio-isotope ^{203}Hg in *Asterias rubens* fed on mussels having been 24 hours in sea water containing 5 ppb ^{203}Hg (see fig. 8.9 for the isotope distribution in the mussel tissues). Accumulation by *Asterias rubens* is particularily noticeable in the pyloric and rectal caeca.

In the case of Cu^{++} , Perpeet and Vloebergh (1972) have shown that copper is much more toxic for starfish than Hg or Pb : a concentration of 0.4 ppm Cu^{++} in sea water affects respiration and produces 100 % mortality within 5 days ; Cu^{++} is principally accumulated in the podia where it produces important tissue damage [Perpeet and Vloebergh (1973)]. Cu^{++} accumulation in pyloric caeca seems not to affect the animals.

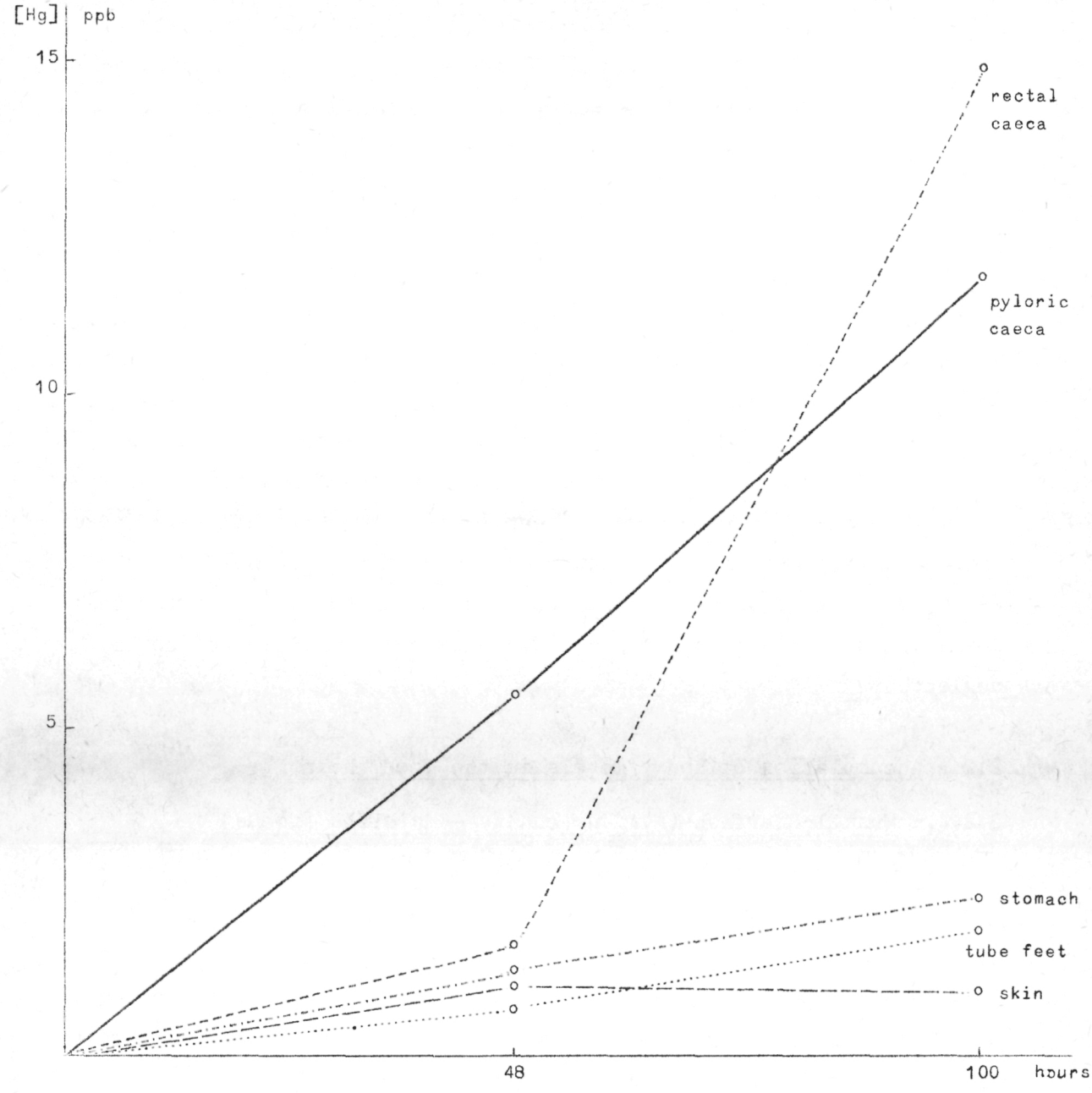

fig. 8.10.

Accumulation of ^{203}Hg in organs of Asterias rubens fed with mussels first intoxicated in presence of 5 ppb ^{203}Hg .

2.2.2.- *Dunaliella viridis* (alga) — *Artemia salina* (Entomostraca) — *Brachydanio rerio* (fresh water fish)

G. PERSOONE and G. UYTTERSPROT (1973c)

The experiments have been carried out as follows :

1) Mass culture of *Dunaliella* in 30 ℓ natural sea water sampled at 22 points of the test region (1.1 to 3.2×10^6 cells/cm^3) .

