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INTERIM REPORT ON THE ICES/IOC BREMERHAVEN WORKSHOP ON BIOLOGICAL EFFECTS TECHNIQUES

by

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Preface

This interim report incorporates most of the data arising from the ICES/IOC workshop on biological effects techniques held at the Alfred Wegener Institute in Bremerhaven, Germany during March 1990. It incorporates background imformation on the workshop, its objectives, the transects used, most of the biological, chemical and hrdrographic data; interim summaries and interpretations of those data, together with some of the problems arising from their interpretation.

The report was assembled following the concluding meeting for the workshop at which all the data were presented and discussed for the first time. The meeting was convened by ICES in Copenhagen 11-13 September 1992. Final publication of all the results of the workshop will be as a Special Volume of Marine Ecology Progress Series in late 1992. Sections 1 to 4, and part of Section 5, are abstracts of the full papers prepared for this Special Volume.

CONTENTS

1. INTRODUCTION (A.R.D. Stebbing and V. Dethlefsen)

Background. The main objective of the ICES/IOC Workshop on Biological Effects was to test and compare biological effects techniques suitable for monitoring environmental quality offshore. The workshop was organised jointly by the Group of Experts on the Effects of Pollution (GEEP), which is part of the Intergovernmental Oceanographic Commission of Unesco, and the ICES Working Group on the Biological Effects of Contaminants.

The workshop was a continuation of a series of practical workshops to deploy biological effects techniques mounted by GEEP in Oslo and Bermuda. The techniques were deployed in the North Sea with the work of the North Sea Task Force (NSTF) and its Master Monitoring Plan in mind, providing the first practical and simultaneous test of all four biological techniques selected by the NSTF.

The techniques were deployed on two transects on known contamination gradients in the southern North Sea. The first was a transect of 9 stations running northwest for 200km from a point off the mouths of the River Elbe and Weser to the eastern side of the Dogger Bank. The second transect ran 5000 m down-current (WNW) of a disused drilling site off the Dutch coast (Figure 1).

Our main objective in selecting contamination gradients for the Workshop was not to identify gradients that required investigation in their own right, but to use gradients that would provide an adequate and realistic test of prospective techniques for North Sea and other European monitoring programmes.

It is impossible on any naturally occurring contamination gradient to eliminate "nuisance variables" that make interpretation of the biological effects and responses to toxic contaminants difficult to interpret. However, such effects can be minimised by the choice of gradients.

While historical data demonstrate stable contamination gradients associated with the Elbe/Weser plumes and the Dutch drilling platform, it was essential to have synoptic chemical data for compounds likely to be toxic on each gradient to help interpret the biological data. The ICES Marine Chemistry Working Group agreed to collaborate and provide appropriate chemical data for tissue, water and sediments, which involved six European laboratories.

German Bight Transect. This transect transverses a known contaminant gradient and is situated between 54^{3}N 07^{3}50'E and 56^{3}N 03^{3}24'E with a total length of approximately 200 km (Fig. 1). The track includes 9 stations from the mouths of the River Elbe and the River Weser to the most distant station located on the northeastern arm of the Dogger Bank. Locations of the stations are given in Table 1. It is entirely located on the German part of the continental shelf in an area routinely monitored and studied by the former German Hydrographic Institute (Bundesamt fur Seeschiffahrt und Hydrographie - BSH) and German North Sea Programmes. Existing knowledge from such programmes was important in the planning stage, and is now important in interpreting the results of the workshop.

