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REPORT ON THE INTERDISCIPLINARY RESEARCH PROJECT "FISH DISEASES IN THE WADDEN SEA"

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ABSTRACT

The 3-year research project "Fish diseases in the Wadden Sea" (1988-1990) was initiated by the Federal Environmental Agency (Umweltbundesamt), and by the coastal states of the Federal Republic of Germany, Schleswig-Holstein (Nationalparkamt) and Niedersachsen. The interdisciplinary research program in the German Wadden Sea and in the adjacent estuaries (Elder, Elbe, Weser, Ems) intends to link biological, biochemical and chemical approaches of environmental research. The aim of the project is to test the response of different biological techniques to environmental stressors and their practicability in order to work out guidelines for the implementation of a biological effect-monitoring in near-shore waters. The project comprises 6 scientific subprojects involving several research institutions (Bundesgesundheitsamt Berlin, Institut für Meereskunde Kiel, Bundesforschungsanstalt für Fischerei, Hamburg/Cuxhaven, Biologische Anstalt Helgoland Hamburg). One of the main biological approaches is the documentation of external dieseases, liver pathology and biochemistry in flounder and their regional and seasonal fluctuations in response to natural and anthropogenic stressors. By interlinking the biological parameters with each other- and to the chemical data of tissue analysis, it is tested whether a complex of corresponding cellular and biochemical phenomena is suitable to reflect sublethal biological effects of contamination in the Wadden Sea. First results on the regional and seasonal variation of the parameters measured will be presented. All data obtained in this study are computed in JMG-format and will be available for ICES.

INTRODUCTION

The 3-year research project "Fish diseases in the Wadden Sea" started in December 1987. The project was initiated by the Federal Environmental Agency (Umweltbundesamt), and by the coastal states of the Federal Republic of Germany, Schleswig-Holstein (Nationalparkamt) and Niedersachsen. The interdisciplinary research program in the German Wadden Sea and in the adjacent estuaries (Elder, Elbe, Weser, Ems) intends to link different approaches of environmental research. It consists of 6 scientific subprojects involving several research institutions, and is coordinated by the Biologische Anstalt Helgoland, Hamburg.

The interdisciplinary investigations concentrate on the simultaneous measurements of putatively anthropogenic stress responses at the different levels of the biological organisation (Fig 1) with the aim to test the sensitivity and practicability of the methods applied. Further aims of the projects are the documentation of external and internal diseases in fish and their regional and seasonal fluctuations in response to natural and anthropogenic stressors. Epidemiological investigations of externally visible fish diseases are carried out in one of the subprojects.Two subprojects deal with the fish liver as the central metabolic and detoxification organ by examination on the organ, cellular and biochemical level. These investigations of sublethal and degenerative changes are carried out on identical organs and include gross and microscopic morphology, cytochemistry, biochemistry. Investigations on the reproductive success of the fish are carried out in another subproject. In two subprojects the concentrations of organic and inorganic contaminants of liver and muscle are determined. Indicator organism for these investigations is the flatfish *Platichthys flesus* (flounder). For epidemiological investigations other fish species are included. All data obtained in this study will be computed in JMG-format. Within the project the question should be answered whether a certain complex of corresponding phenomena is suitable to detect biological effects of contamination in the Wadden Sea and can be consequently used for biological monitoring. The main aim of the project is to work out guidelines for the implementation of an effect-monitoring in near-shore waters.

Subproject 1: Epidemiological and histopathological investigations

(H Möller and K. Anders, Institut für Meereskunde,
Universität Kiel, Düsternbrooker Weg 20, 2300 Kiel, FRG)

Aims of the project are:

(1) Documentation of the varieties of externally visible disease types in fish from the Wadden Sea,

(2) farest possible identification of desease origins,

(3) sampling of flounders for other subprojects.

The sampling of fish material for this subproject was mainly done with shrimp trawlers, using commercial gear. Samples were taken monthly in the estuaries of the Elbe (stats.1-5) and Eider (stats.11-14), as well as every three months in the estuaries of Ems (stats.51-55) and Weser (stats.31-35), and on transects from the coast towards the open sea starting from Büsum (stats.15-19,Meldorfer Bucht), Husum (stats.41-45) and Dagebüll (stats.61-64) (Fig.2).

With the exception of clupeids and small fish below 10cm length, the occurrence of all fish in the samples was quantified. Altogether,more than 127,000 specimens have been examined for diseases (Tab.1). So far,22 different Types of diseases have been recorded,which will be described in an "Atlas of fish diseases from the Wadden Sea".This atlas will serve as a help for disease identification,including relevant information on disease etiology,distribution,seasonality,enviromental relations and pathogenicity.

Disease types which are considered both common and conspicuous, were found in flounder, smelt, dab, cod,whiting and eel. "Common" in this respect are diseases which are found in at least 1% of fish older than one year on average of all samples. As the prevalence of most diseases is related to season and region as well as to the size/age of fish, final statements still need the standardisation of true data. However, some trends are evident already now:

(1) The ulcerative "yellow pest" of young cod, first reported during this survey, mainly was found in the estuaries of Weser, Elbe and Ems and preferably occured from January to March. In some hauls, more than 15% of the cod were found to be affected. The disease is obviously lethal. Flexibacter and Pseudomonas bacteria as well as fungi have been demonstrated in and partly isolated from the wounds, however, the cause of the disease still remains is not yet clear.

(2) Viruses and bacteria are known or supposed to be the causative agents of lymphocystis disease, fin rot and skin ulcer of flounder.Relatively high disease prevalences were found on stations with relatively low average salinities and high tidal fluctuations in salinity (Tab. 2). Total disease prevalences above 10% were recorded from the innermost stations on the Eider Rivers (Fig. 3).

(3) The occurrence of buccal granulomatosis of smelt as well shows distinct regional fluctuations.Highest prevalences and largest granuloms were found in smelt from the Elbe estuary.

Skeletal deformities and deformities of the gill covers in cod and smelt, skin lesions in dab, spawning papillomatosis in smelt and infections with the copepod *Lernaeocera branchialis* in whiting and cod are other common diseases, but so far their dynamics have not yet been evaluated finally. The same is true for a number of newly found tumour-like diseases in different fish species from the Wadden Sea.

By affiliating serveral doctor and master theses and by involving guest scientists, additional topics could be studied, such as the age distribution and growth of flounder, the virology of smelt diseases, the bacteriology of flounder diseases, the parasites occurring in the flesh of smelt and sea scorpion, and various biological aspects of sand goby, hooknose, and eelpout.

Subproject 2 Investigations on the fish liver as central metabolic a detoxicating organ for the biological effect-monitoring

(A. Köhler-Günther/ M. Hoppenheit, Biologische Anstalt Helgoland, Notkestrasse 31, 2000 Hamburg 52, FRG)

The aim of subproject 2 is to develop and to test fast and practicable cytochemical techniques which are able to reflect sublethal and degenerative changes of the flounder liver for the biological effect-monitoring. Methods include the measurements of lysosomal stability, size and number (cytochemistry) and parallel gross and microscopic liver pathology (LM/TEM) in order to affirm the assertions of the lysosomal tests.

First responses to environmental stressors are detectable at the cellular level of the central metabolic organs. These are linked to the induction of detoxifying cellular mechanisms, such as an activation of the intracellular digestive system (lysosomal system) or detoxifying enzymes (MFOs, subproject 3). The acticity of detoxifying systems, measured by biochemical and cytochemical techniques is reflected by the ultrastructure of cell components as sites of enzyme syntheses.

Damage of the liver detoxifying capacity lead to severe liver lesions ranging from reversible alterations (stage 1,2), to degenerative and preneoplastic changes of single cells (stage 3) and parts of liver tissue (stage 4-6) and benign and malignant neoplasms (stage 7 and 8) (Table 3). These pathological phenomena, which affect the whole organism during their progression can be clearly identified by more sophisticated techniques of cell and tissue pathology. Parallel ultrastructural findings in fish livers from field and experimental studies evidenced that the lysosomal detoxifying and digestive system play a key role in the response of the fish liver to different contamination situations. The lysosomal system (Lysy) accumulates and sequesters a wide range of anthropogenic substances, e.g. heavy metals, as well as PAHs, organochlorines or asbestos. Every eucaryotic cell possess a lysosomal system, representing the unspecific immune system of the cell. Damage to the lysosomal membrane or overloading of the storage capacity by toxic compounds leads to increased fragility of the lysosomal membrane with subsequent release of degrading enzymes to the cyto-and nucleoplasm leading to cell damage and death. Therefore, disturbances of the lysosomal integrity represent an integrative parameter of the toxic effects of different classes of contaminants.

The cytochemical/biochemical methods tested for the biological effects monitoring included the measurement of lysosomal perturbations (number, size, membrane stability), the accumulation of unsaturated neutrallipids and parallel gross and microscopic liver pathology (LM/TEM). Lysosomal size and number was morphometrically measured with a MOP Kontron Videoplan and the numerical and volume density calculated. Recently measurements of the total enzyme activity of liver cell lysosomes are carried out by photomicrodenistimetry (Zeiss).The lysosomal stability test, established in mammalian research and already applied for measuring stress responses in marine invertebrates, was transferred to the fish liver (flounder, dab, plaice) in the laboratory of the Biologische Anstalt Helgoland.

Samples are taken monthly in the estuaries of the Elbe and Elder and every 3 month over the whole Wadden Sea area with the research vessel Uthörn (Biologische Anstalt Helgoland) or a shrimp cutter; for further sampling strategies see subprojects 1 and 3. Only female flounders without externally visible diseases are examined.

A Two-Step Response of the lysosomal system in flounder liver could be identified during this study. In normal liver cells only few lysosomes can be identified involved in autophagic processes during normal turnover of cell components. The first step observed consisted of an increase in number and size of the lysosomes representing the activation of the detoxification processes as an adaptive response to an sublethal stimulus. Therefore, the measurements of the activation of the lysosomal detoxifying system is comparable to those of the mixed function oxidative system.