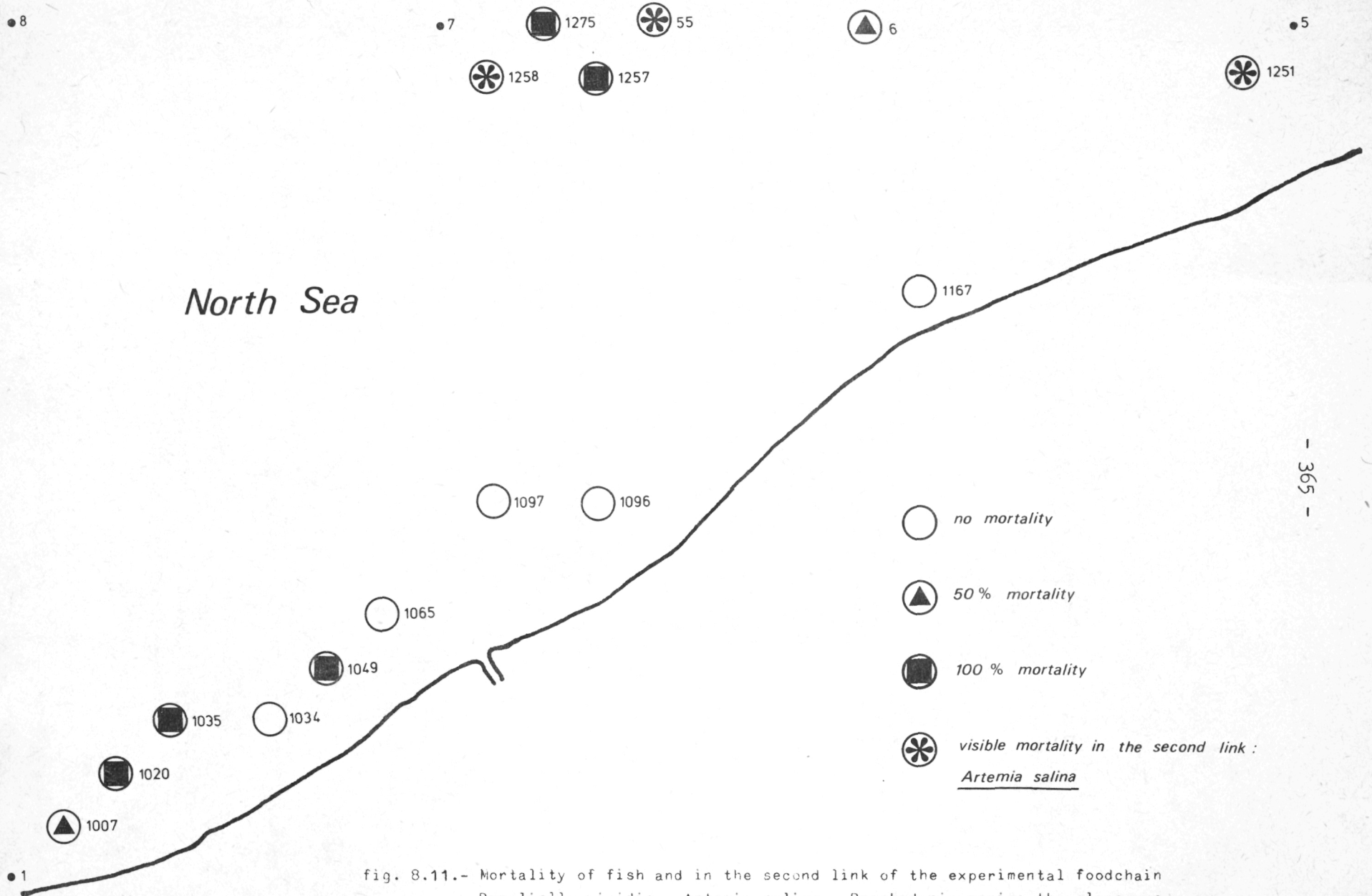

fig. 8.11.- Mortality of fish and in the second link of the experimental foodchain Dunaliella viridis - Artemia salina - Brachydanio rerio; the algae are grown on sea water sampled as indicated on the map (see fig. 8.4 for location of points 1 and 5 to 8).

2) Feeding of 100.000 *Artemia salina* larvae during 2 days with the harvested algae.

3) Feeding of two fishes for about one month with the deep frozen *Artemia* larvae.

Controls are carried out starting with artificial sea water ($1.6 \times 10^6/cm^3$ algae cells).

The results obtained are still in a very preliminary stage and only refer to the observed fish mortality as seen in figures 8.11 and 8.12 where the sites of sea water sampling are clearly indicated, if one further refers to figures 8.4, 8.5 and 8.6 to locate points 1 and 5 to 8 . No chemical data are so far available except in a few cases for the composition of the water samples, that of the algae, the artemia or the fish.

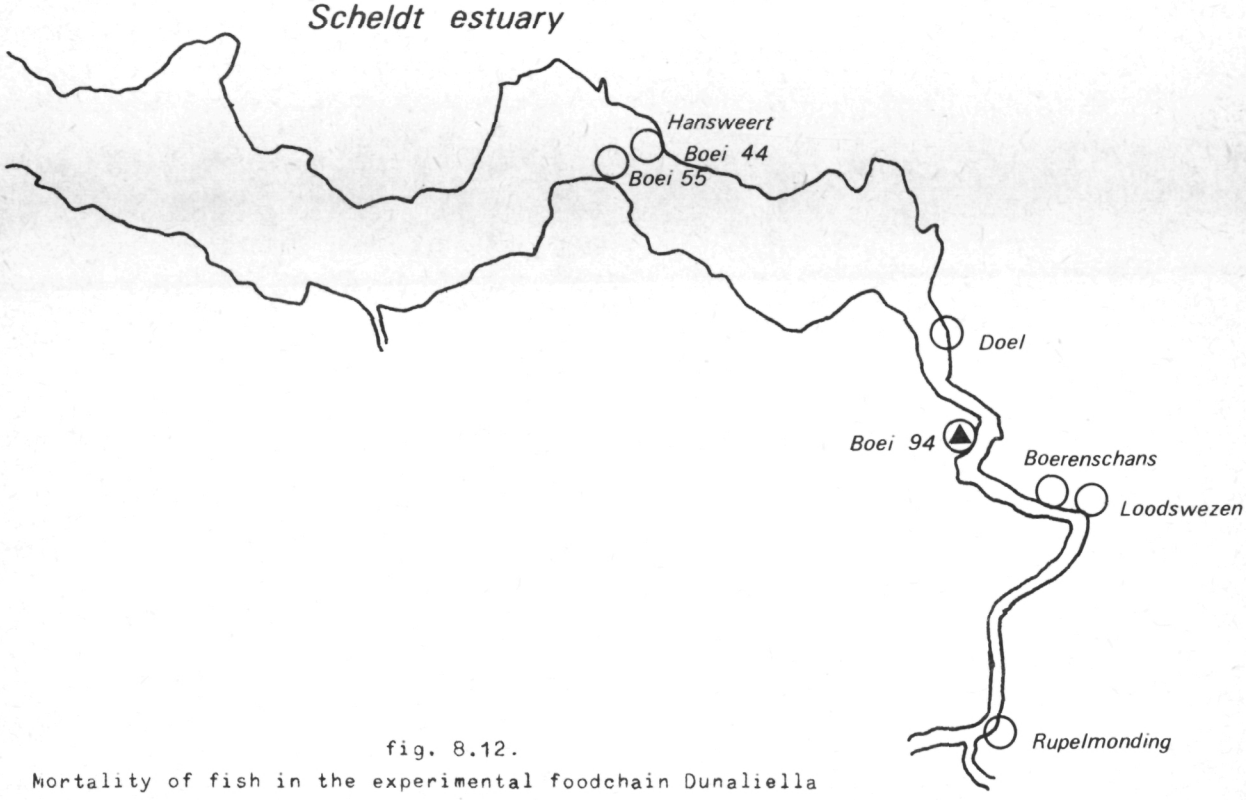

fig. 8.12.
Mortality of fish in the experimental foodchain Dunaliella viridis - Artemia salina - Brachydanio rerio; the algae are grown on water sampled in the Scheldt estuary as indicated on the map.

Generally speaking the Scheldt water samples seem less toxic than the coastal sea water samples and among these higher toxicity was found between Nieuwpoort and Oostende. High mercury level (3.5 ppm) in the algae was detected at point 1035. Water from points 6 and 7 and neighbouring sites is toxic either at the second or the third trophic level. They lie in zones where large amounts of organic and mineral waste are dumped (H_2SO_4 and $FeSO_4$ solutions from titanium industry and industrial yeast and fungi residues).

Correlation with the *in situ* measurements regarding primary and secondary production, fish and benthic fauna is by no means evident. Coastal eutrophisation, increase of biomass of resistant species, effects of predation in complex communities, food selection are factors among many others which cannot be simulated in short artificial food chains of the type used by Persoone and Uyttersprot and where extreme conditions exist.

It is felt by the author of this report that designing a bioassay as complicated as a three stage food chain to test water quality is practically hopeless : the amount of chemical analysis is enormous to trace all possible pollutants, the results remain far from representing what really happens in the true ecological system.