The second step consisted of alterations of the membrane properties of lysosomes by the accumulated substances, representing an overloading of the storage capacity or direct damage to the lysosomal membrane by different groups of foreign substances The damage of the lysosomal detoxifying system was measured by the lysosomal stability test

In flounder with "healthy" livers, which could be caught at the stations 61 and 15, a few small lysosomes considerable membrane stability of up to 40 minutes in single individuals (Fig 4 a,b). No pathological alterations could be identified in these livers. In contrast, liver cell lysosomes of flounder caught in the Elbe estuary for example drastically increased in number and size. There was a considerable accumulation of pigmented material (melanin, lipofuscin), injured cell components and unsaturated neutral lipids (Fig.4 b,c). A considerable accumulation of pathological lipids could be noted during the feeding perid between May and September (Fig.5)

A highly significant negative correlation ($r = -0.63$ to $r = -0.80$, $n=25$) was found between the enlargement of lysosomes and the number of lysosomes at Stations 03 and 15 (Fig.1 a,b), indicating that new lysosomal formations were retarded and that lysosomes displayed an increased rate of fusion. Also, highly significant but positive correlations were calculated for lysosomal size and for pathological accumulation of unsaturated neutral lipids ($r = +0.68$, $n=25$), confirming our anticipation that lipid accumulation was intralysosomal.

Highly significant correlations were found between the lysosomal membrane stability and the degree of histopathological liver lesions (Fig. 6,7) ranging from normal and minor reversible changes (stages 1 and 2) to necrosis, fibrosis, cytoskeletal changes, megalocytic hepatosis, caryomegaly, lipid accumulation (stages 3 and 4) to heavy staetosis, fat necrosis and cirrhosis (stage 5) and eosinophilic, clear cell and basophilic foci (stage 6),(Table 3).

According to our preliminary estimations healthy flounder livers showed a membrane stability ranging from 28 up to 45 minutes during the feeding period from may to october. In slightly affected livers the mean labilisation period varied from 15 -18 minutes. Liver lesions of a higher degree are accompanied by drastically reduced mean labilisation periods (2 -5 min.).

The results of the livers of flounder caught from the mouth of the Elbe estuary along the northern Wadden Sea coast and the Eider estuary in May 1989 showed striking differences (Fig 5). In the Elbe estuary, the early breakdown of the lysosomal membranes of flounder liver cells reflected by short labilisation times indicated serious damage of the lysosomal system In contrast, in the Wadden Sea (St. 15) the labilisation periods show a significantly higher lysosomal stability indicating the unabated function of the lysosomal detoxifying system. Also in the Weser estuary which is chracterised by extremely high level of Pb and Cd (subproject 5), a dramatic impairment of the adaptive detoxifying liver function could be noted and an influence towards the eastfrisian coast. Completely in contrast to the preliminary expectations are the findings at the station near the Island of Amrum in the northern Wadden Sea area (St 41). The dramatic findings with respect to liver damage at the cytochemical/biochemical and morphological level do not coincide with the levels of selected contaminant measured during this study. This event shows that fast applicable techniques of the biological effects monitoring could be also useful as a screening tool for detection of new " areas of special concern". The considerable value of the test battery for lysosomal perturbations in fish liver is the fact that activation and damage as well of a basic detoxification system responding to different classes of pollutants can be measured. At present the lysosomal tests can be applied to 1500 individuals/ 1 man year (consumables of 80 DM/50 animals) with costs of equipment present in every histological lab (e.g.60.000 DM).

The first results of linking the data of the lysosomal stability test to the findings observed in tissue pathology indicated that the integrity of the lysosomal membrane in fish liver sensitively reflect the breakdown of the adaptive detoxifying capacity of liver cells leading to damage of cell function and progression towards preneoplastic and neoplastic transformation.

Subproject 3: Investigations on the biotransformation (detoxication activity of mixed functi on oxidases, MFOs) in the fish liver

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For determination of detoxication activity (biotransformation) in the fish liver the activities of mixed function oxidases (MFOs) are meas- ured. 7-Ethoxyresorufin, 7-Pentoxyresoru- fin and 7-Ethoxycoumarin are used as substrates. Usually female flounders of 18 - 25cm total length are examined.

Seasonal sampling

Since February 1988 samples have been taken monthly at two stations in the Wadden Sea (within the Elbe estuary and a reference site, Eider estuary). The livers of 25 flounders ta- ken from each sample are dissected into several parts and serve not only for measurement of MFOs, but also for measurement of heavy metals (subproject 5) and of organochlorine com- pounds (subproject 6). In the first months only pool-samples were used. Since September 1988 usually the MFO-activities of single livers have been determined in pool-samples, too.

Regional sampling

Every third month samples are taken at stations in a rough grid (see subprojekt 2). Addi- tionally, at these times one part of each liver serves for histopathological investigations. Furthermore comparisons between pool- and individual samples of flounders of different length-classes are carried out.

Once a year sampling is done over the whole area (42 sites). The livers of all flounders examined are worked on individually, and values of pool samples are calculated. The dissecti- on of the samples is done as explained above.

Some preliminary results are the following:

Generally

- The strength of MFO-activities seems to be dependent on the length/ age of the fish, bigger flounders showing relatively higher activities.
- Males show higher MFO-activities than females of the same length, at least temporarily.
- MFO-activities of juveniles are comparable or higher than those of males.
- During the spawning season the MFO-system of female flounders is diminished. Just before spawning there is nearly a complete breakdown, but afterwards it recovers relatively quickly to its former level.

Seasonal samples

- During the 23 months of investigation, the MFO-activities were higher in the Elbe estuary than at the reference site (Fig. 5).

Regional samples

- In 1988 the highest activities were found in the Elbe estuary, relatively low activities in front of the East Frisian Islands, in front of Amrum and southwest Pellworm, that means at the offshore stations Striking differences in MFO-activities exist in the estuaries of Weser and Ems in comparison to those of the Elbe. In the Elbe estuary the MFO-activities decrease with increasing distance from the shore (Fig. 6). It is not like that in Weser and Ems. In the Weser estuary MFO-activities first increase and than slightly decrease only at the outermost station. The Ems is considered to show a similar pattern, although no samples could be taken at the furthest stations.

- In 1989 the results from the regional samples gave a similar picture except on nearshore sampling site close Husum (sampling 41). In spring as well as in autumn relatively high induction were found at that site. Data of histopathological investigations from the same individual fish livers fit into these results. At moment an interliking procedure with the data of concentrations of heavy metals and organochlorine compounds from the same fishes is carried out.

Laboratory experiments

- Laboratory experiments were performed to test the influence of salinity on MFO-activities. ß-Naphtoflavone-treated fish and control groups were used in these tests No salinity-dependent differences in MFO-activities could be traced (Fig. 7a, b).

As toxic injury of the organisms measured at the biochemical and cellular level tend to be more contaminant specific, clear signals are achieved already with relatively low numbers of individuals investigated, as became evident from the results of subprojects 2 and 3.

Subproject 4 Investigations on the reproductive success of flounders from the Wadden Sea

(P. Cameron/ H. v. Westernhagen, Biologische Anstalt Helgoland, Notkestrasse 31, 2000 Hamburg 52, FRG)

In subproject 4 "Investigations on the reproductive capacity of flounders from the Wadden Sea" (P. Cameron, Biologische Anstalt Helgoland) experiments with 78 pairs of flounder (1989: 41 pairs; 1990: 37 pairs) were carried out in order to determine the reproductive success in correlation to the contamination of the parental fish with organochlorines Mature flounders were caught north-west of Helgoland, eggs and sperm were stripped from running ripe parental fish and mixed for fertilization. Incubation was carried out, dead eggs were removed and recorded daily until hatching of larvae. And the end of each experiment the number of normal, curved and severe malformed larvae was determined. Gonad tissue was cut from female parental fish and contamination with organochlorines was analyzed by gaschromatographie. In the followin, first results from the experiments with flounders caught in 1989 will be shown. Viable hatch ranged from 16% to 96%, with a mean value of 64.4%, the distribution of the hatching success was shifted to the lower ranges. The same pattern is known from former similar experiments and may serve as an indication of impaired reproduction caused by pollutant During embryonic development only a few embryos were dying, resulting in a high mean value for total hatch of 87.9%, while a lot of curved, non-viable individuals were found among the hatched larvae, therefore reducing viable hatch. Highest residue levels were determine for PCBs (CB 138, 153 > CB 118, 101 > CB 180 > CB 170 > CB 52, 28),followed by DDE and dieldrin; high contamination was also found for endosulfansulfat, DDD, HCB, DCT and lindane. PCB-content (as Clophen A60) ranged from 532 to 12700 µg/kg fat, with a mean value of 252 µg/kg fat. DDE occurred up to 926 µg/kg fat, the mean value was 144 µg/kg fat, the mean for dieldrin was 126 µg/kg fat, values reaching from 13 to 1004 µg/kg fat. Mean contamination by all the other substances lay below 50 µg/kg fat.

When trying to correlate tissue contamination with hatching success, there is a significant negative correlation to be found between ovary burden of PCBs (as Clophen A60), HCB, as well as alpha- and gamma-HCH and total hatch. In addition it can be observed, that for PCBs as well as DDE, extremely high residue levels reduce viable hatch to under 50%, where only one quarter of all values can be found. Because there is only a relatively low number of analyzed fish until now, only one flounder could be found to be extremely high contaminated. The fact that a large number of low hatch events cannot be explained by simultaneously occuring high concentrations of the analyzed contaminants suggests that an additional array of substances may be responsible for the impairment of reproduction. The same pattern was found in similar experiments with other fish species.

Subproject 5: Investigations on contamination with heavy metals

(U. Harms, Bundesforschungsanstalt für Fischerei,
Wüstland 2, 2000 Hamburg 55)

The developing concern about the Wadden Sea has also increased interest in the occurrence and circulation of inorganic contaminants that could have biological effects.