The fact that some laboratories are capable of maintaining experimental food chains on a large scale, is however of greatest interest because it opens large possibilities in the study, under different conditions, of rates of uptake and release of various pollutants, added one by one or simultaneously to sea water. One should however be careful in selecting the test organisms and try different ones. It is for instance important to know that Hg is lethal at some level on fresh water fish because it stops respiration, and that it is toxic for sea water fish because it acts on osmoregulation in the gills before it interferes with respiration. It would certainly be more realistic to use *Cottus scorpius* for example instead of *Brachydanio rerio* (tropical fresh water fish), as is done by Persoone and Uyttersprot.

2.3.- Accumulation and release of Hg in fish [sea water adapted eels (*Anguilla anguilla*); plaice (*Pleuronectes platessa*), dab (*Limanda limanda*); *Cottus scorpius*]

J.M. BOUQUEGNEAU

2.3.1.- Sea water adapted eels

Bouquegneau has shown (1972) that Hg accumulates in the different organs of eels exposed to natural sea water containing controlled amounts of Hg . The main site of entry (the animals normally do not feed in sea water) is located at the gills, which rapidly accumulate large amounts of Hg . At 10 ppm Hg in the sea water, lethal effects are observed within a few hours and the osmotic balance of the animal is totally upset; at 0.1 ppm , sublethal effects are noticed. Adaptation occurs : the animals maintain their osmotic balance but accumulate in the gills amounts of Hg larger than those found to be lethal when the fish is exposed to 10 ppm . Figure 8.13 shows typical mortality curves for both the lethal and sublethal effects and it is clear that fish first exposed to 0.1 ppm are more resistant when in presence of 10 ppm and CH_3HgCl is more toxic than $HgCl_2$.

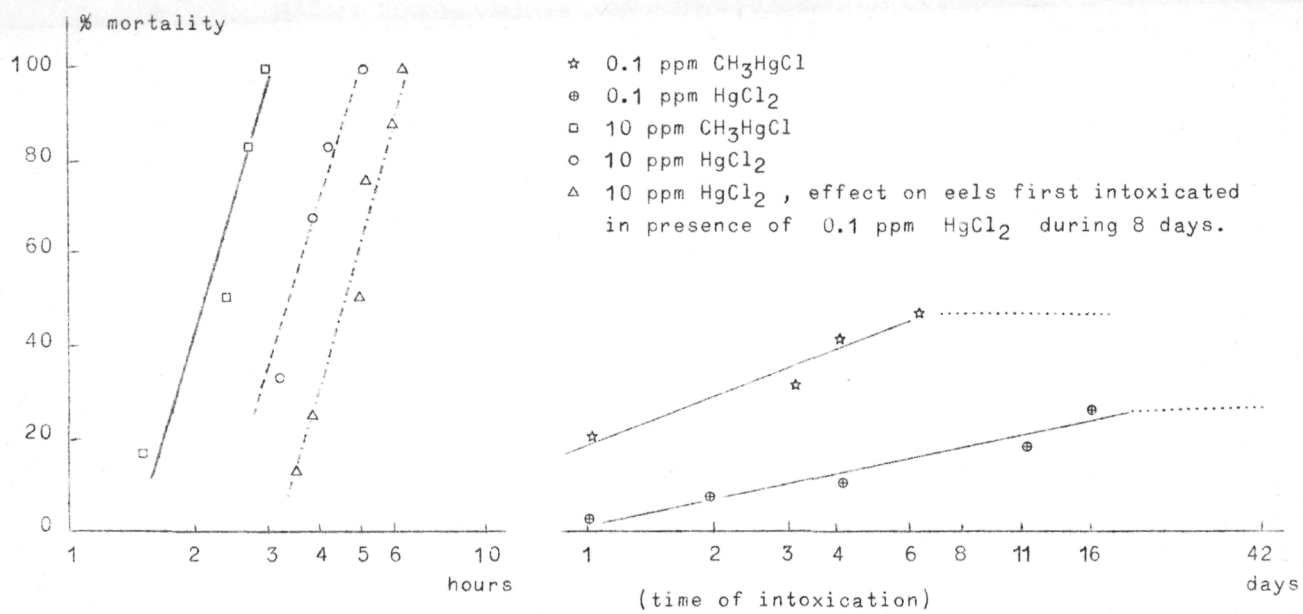

fig. 8.13.

Mortality of sea water adapted eels exposed to constant concentrations of $HgCl_2$ and CH_3HgCl in natural sea water.

The kinetics of accumulation of mercury and subsequent release in Hg free sea water has been followed in sublethal conditions at the 0.1 ppm level, either for $HgCl_2$ or CH_3HgCl . The concentration factors (ppm Hg in tissue/ppm in water) for the different organs at a given intoxication time (8 days) are listed below in the order of increasing importance :

$HgCl_2$	CH_3HgCl
Gas bladder and muscles	Gas bladder and muscles
Bile and digestive tract	Bile
	Digestive tract
Skin	
Brain	Brain
	Skin
Liver	Liver and kidney
Kidney	
Spleen	Spleen
Gills	Gills

Accumulation is faster in the gills during the first days, but after 12 days higher concentrations are found in the spleen and kidney in the case of $HgCl_2$.

Figures 8.14 to 8.18 show the curves obtained respectively for muscle, gills, kidney, liver and brain for accumulation and subsequent release.

In blood, after 8 days intoxication in presence of 0.1 ppm , the mercury is distributed as indicated in Table 8.2 :

Table 8.2

	CH_3HgCl		$HgCl_2$	
	ppm Hg	µg Hg/g blood	ppm Hg	µg Hg/g blood
Blood	31.2	31.2	2.7	2.7
Plasma	2.7	1.8	2.1	1.2
Erythrocytes	90.3	29.4	3.5	1.5

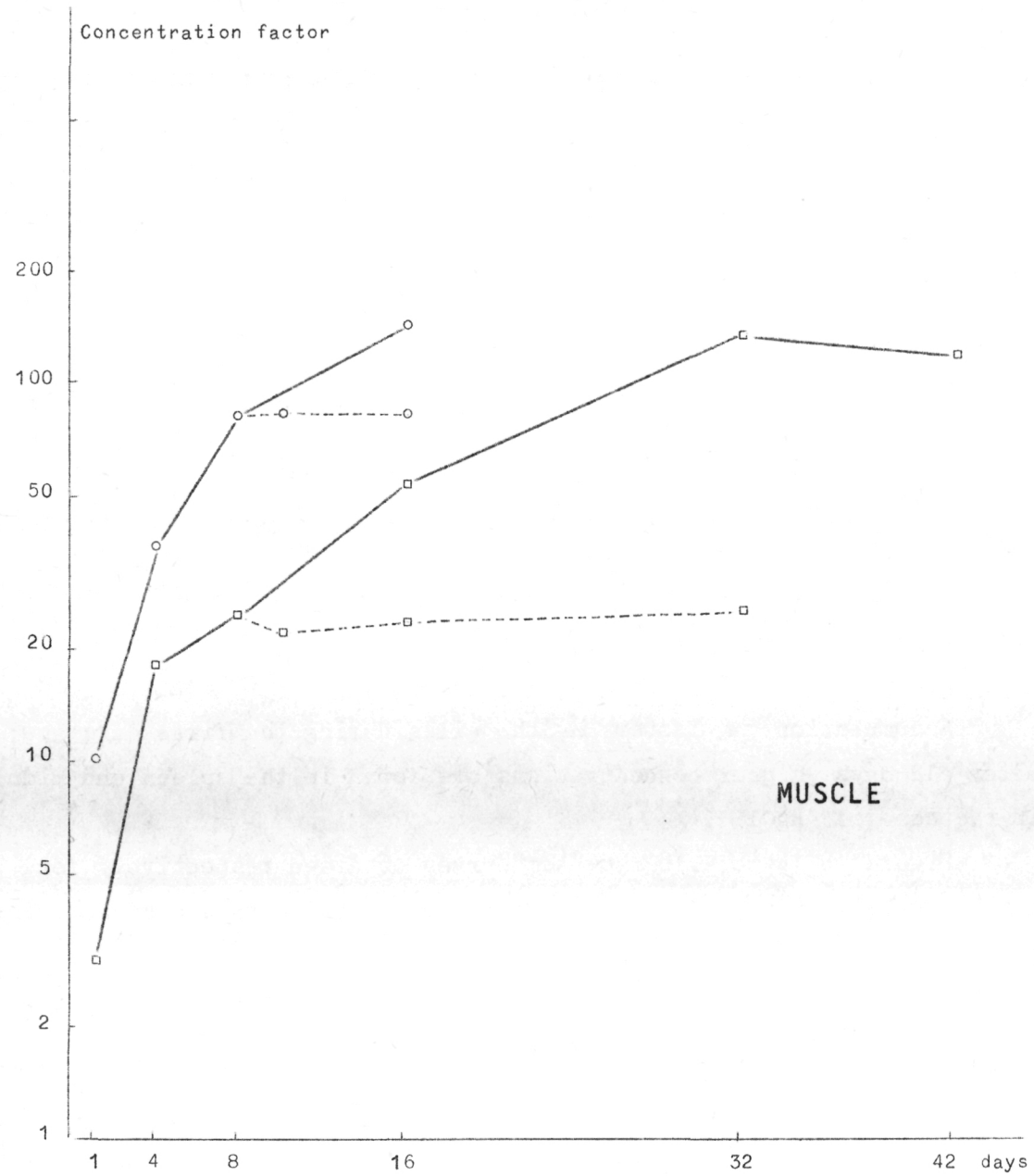

fig. 8.14.- Concentration factor in function of time ppm Hg in tissue / ppm Hg in water for mercury in eel muscles; the animals are exposed (heavy curve) to 0.1 ppm $HgCl_2$ (o) and 0.1 ppm CH_3HgCl (□) in natural sea water; dashed curves show release of Hg when the animals intoxicated during 8 days are exposed to mercury free sea water.

The difference between CH_3HgCl and $HgCl_2$ is striking : ten times more mercury is transported by blood in the case of CH_3HgCl poisoning, most of it attached to red cells. Precipitation of plasma

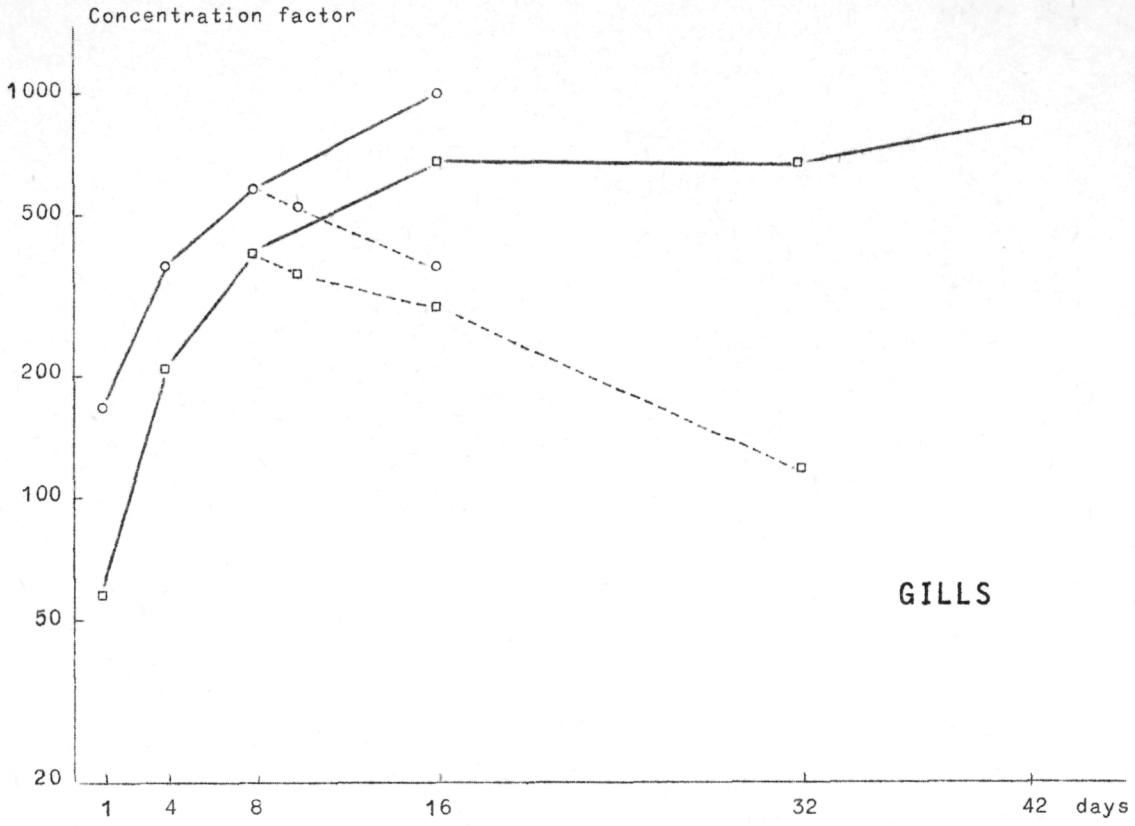

fig. 8.15.- Concentration factor for mercury in eel gills (see legend of fig. 8.14 for explanation).

proteins with trichloracetic acid shows that mercury from $HgCl_2$ stays practically attached to the precipitate and that mercury from CH_3HgCl remains almost completely in solution.

The differences observed between $HgCl_2$ and CH_3HgCl intoxication at the level of the organs and of blood suggest that, at least in first approximation, these toxic substances keep their initial chemical speciation and that probably very little undergoes metabolic changes (methylation of Hg^{++} for instance).

From the accumulation and release curves of the different organs and the weight fractions of these, total Hg-load of the animal can be calculated as a function of time. The result is given in figure 8.19. The calculations show that 87 % of the load is carried by muscle, skin and gills. It is also of interest to notice that the total load curves are mathematically much simpler than the curves for the individual organs.