In this context, potentially toxic heavy metals such as cadmium, lead, mercury, copper, and zinc require predominant consideration. They pose a constant threat to natural living resources, since they are introduced through various sources into the coastal zones and are accumulated by organisms living there. Admittedly, copper and zinc are essentials to life, since they are components of enzymes, proteins and other substances which maintain or regulate vital functions (elements with nutritional significance). However, they may become hazardous, if they reach the marine environment in substantial amounts via rivers, direct discharges and atmospheric deposition, thereby exceeding suitable concentration levels.

Lead, cadmium and mercury have no known necessary functions in living organisms. They can cause harmful effects in fauna and flora even if they are present in comparatively small quantities

As a contribution to the interdisciplinary research project the aforemented elements are analysed in relevant sub-samples collected according to the sampling scheme outlined above (see subproject 3).

Mercury is determined in muscle tissue by cold vapour atomic absorption spectrometry. Cadmium, lead, copper and zinc are determined in liver-subsamples, using flame (Cu and Zn) respectively graphite furnace (Cd and Pb) atomic absorption spectrometry after appropriate sample pretreatment. Control to confirm accuracy of analytical results consist of intralaboratory tests using certified reference materials, the checking of analytical performance using radiotracers, and participation in intercomparison exercises.

Analytical results gained so far indicate element-specific differences in concentration levels between the different subareas investigated. Samples from the estuaries of the rivers Weser and Ems reveal elevated cadmium concentrations. The same is true for the Wadden Sea between these two estuaries. The mercury data obtained are drastically enhanced within the estuarine system of the river Elbe with a clear concentration gradient in northernly direction along the North Frisian Wadden Sea.

It seems that the observed structured distribution of inorganic contaminants analysed is to a large extent influenced both by the inflow of waters from the aforementioned rivers and by the prevailing residual currents in the German Bight. Apart from the significance of hydrographic conditions, an explanation for the spatial changes in the contaminant pattern may be found in specific differences in the mobility of each single element in the area under study.

Subproject 6: Investigations on organohalogen contamination

(K. Söffker, Bundesforschungsanstalt für Fischerei, Außenstelle Cuxhaven, Niedersachsenstraße, 2190 Cuxhaven)

In subproject 6, the contamination of liver and muscle tissue of the flounder from the area of the German Wadden Sea with organochlorine compounds like polychlorinated biphenyls (PCBs), DDT and its metabolites DDE and DDD (DDTs), hexachlorocyclohexanes (HCHs, e.g. lindane), hexachlorobenzene (HCB), pentachlorobenzene (PeCB) and octachloro-styrene (OCS) is investigated. The flounders used are normally female and they belong to the length class 17-25cm, which corresponds to an age of about two years. The dissected livers are shared as outlined above; muscle tissue is shared between subproject 5 and 6.

The following points of emphasis are under investigation:

1. Analysis of individuals - histopathological changes of the liver

In this section, the pollutant concentrations in the fish will have to be correlated with pathological changes of the flounder liver, the lysosomal stability and the MFO activity. This is to be done in cooperation with the subprojects 2, 3 and 5. Yet two sampling campaigns have been carried out, in July and August 1988. In July the stations were situated in the Elbe estuary and in the Wadden Sea in front of the Eider estuary, while in August the Elbe estuary and a station southwest of the isle Nordstrand were fished.

2. Seasonal samples

Since February 1988 monthly sampling has been carried out at two stations, one in the Elbe and one in the Eider estuary, to investigate the seasonal variability of organochlorine compound concentrations in the flounder over a time period of two years. The flounders from the Elbe area are twice as highly contaminated as those from the Wadden Sea near the Eider estuary (Fig. 7). The concentration patterns are approximately the same in both areas.

During the course of the year, differences in the behaviour of certain pollutant groups were detected in liver and muscle of flounders from both areas. While the concentrations of the hexachlorocyclohexanes were determined by the seasonal changes of the tissue fat contents, the concentrations of hexachlorobenzene, octachlorostyrene, and DDTs were in contrast only low at the end of the year in spite of high fat contents.

3. Regional samples

To investigate regional differences of contamination with organochlo- rine compounds in fish from the german part of the Wadden Sea, flounders were sampled at 42 stations in the summers of two consecutive years (1988 and 1989). First results of the campaign in September 1988 are available. The flounders from Ems, Weser and Elbe were approximately equally contaminated with PCBs, while the concentrations of DDT and especially of its metabolites DDD and DDE increased from the Ems over the Weser to the Elbe. Similar gradients were also detected in the other organochlorine compounds (HCB, OCS, HCHs). Going from the Elbe to the North Frisian Wadden Sea, all investigated compounds showed strongly depleting concentration gradients.

These preliminary results must be complemented by the results of ana-lyses still outstanding before final conclusions can be drawn. Further factors which influence the contamination levels are the colour of the liver, which is correlated with the fat content, and partly the age and the will beworked out of the fish. This is taken into consideration for the grouping of pool samples in the analytical subsamples.

CONCLUSIONS

A final report on this interdisciplinary project will be available in the middle of the year 1991. The interpretation of the data of the subprojects and the recommendations for a biological effect monitoring will be based on the interlinkage of chemical and biological data. A set of biological methods will be proposed which are able to reflect injury by anthropogenic sources at different biological levels from the cell to the population. A battery of techniques for the biochemical measurements of detoxifying enzymes is developed which do not only reflect an adaptive response to a specific group of pollutants, such as mixed function oxidases or metallothioneins. Additionally, techniques will be included which are able to measure the damage of basic protective mechanisms by different groups of toxicants, as could be evidenced for the lysosomal detoxifying system in fish liver. For the monitoring of fish diseases, it is recommended to include the macroscopic inspection (colour, nodules) and histological case studies of the liver as one of the main target organs of toxicants, additionally to the registration of externally visible fish diseases. Based on the critical analyses of corresponding indices reflecting injury of marine fish populations by anthropogenic substances, guidelines will be worked out for the implementation of a comprehensive biological effects monitoring.

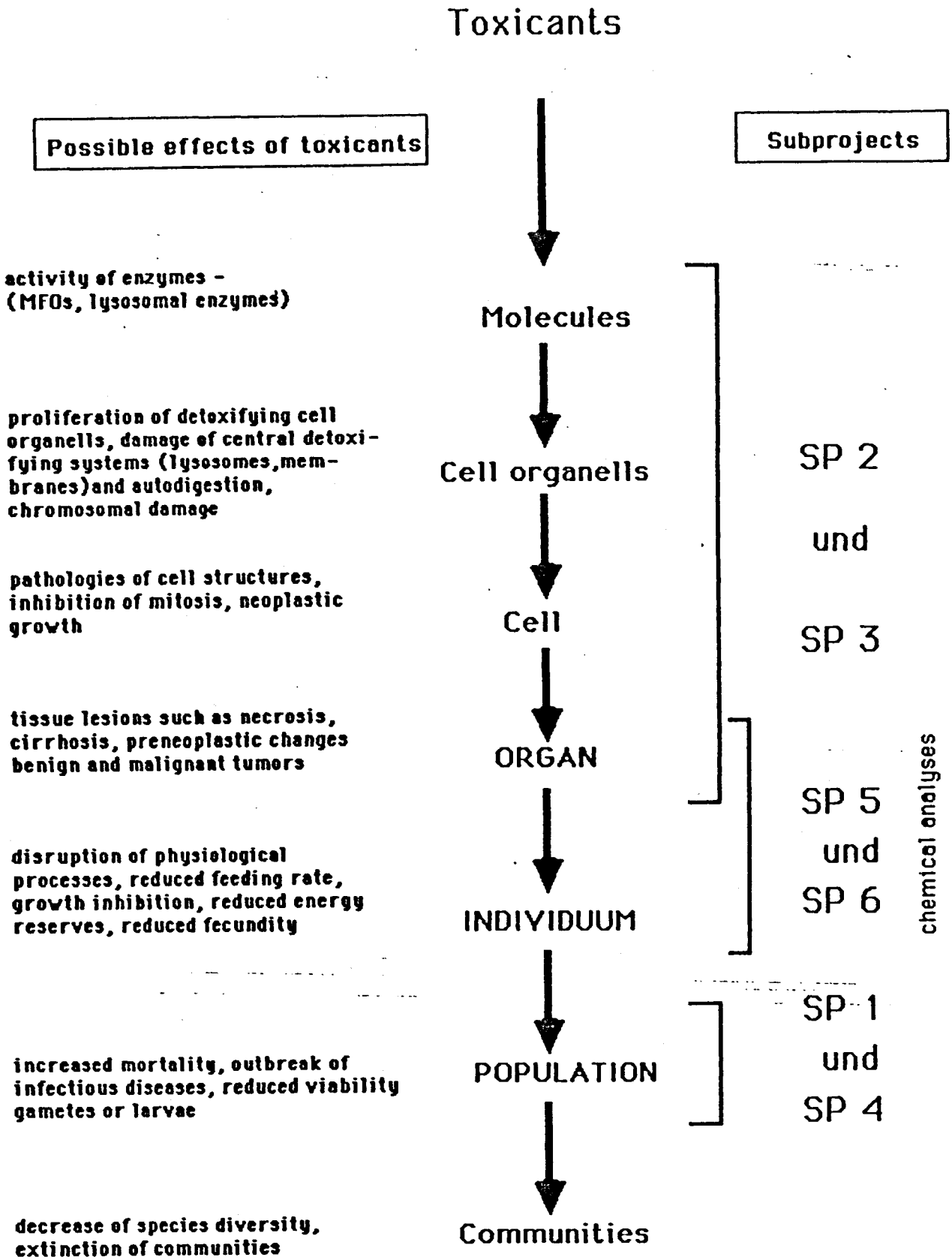

Toxicants
Possible effects of toxicants
Subprojects
activity of enzymes - (MFOs, lysosomal enzymes)
Molecules
proliferation of detoxifying cell organells, damage of central detoxifying systems (lysosomes, membranes) and autodigestion, chromosomal damage
Cell organells
SP 2
und
pathologies of cell structures, inhibition of mitosis, neoplastic growth
Cell
SP 3
tissue lesions such as necrosis, cirrhosis, preneoplastic changes benign and malignant tumors
ORGAN
SP 5
und
SP 6
chemical analyses
disruption of physiological processes, reduced feeding rate, growth inhibition, reduced energy reserves, reduced fecundity
INDIVIDUUM
SP 1
und
SP 4
increased mortality, outbreak of infectious diseases, reduced viability gametes or larvae
POPULATION
decrease of species diversity, extinction of communities
Communities

Fig.1

By affiliating several doctor and master theses and by involving guest scientists, additional topics could be studied, such as the age distribution and growth of flounder, the virology of smelt diseases, the bacteriology of flounder diseases, the parasites occurring in the flesh of smelt and sea scorpion, and various biological aspects of sand goby, hooknose and eelpout.