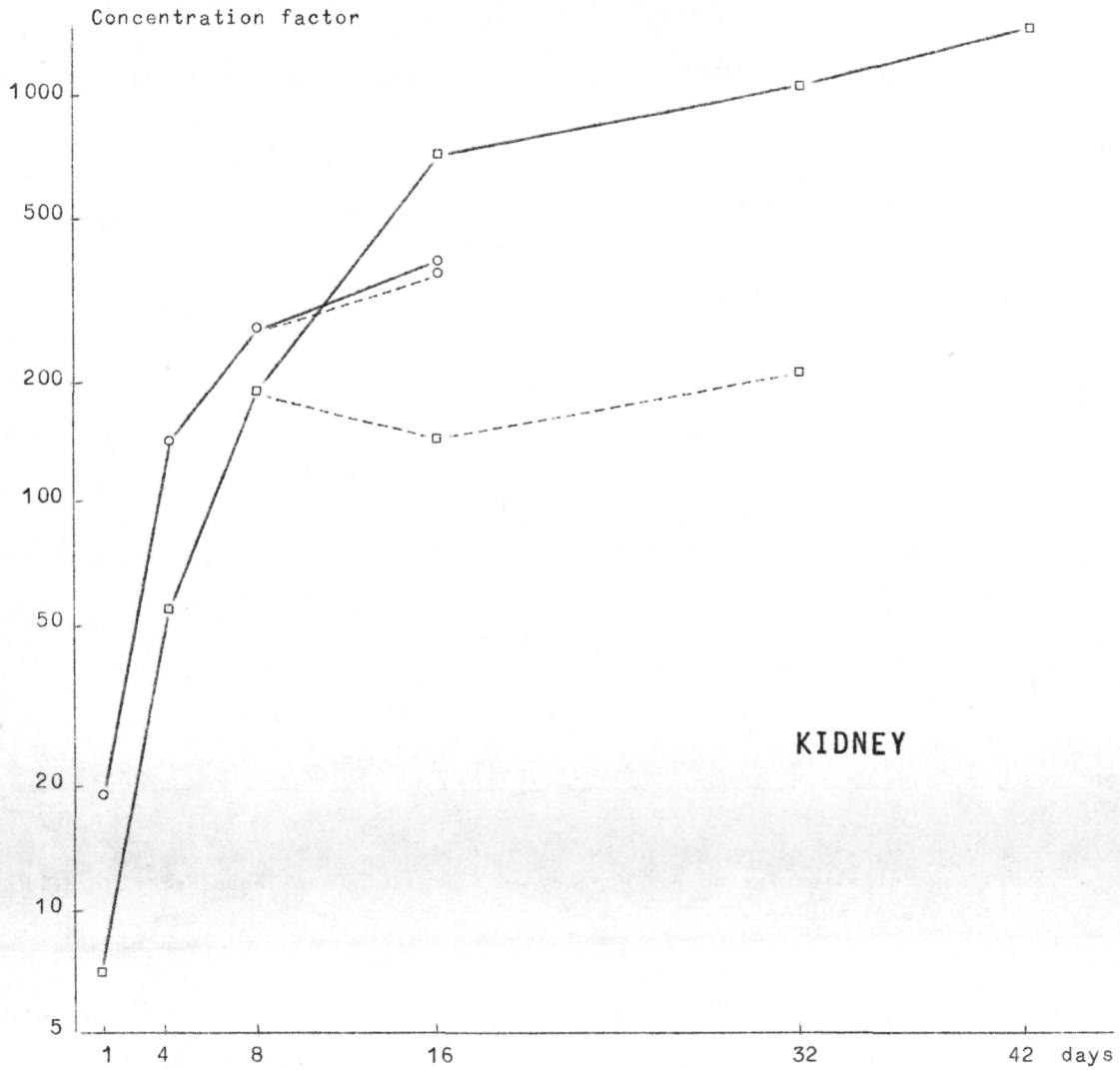

fig. 8.16.- Concentration factor for mercury in eel kidney (see legend of fig. 8.14 for explanation).

It is clear that global elimination is a slow process compared to accumulation. When one compares what happens at the level of the different organs differences do appear which allow to classify them in three categories :

1) Organs where Hg has a short half-life :
   gills, skin (in the case of $HgCl_2$)
   liver, air bladder (in the case of $HgCl_2$)
   spleen, brain (in the case of $HgCl_2$)

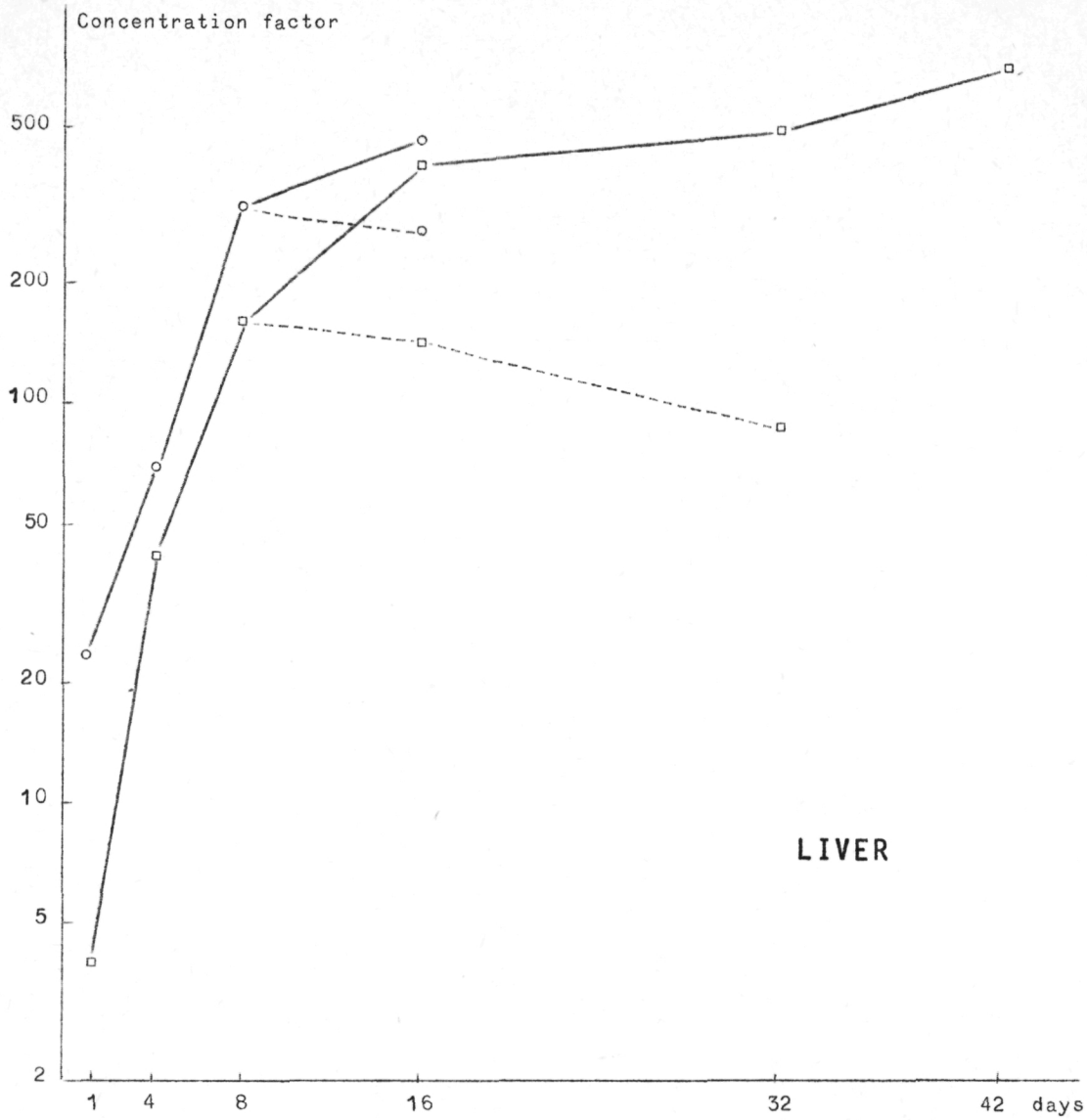

fig. 8.17.- Concentration factor for mercury in eel liver (see legend of fig. 8.14 for explanation).