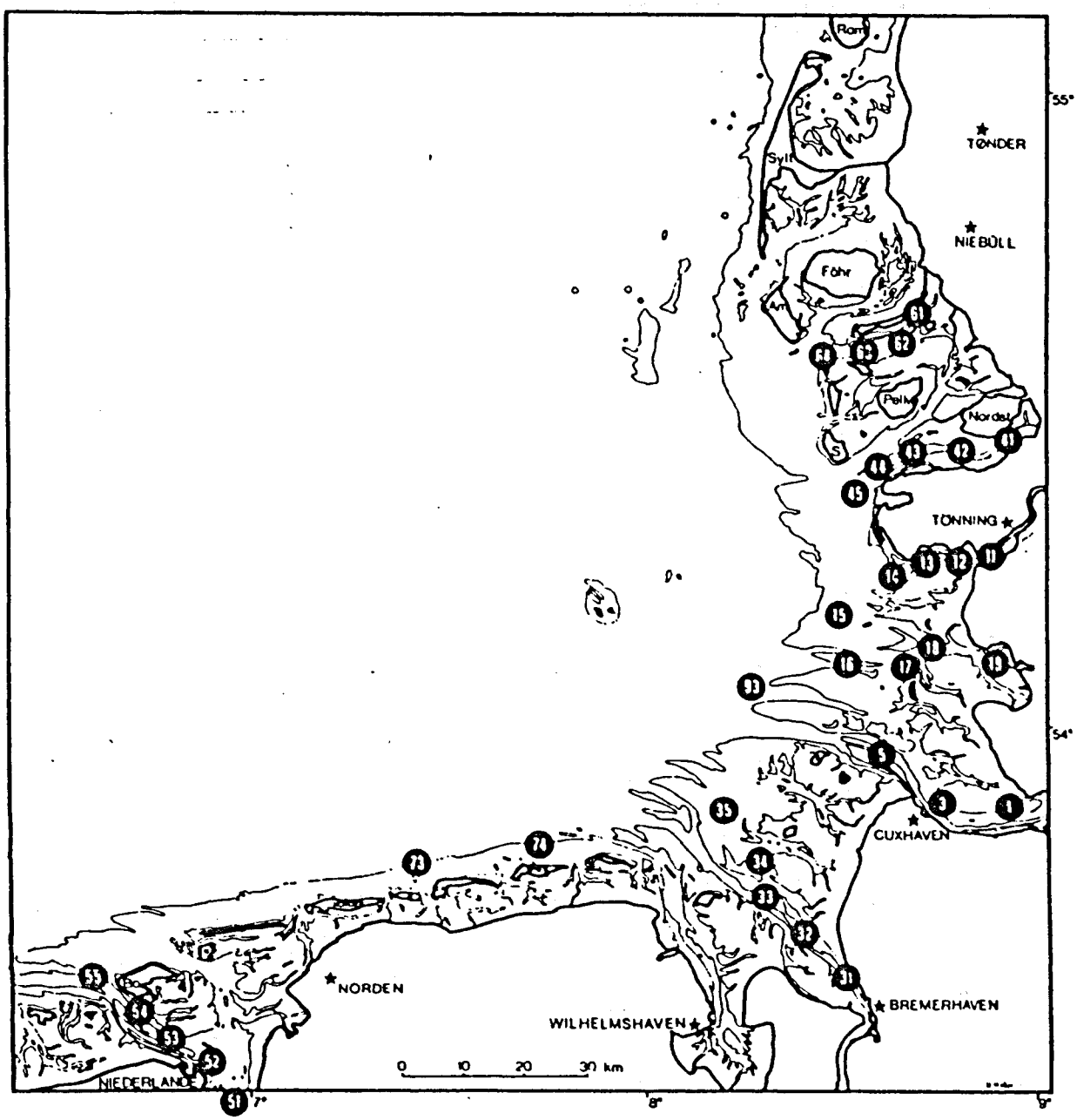


Fig.2 Station map of the survey on "Fish diseases of the Wadden Sea", 1988-1989.

Table. 1. Numbers of Wadden Sea fish examined for external diseases in 1988-1989.

Species	Length in cm	n examined
flounder	5-11	13,979
flounder	12+	20,347
dab	12+	18,034
plaice	12+	8,775
sole	12+	2,615
smelt	10+	40,011
cod	10+	10,689
whiting	10+	7,885
eel	10+	494
sea scorion	5+	401+
hooknose	5+	1,105+
eelpout	5+	1,057+

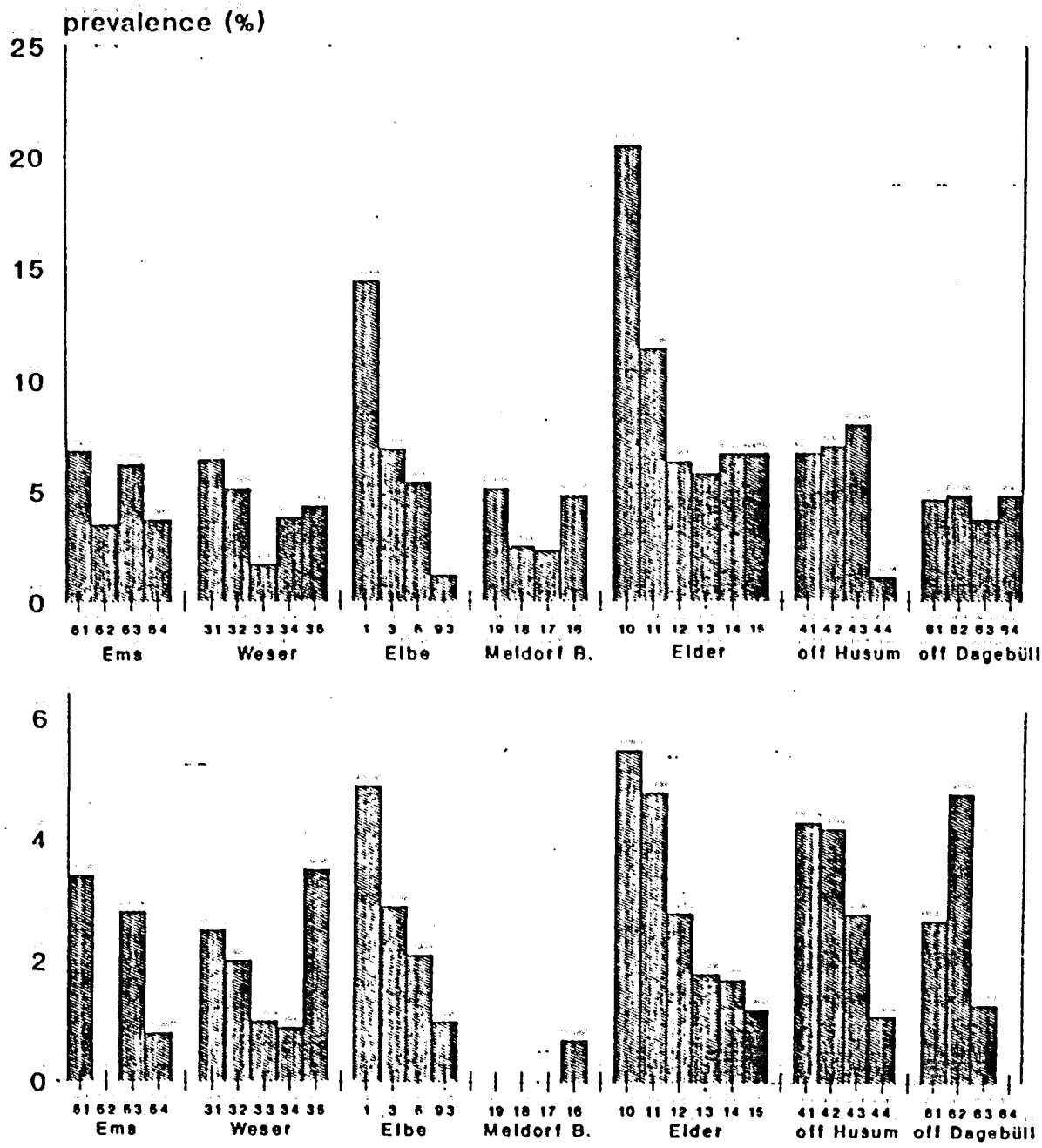


Fig. 3 Disease prevalence of flounder (18 cm) on stations in the Wadden Sea, average of all samples taken in 1988-1989. Top: total disease prevalence, below: only lymphocystis disease.

Table 2. Average salinity on fishing stations, catch per hour (12-25 cm), and disease prevalences of flounder in the Wadden Sea. Averages of all samples taken between January 1988 and December 1990. Data of flounder are from animals of 12-25 cm length and are adjusted for the 18 cm group.