2) Organs where Hg has a long half-life :

muscles

skin (in the case of CH_3HgCl)

air bladder (in the case of CH_3HgCl)

3) Organs where accumulation continues after return in the Hg-free sea water :

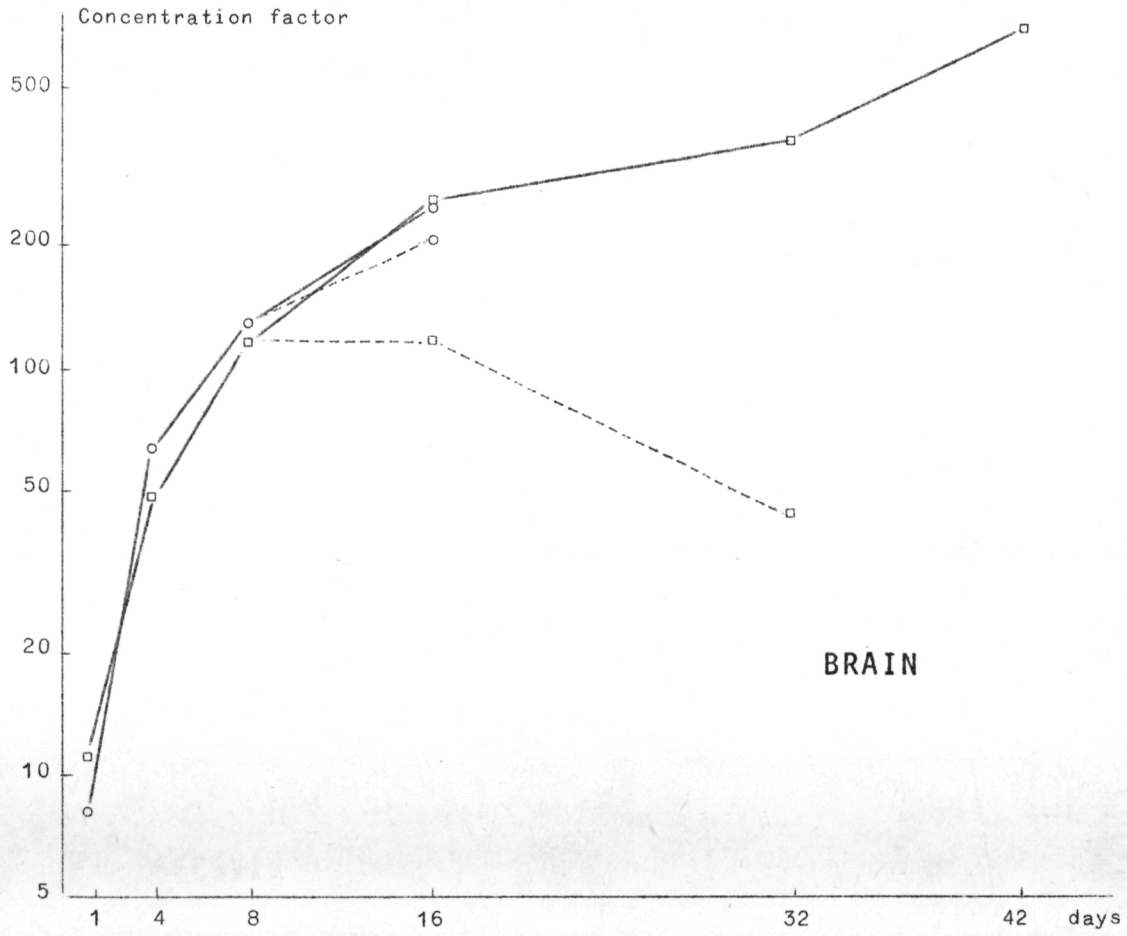

fig. 8.18.- Concentration factor for mercury in eel brain (see legend of fig. 8.14 for explanation).

digestive tract
kidney } involved in excretion
bile
brain (in the case of CH_3HgCl)

These observations fit with the results of Järvenpää, Tillander and Miettinen (1970) who injected $CH_3{}^{203}Hg^+$ in eels through the mouth and followed the elimination of the radio-isotope : 2 compartments were found one with a falf-life of 10 days , another with a half-life of 1000 days and involving 70 % of the total radioactivity. The first would correspond to the gills and liver the second to muscles according to Bouquegneau.

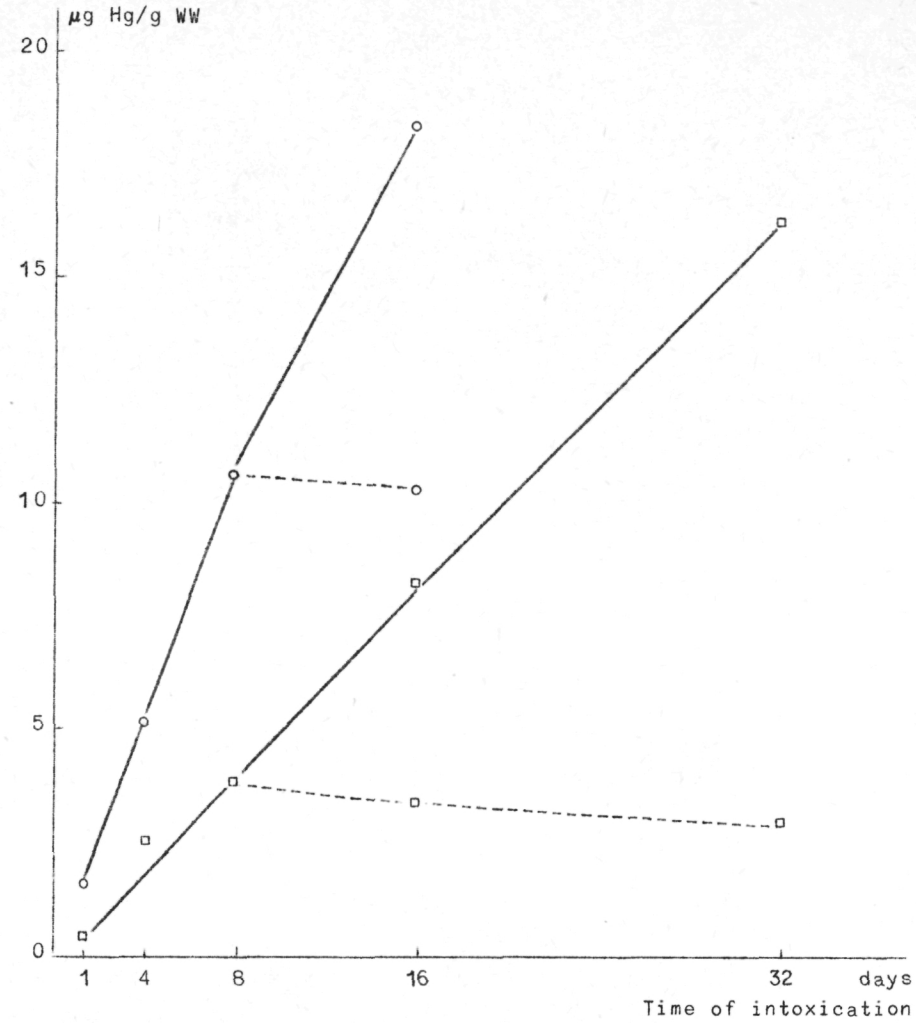

fig. 8.19.- Calculated total load of mercury (µg/g wet weight) in sea water adapted eels exposed to 0.1 ppm Hg (o $HgCl_2$, □ CH_3HgCl) as a function of time (heavy curve); dashed curve corresponds to the release of Hg when the animals intoxicated during 8 days are exposed to mercury-free water.

His findings are summarized in figure 8.20 representing a model of the different compartments and exchanges to be considered in eels intoxicated with $HgCl_2$ or CH_3HgCl at sublethal levels.

Methyl mercury is incorporated at twice the rate of $HgCl_2$. The mercury penetrates principally via the gills, but also through the skin and the digestive tract. In the case of CH_3HgCl blood red cells accumulate Hg to a great extent but free CH_3HgCl seems to be present in blood. In the case of $HgCl_2$ most of the Hg^{++} is attached to plasma proteins, and the total blood content is ten times less than in the case

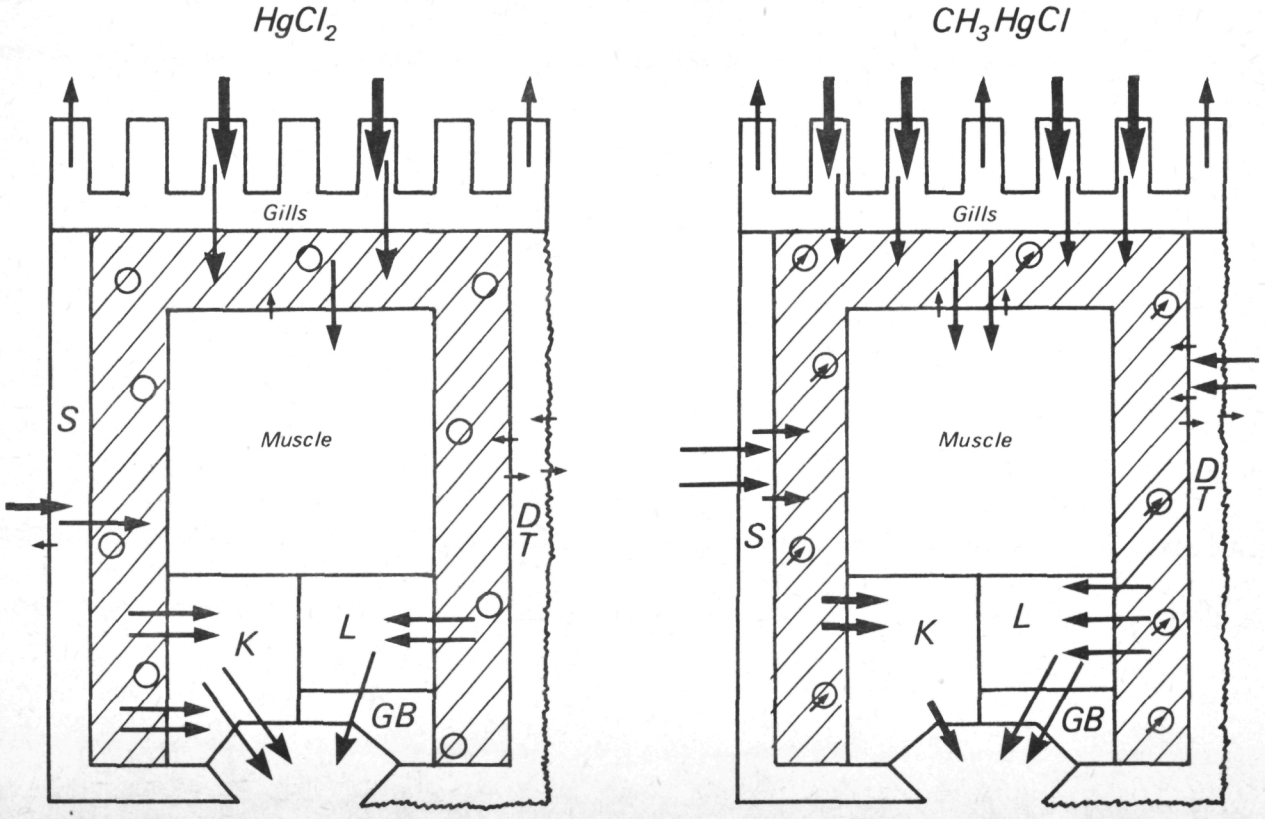

fig. 8.20.- Schematic representation of the various compartments corresponding to the organs of the eel and of the pathways leading to accumulation, release and excretion of mercury.
S = skin , K = kidney , L = liver , GB = gal bladder , DT = digestive tract , O = erythrocytes , /// = plasma ; number and size of arrows indicate approximated relative fluxes.

of CH_3HgCl . Hg is transported by blood to the different organs. Nearly 70 % of the total load is in the muscles and removal from this tissue is very slow. The kidney and the liver eliminate the mercury and the kidney is more efficient; skin and the digestive tract participate to a much lesser extent in this elimination. The continued accumulation observed in some tissues during leaching-out period in Hg-free water is obviously linked to redistribution among the various organs depending on their respective half-lives for Hg and the volume of blood circulation.

The results obtained by Bouquegneau indicate clearly that direct contamination by water containing Hg results in high accumulation

levels in eels. This is in agreement with the observations of Hannerz (1968) who estimates that in pike poisoning from water is more effective than through food.

The fact that muscle releases Hg at a much slower rate than liver and that liver accumulates faster than muscle, explains that the ratio Hg in muscle/Hg in liver will be small for fish living in highly polluted water [as observed by Cumont *et al.* (1972)] and will increase when the fish lives in less polluted regions; the value of the ratio is therefore linked to the history of the fish.

Another interesting practical conclusion is that a control of the Hg level in red blood cells might prove a useful test to distinguish between organic and inorganic mercury intoxication.

2.3.2.- Plaice, dab, cottus

Table 8.3 indicates how mercury ($HgCl_2$) is accumulated in the various organs of plaice, dab and cottus having lived 4 , 8 and 16 days in natural sea water containing 0.1 ppm $HgCl_2$. The fish received no food during the test. The results are very similar to those obtained for sea water adapted eels. The turnover however seems faster in some organs with low half-lives : after 16 days the Hg concentration in the muscle of cottus rises from 0.5 to 5 ppm for both fishes but the level in the gills are respectively ~ 40 and ~ 68 ppm in cottus and in eel.

It seems thus that the observations of Bouquegneau, made mainly on eels, are also valid for other marine teleosts, at least for the accumulation processes.

2.3.3.- Interpretation

According to Bouquegneau, Hg at lethal doses (10 ppm) inhibits the active transport of Na^+ in the gills at the level of the enzyme Na^+K^+ATPase , but interestingly enough seems to decouple the Na and K transport. No effect is observed on respiration, so that marine teleosts behave differently from fresh water species, where respiration [Lindahl and Hell (1970)] is affected.

Table 8.3

Accumulation of mercury in different organs of eels, plaices, dabs and Cottus living for 4 , 8 or 16 days in sea water containing 0.1 ppm Hg ($HgCl_2$).