Station	Lowest salinity ‰	Highest salinity ‰	Deviation in salinity ‰	n/h	Total disease rate %	Lymphocystis %	Skin ulcer %	Fin rot %
01	4	16	12	307	14.4	4.9	6.5	2.9
03	9	20	11	278	6.9	2.9	2.6	1.4
05	15	25	10	69	5.4	2.1	1.9	1.4
10	9	28	19	130	20.5	5.5	9.5	5.9
11	6	29	23	98	11.4	4.8	4.5	2.2
12	15	29	14	70	6.3	2.8	1.9	1.5
13	20	28	9	87	5.8	1.8	2.3	1.8
14	20	31	11	33	6.7	1.7	2.1	3.0
15	21	31	10	16	6.7	1.2	3.3	2.2
16	24	28	4	69	4.8	0.7	1.1	3.1
17	23	28	5	23	2.3	0	0.7	1.6
18	24	31	7	52	2.5	0	2.5	0
19	15	28	13	48	5.1	0	2.3	2.8
31	14	22	8	115	6.4	2.5	2.8	1.2
32	11	25	14	94	5.1	2.0	1.2	1.9
33	16	27	11	45	1.7	1.0	0.4	0.4
34	26	28	2	28	3.8	0.9	2.1	0.9
35	25	29	4	56	4.3	3.5	0.3	0.5
41	22	31	9	69	6.7	4.3	1.4	1.0
42	23	31	8	47	7.0	4.2	0.3	2.5
43	23	32	9	32	8.0	2.8	2.4	2.7
44	24	31	7	17	1.1	1.1	0	0
45	25	32	7	18				
51	21	27	6	91	6.8	3.4	1.3	2.2
52	22	28	6	55	3.5	0	1.2	2.2
53	25	29	4	43	6.2	2.8	1.8	1.5
54	26	29	3	60	3.7	0.8	1.4	1.6
55	25	30	5	28				
61	23	30	7	57	4.6	2.7	1.5	0.5
62	24	31	7	25	4.8	4.8	0	0
63	25	30	5	26	3.7	1.3	1.0	1.4
64	25	30	5	19	4.8	0	0.9	3.9
72	30	30	0					
73	27	32	5					
74	30	32	2					
79	29	29	0					
93	29	30	1	-	1.2	1.0	0.2	0

Stages of liver lesions in flounder from the German Wadden Sea

1. Normal liver parenchyma

2. Necrosis of single hepatocytes, perisinusoidal lipid accumulation

3. Focal necrosis, nuclear polymorphism, paracristal inclusions single-cell megalocytosis, moderate but homogeneous lipid accumulation

4. Expansion of focal necrosis around the blood vessels, caryomegaly, megalocytic hepatosis, increased eosinophilia with hypertrophy, hyperplasia, focal spongiosis, paracristals

5. Dissolution of parenchymal structure, network of dark,shrunken hepatocytes, lytic necrosis, homogeneous steatosis,pycnosis, nuclear polymorphism, caryomegaly, nuclear lipid inclusions, fibrosis and occasional cirrhosis, megalocytic hepatosis, spongiosis

6. "Clear cell", eosinophilic and/or basophilic foci, "mixed" cell foci, hyperplastic foci associated with lesions of the stage 4 and 5, no compression of the surrounding tissue, fibrosis and partial cirrhosis, increase of melano-macrophage centers

7. Benign neoplasms such as liver cell adenoma, hemangioma, cholangioma, cholangiofibrosis, clear defined areas in liver tissue displaying lesions of the stages 4, 5 and 6 with extreme proliferation of macrophage-centers, compression of the surrounding tissue

8. Malignant neoplasms such as liver cell carcinoma, cholangiocellular carcinoma, "mixed" hepatocholangiocellular carcinoma, "mixed" hemangioma with liver cell carcinoma, angiosarcoma and hepatoblastoma associated with above carcinomas, invasive growth into surrounding tissue of stage 6, extensive proliferation of macrophage centers, cells drastically depleted of reserve substances substances

Table 3 : subproject 2

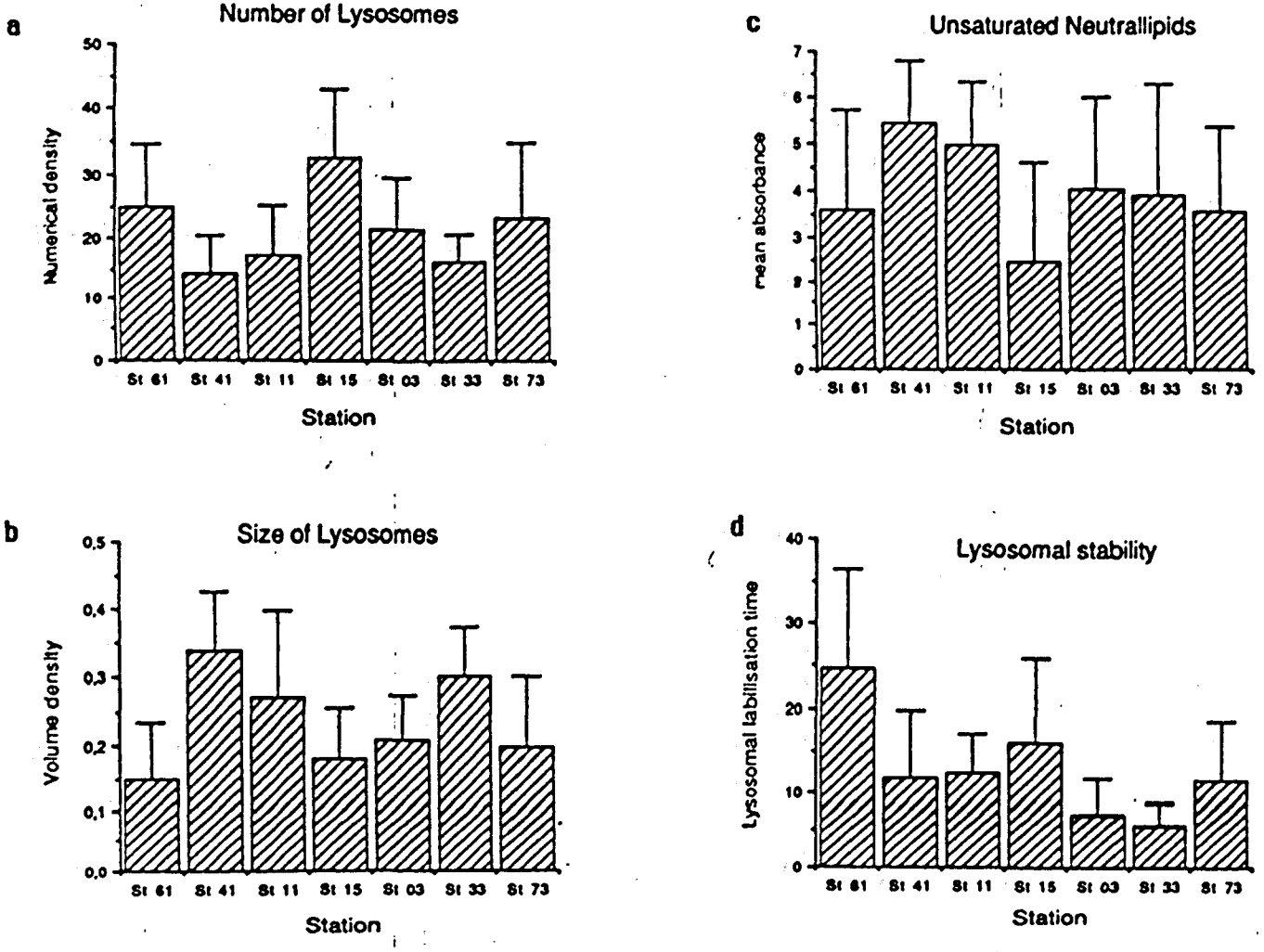

a
Number of Lysosomes
Numerical density
50
40
30
20
10
0
St 61
St 41
St 11
St 15
St 03
St 33
St 73
Station
c
Unsaturated Neutrallipids
mean absorbance
7
6
5
4
3
2
1
0
St 61
St 41
St 11
St 15
St 03
St 33
St 73
Station
b
Size of Lysosomes
Volume density
0,5
0,4
0,3
0,2
0,1
0,0
St 61
St 41
St 11
St 15
St 03
St 33
St 73
Station
d
Lysosomal stability
Lysosomal labilisation time
40
30
20
10
0
St 61
St 41
St 11
St 15
St 03
St 33
St 73
Station

Fig. 4

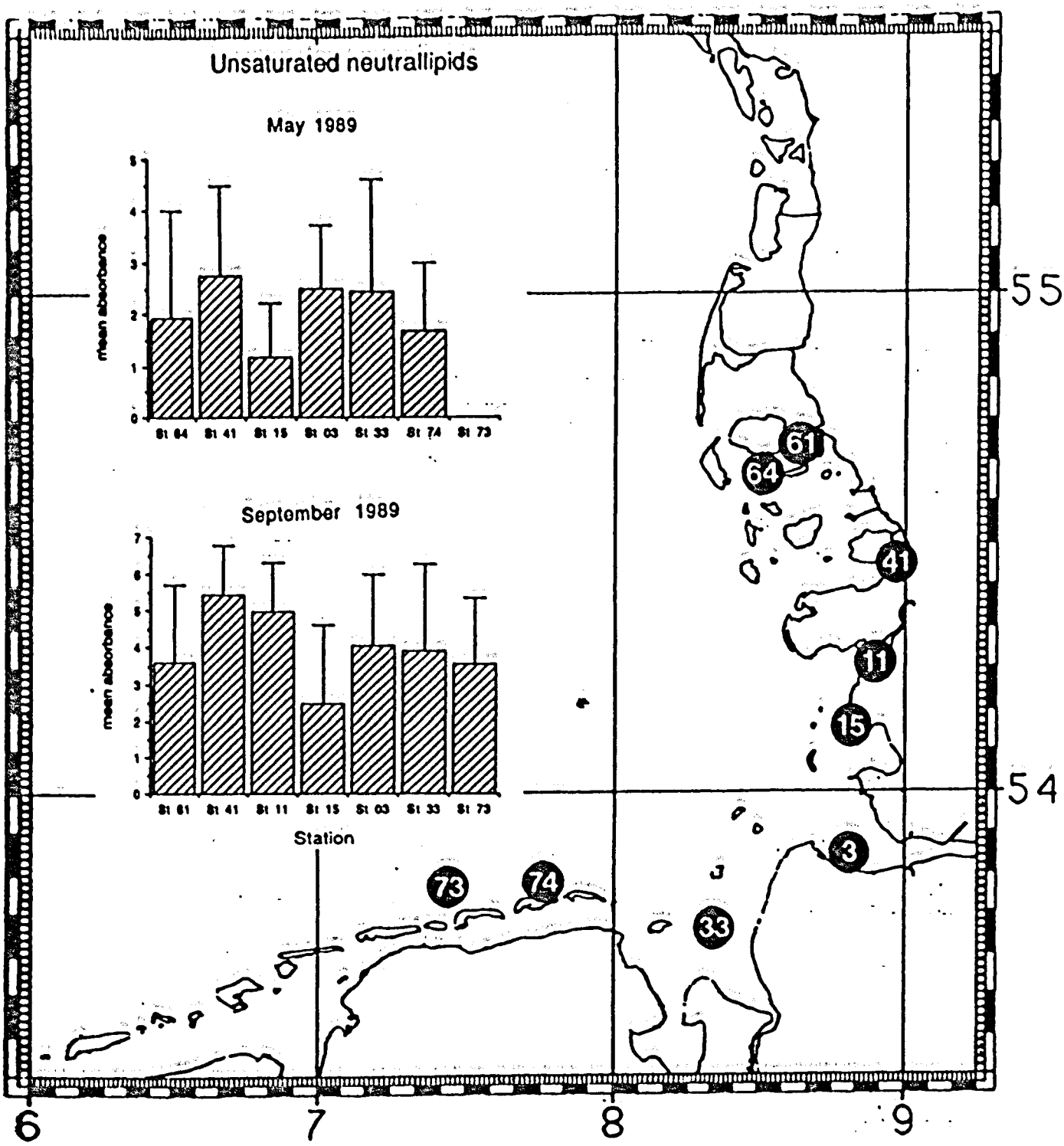

Unsaturated neutrallipids
May 1989
mean absorbance
St 64
St 41
St 15
St 03
St 33
St 74
St 73
September 1989
mean absorbance
St 61
St 41
St 11
St 15
St 03
St 33
St 73
Station
55
54
6
7
8
9
61
64
41
11
15
3
73
74
33

Fig. 5

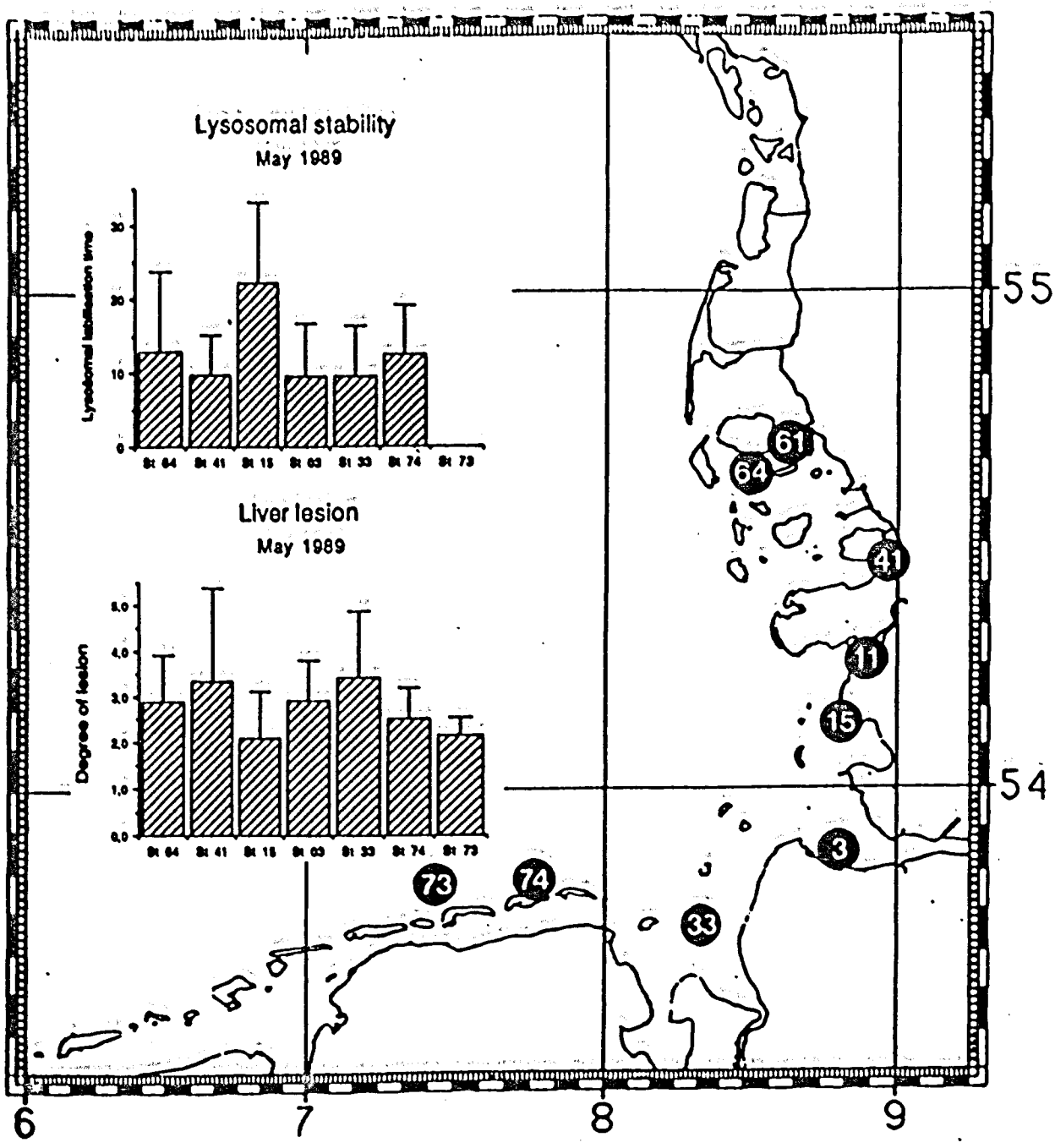


Fig. 6 : Regional investigations of lysosomal stability and liver lesions in flounder (17-25 cm) in identical individuals.

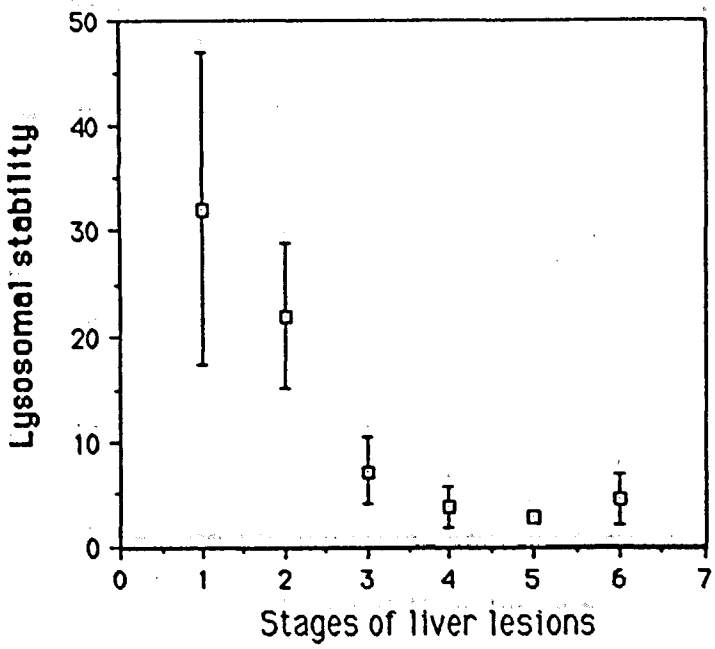


Fig. 7 : Mean lysosomal stability at different degrees of liver lesions (Tab. 3) (N= 75)