	Fishes intoxicated by 0.1 ppm Hg in seawater for 4 days		
	Eel	Dab	Cottus
Gills	21.1	21.4	25.7
Kidney	5.4	38.6	13.3
Spleen	13.9	38.6	12.0
Skin	4.3	-	3.4
Muscle	1.8	2.1	2.5
Liver	4.1	11.6	7.9
Digestive tract	2.1	4.7	2.1

	Fishes intoxicated by 0.1 ppm Hg in seawater for 8 days		
	Eel	Plaice	Dab
Gills	40.4	35.0	40.1
Kidney	18.9	39.5	63.9
Spleen	27.1	22.2	47.7
Skin	6.7	3.2	-
Muscle	2.4	1.1	2.1
Liver	16.3	16.6	16.3
Digestive tract	3.4	4.8	11.1

	Fishes intoxicated by 0.1 ppm Hg in seawater for 16 days		for 24 days
	Eel	Cottus	Cottus
Gills	67.8	42.6	52.0
Kidney	70.1	38.8	54.1
Spleen	87.5	20.3	23.8
Skin	13.0	8.6	15.1
Muscle	5.4	5.1	6.6
Liver	39.6	20.7	46.3
Digestive tract	8.9	2.3	5.3

Fig. 8.21 gives the composition of gills of eels intoxicated in presence of 10 ppm Hg , regarding H_2O , K^+ and Na^+ content, for $HgCl_2$ and CH_3HgCl respectively.

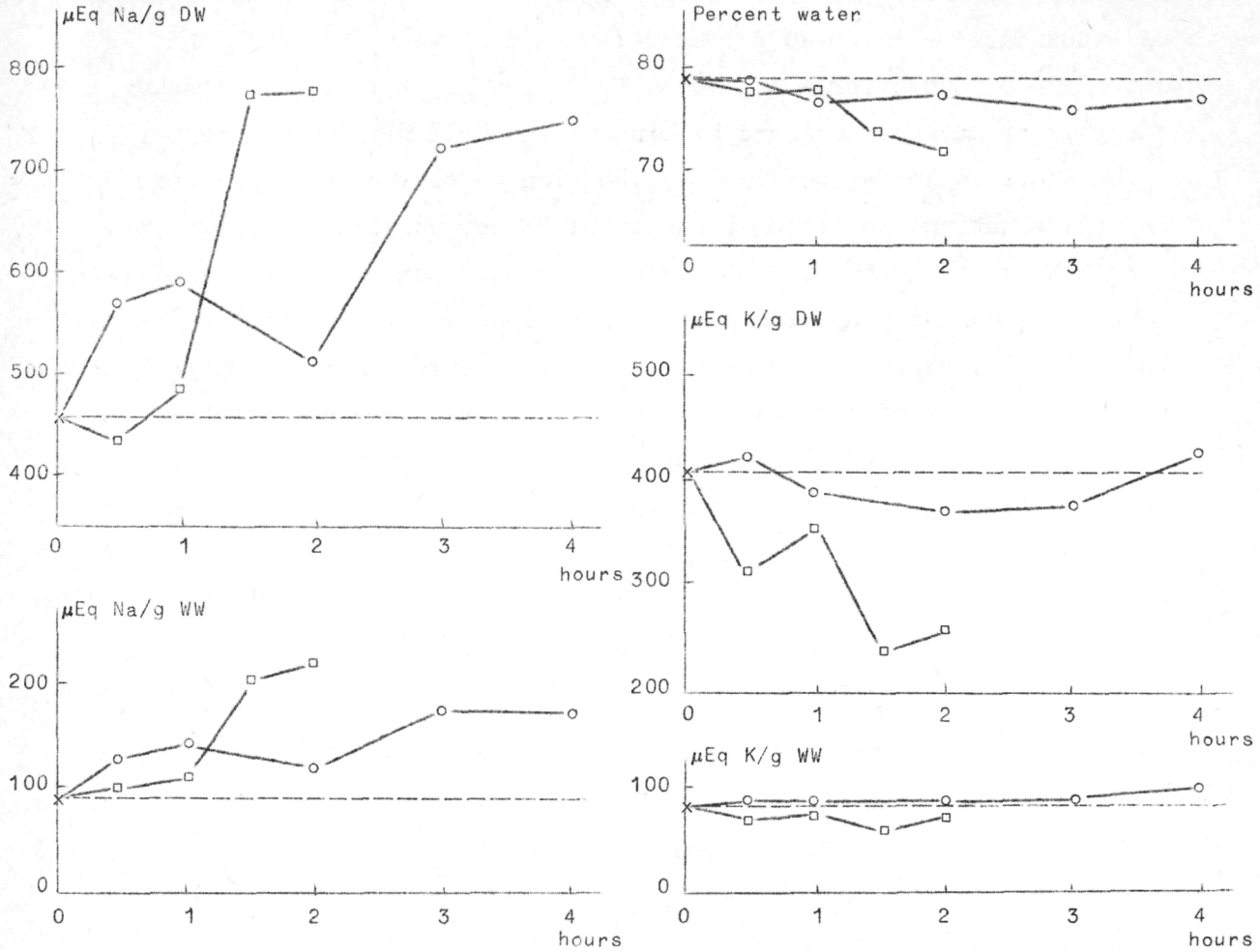

fig. 8.21.- Change in function of time of the concentration of Na^+ , K^+ and water in the gills of eels intoxicated in natural sea water in presence of 10 ppm Hg (o $HgCl_2$, □ CH_3HgCl); WW = wet weight; DW = dry weight.

In sea water containing $HgCl_2$ there is a slight loss of water, a large increase of Na^+ , the K^+ concentration remains constant, any change being explained by the water loss. When CH_3HgCl is used after 1 hour there is a slight loss of water and K^+ , an increase of Na^+ , but after 2 hours a large loss of water is observed, a fall of K^+ which is partly explained by the water movement, a further increase in Na^+ .

The osmotic balance is thus completely upset and similar results are obtained when isolated gills are exposed to mercury poisoning. This

confirms the observations made by Bouquegneau on the plasma composition of intoxicated eels in 1972. The difference between CH_3HgCl and $HgCl_2$ lies in the sudden large loss of water which might reflect necrosis of the gill tissues as observed by Lindahl and Hell with phenylmercury.

There is so far no final explanation as to the mechanism which makes adaptations possible at sublethal doses, while accumulation proceeds in the gill itself. Bouquegneau suggests that the most likely system is the building up of high concentrations of SH-bearing substances in the gills, which would compete for the mercury otherwise attracted to the active sites of the ATPase. The gills would then be protected as the heart is protected by plasma proteins, as indicated by Marcq's results on the isolated eel atrium. One might also think of a higher turnover in the biosynthesis of the enzyme itself.

3.- General conclusions

It seems to the author of this report that the contribution of physiologists within the framework of the modelling of a sea region must be more and more directed towards the understanding of the kinetics of uptake and release of pollutants, either in the food chain or because of direct water contact.

The often encountered great resistance of many marine animals and even of their isolated tissues to heavy metals and pesticides explains their potential danger for human consumption, but makes work on bioassay methods unrealistic because of the high doses to be used to observe some effects. It seems too that water quality tests based on the sensitivity of some organism of even experimental food chains are in the realm of utopia, and that the efforts to the experimenters will be better repaid by turning their full attention to the dynamics of accumulation and release processes.

It might turn out from these laboratory observations that substances like Hg for example show such a high affinity for SH-groups that in oxic basins there is nowhere to go for these toxic substances than to stay in the food web. To try and find out the fate of such

poisons, their rate of accumulation, their transit time in the food chain, their eventual removal in particular environmental conditions is of the greatest importance for the study of pollution and its possible regression.

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