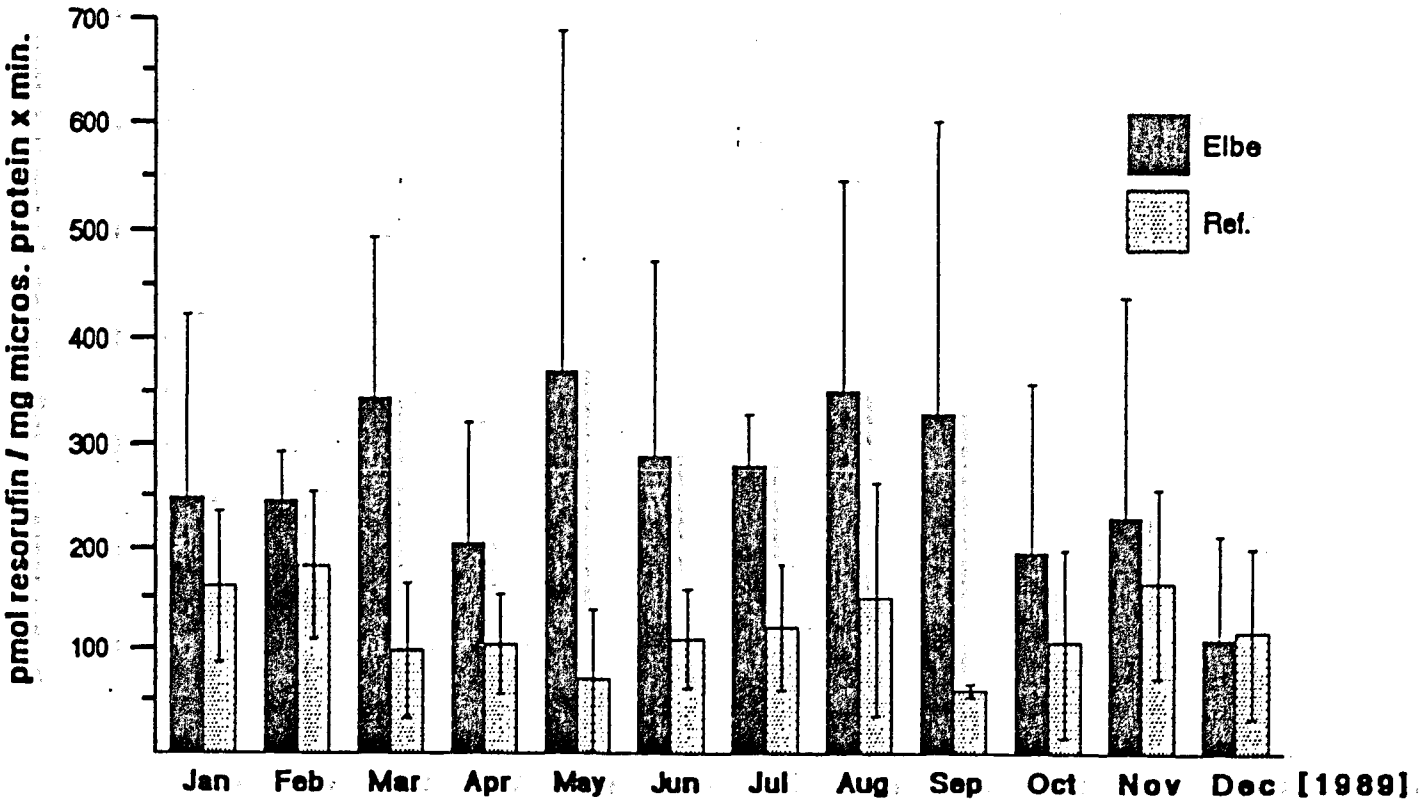


Fig. 8 **Saisonal changes in EROD-activity (7-Ethoxyresorufin-O-de-ethylase-activity) in the river Elbe and at the reference site during 1989**

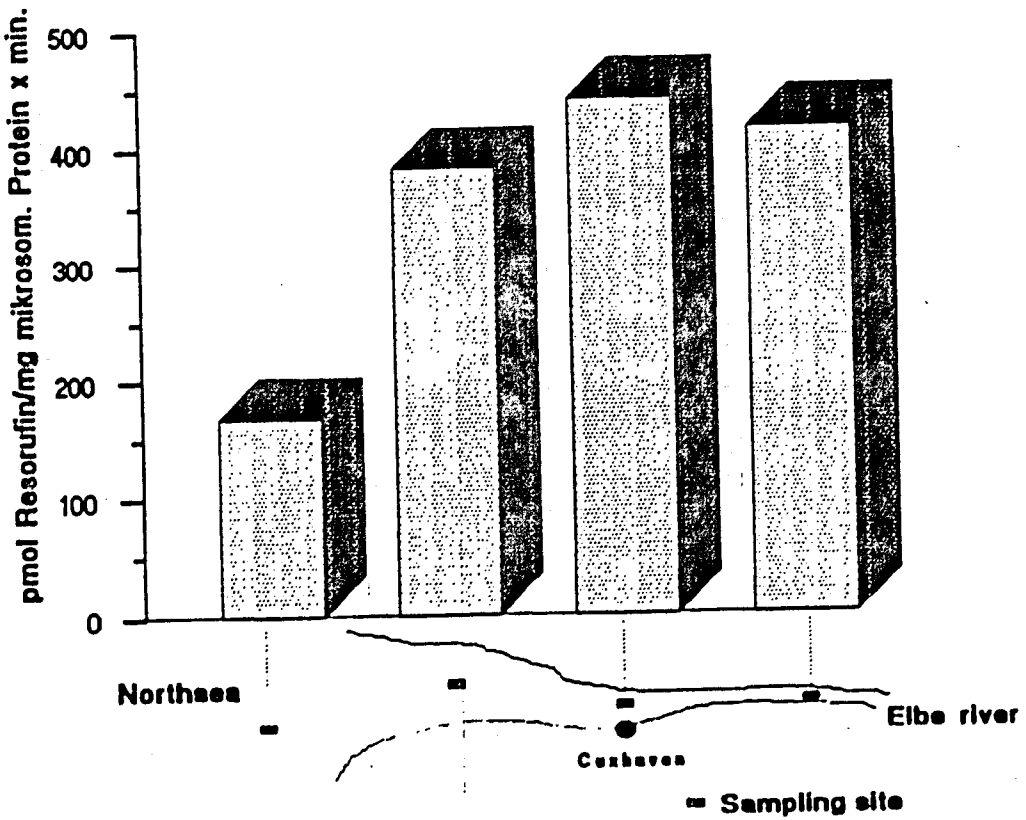


Fig. 9 Activity of mixed function oxidases (MFO), measured as 7-Ethoxyresorufi-O-de-ethylase-activity (EROD) in the Elbe estuary, Aug. 1988

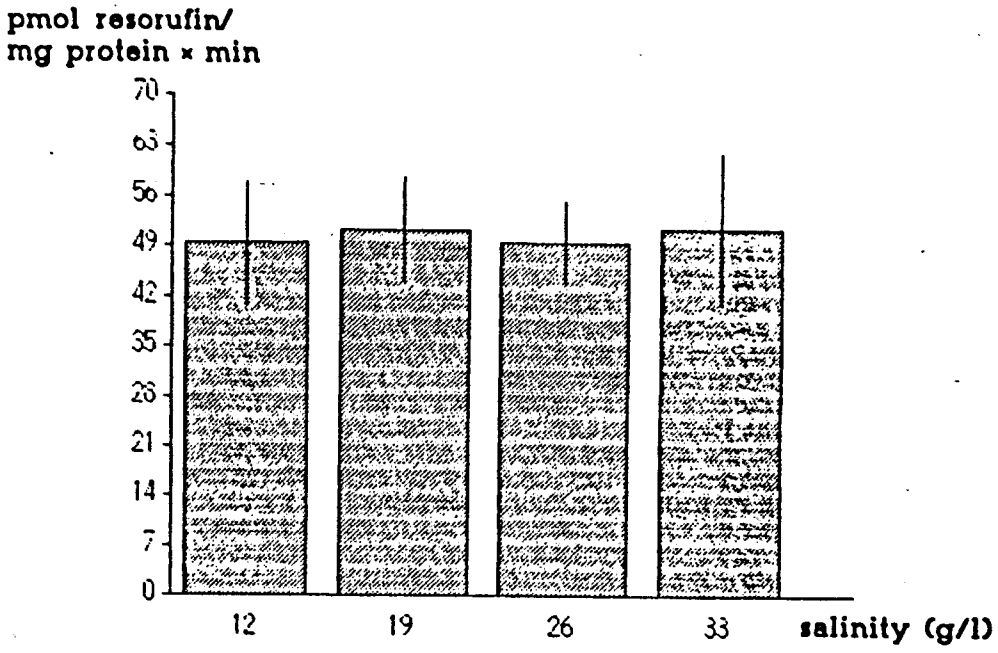


Fig.10 a : 7-Ethoxyresorufin-O-De-Ethylase-activity from flounder livers at different salinity, control group

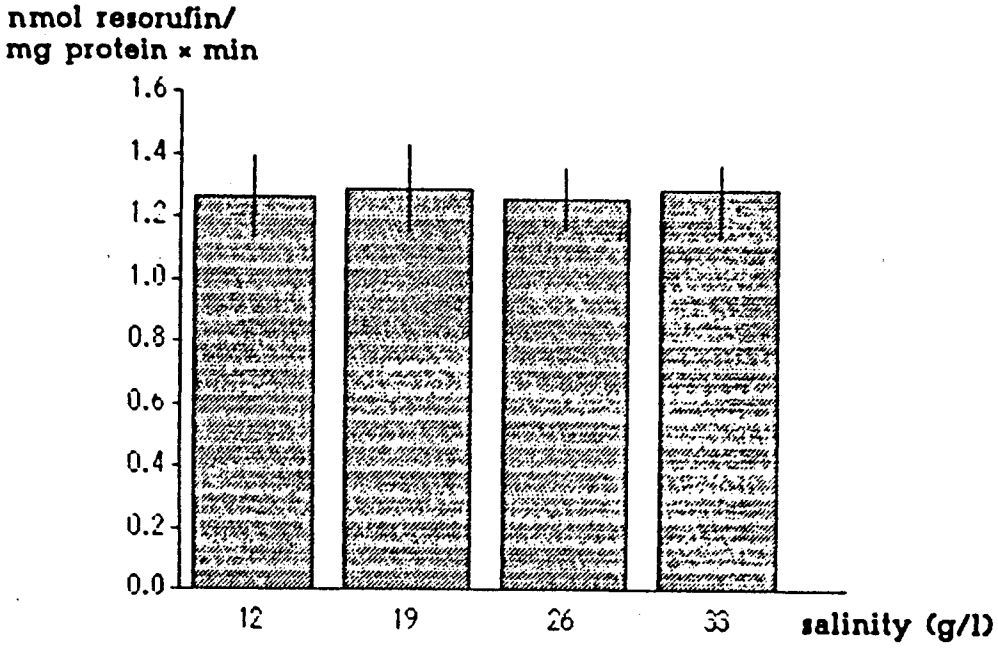


Fig.10 b : 7-Ethoxyresorufin-O-De-Ethylase-Activity from flounder livers at different salinity, Beta-Naphtoflavone-treated group

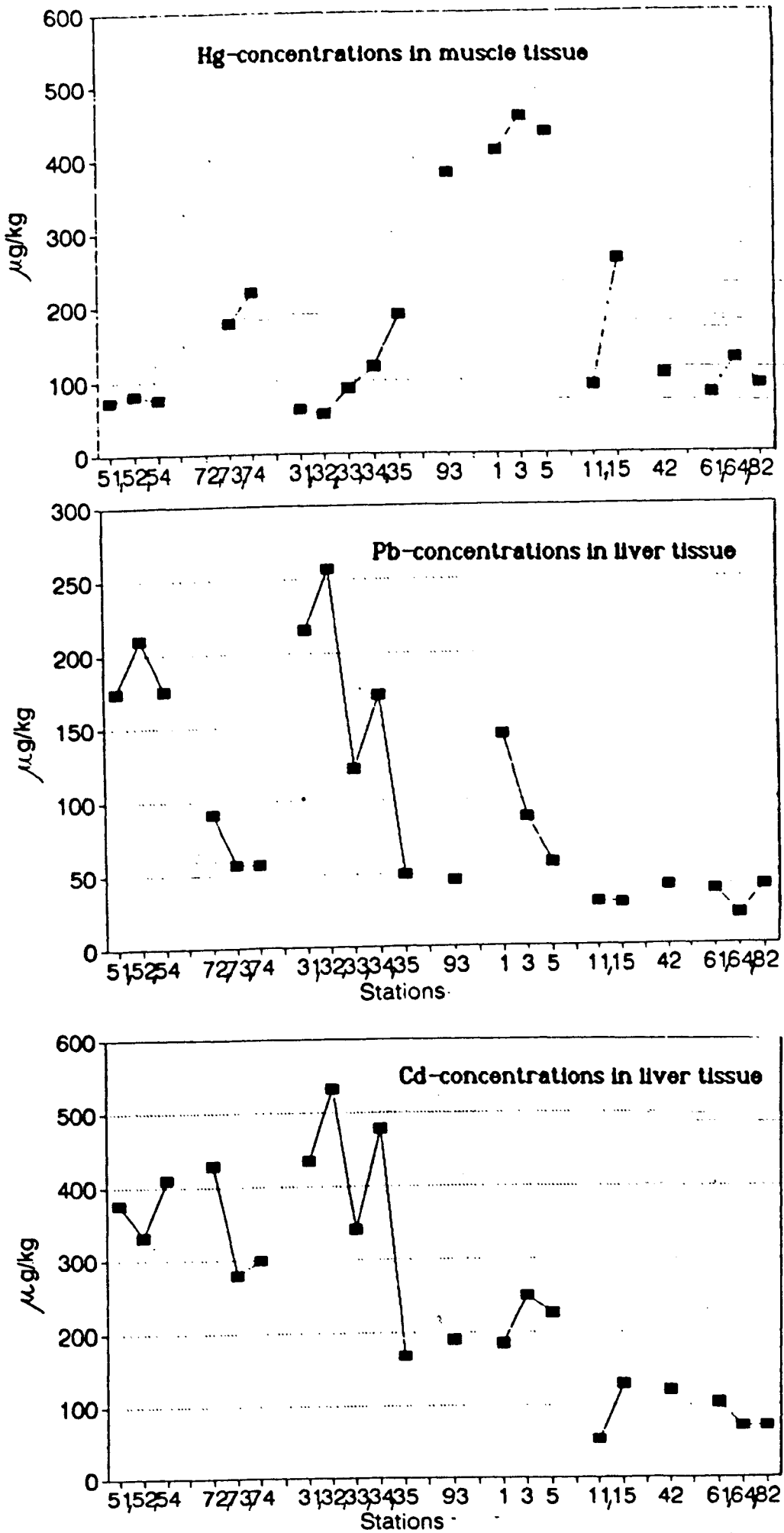
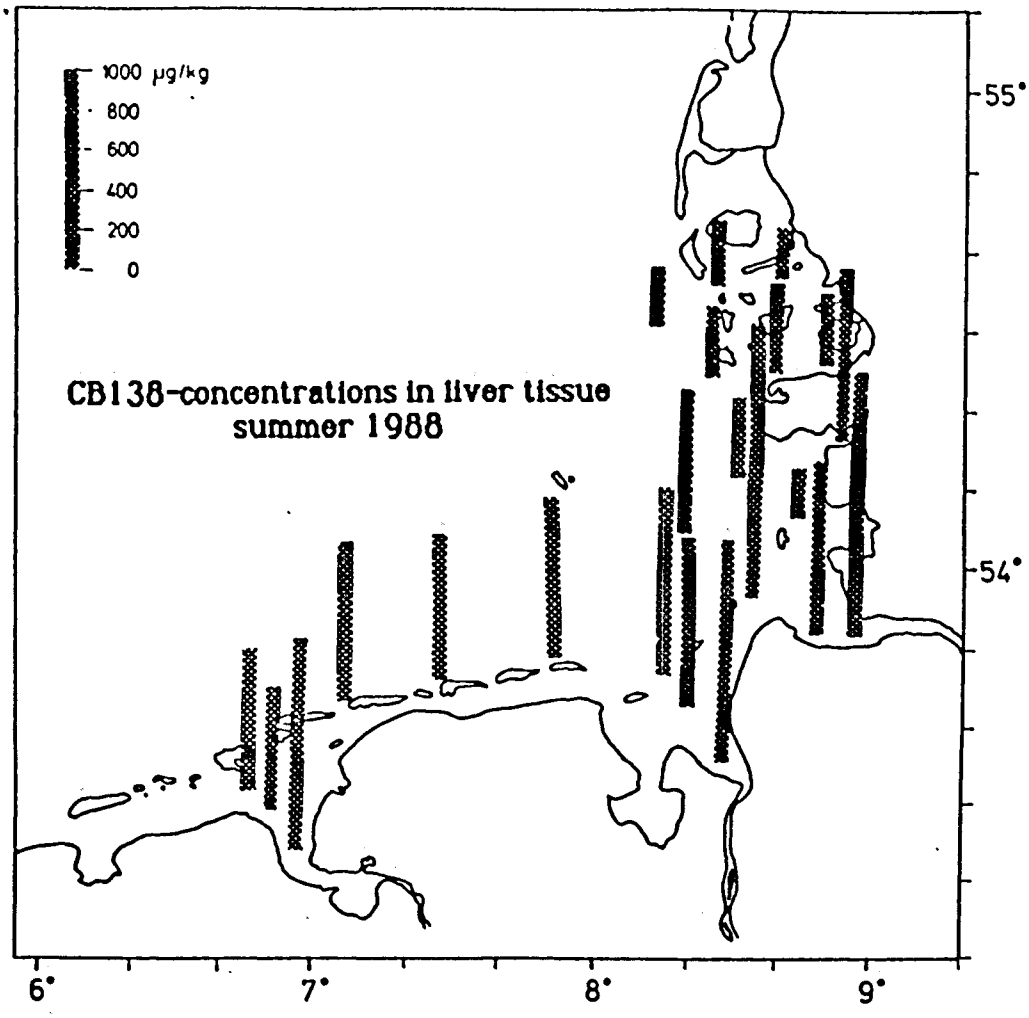
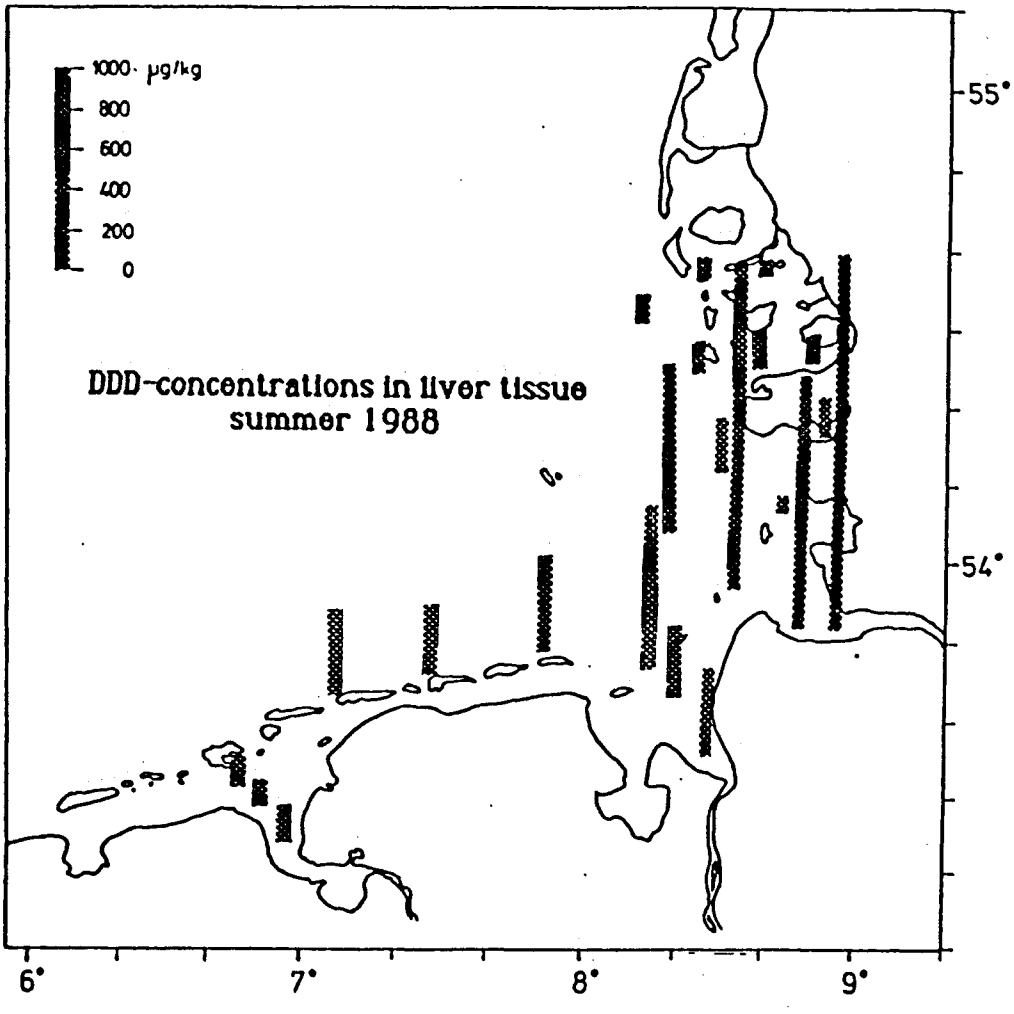


Fig. 11 Regional samples from the Wadden Sea and adjacent estuaries

Fig. 12


1000 µg/kg
800
600
400
200
0
CB138-concentrations in liver tissue
summer 1988
55°
54°
6°
7°
8°
9°


1000 µg/kg
800
600
400
200
0
DDD-concentrations in liver tissue
summer 1988
55°
54°
6°
7°
8°
9°