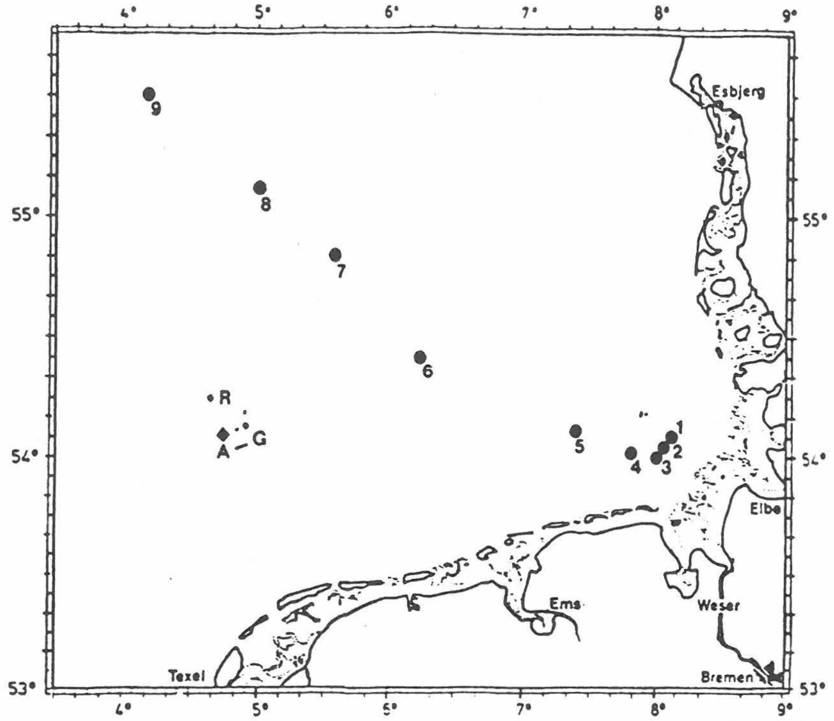


Figure 1. The German Bight showing the main transect (Stations 1 to 9) and the drilling site transect (Stations A to G) with its reference site (R).

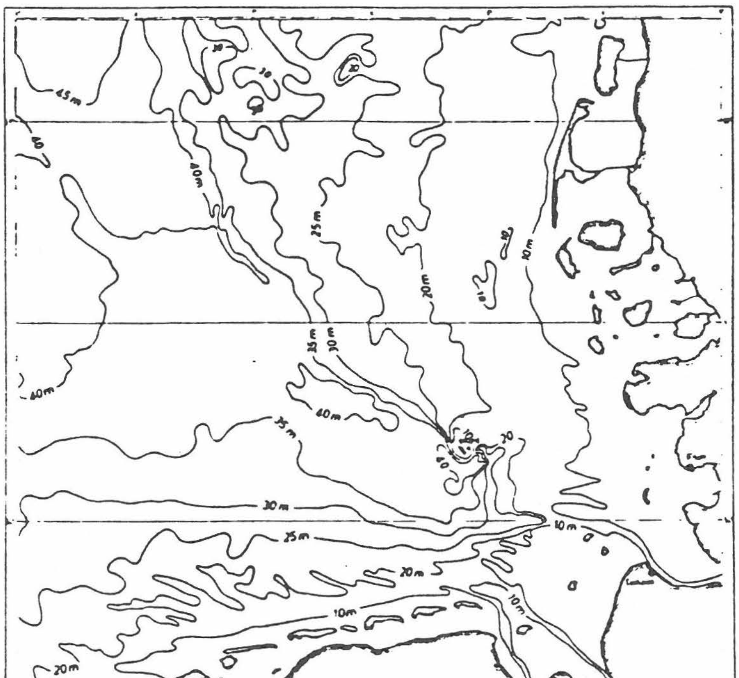


Figure 2. Bathymetry of the German Bight.

Table 1. Locations of German Bight sampling stations

No.	Sediment	Co-ordinates	Water depth (ca)
1	mud	54^{3}04'N 08^{3}07.5'E	19 m
2	mud	54^{3}02'N 08^{3}03'E	27 m (no fishing)
3	muddy sand	54^{3}00'N 08^{3}00'E	29 m
4	muddy sand	54^{3}01'N 07^{3}49'E	36 m (no fishing)
5	muddy sand	54^{3}06.5N 07^{3}24'E	35 m
6	muddy sand	54^{3}25'N 06^{3}15'E	40 m
7	muddy sand	54^{3}50'N 05^{3}35'E	43 m
8	muddy sand	54^{3}06'N 05^{3}00'E	42 m
9	sand	55^{3}30'N 04^{3}10'E	30 m (no benthic sampling)

Stations were selected to minimise variations in depth, their known differences in contaminant concentrations in sediments, a variety of contaminants (OCs, metals, PAHs petroleum hydrocarbons), and proximity to Bremerhaven. Some of the stations coincided with those used for monitoring purposes by the BSH. Additional stations were situated inshore of this transect to detect the effect of the river Elbe plume on the benthic community structure.

Drilling Site Transect. A drilling site was selected because it minimises the effects of factors unrelated to the contaminants that the biological techniques were deployed to detect, by providing a point source and sharp decline in concentrations with distance. This area off the coast of the Netherlands is a site of exploratory drilling which was abandoned in 1987.

Water and oil-based drilling muds are the major contaminants which provide a clear gradient of biological impact on benthic community structure when studied by the Dutch in 1988 (see Section 3).

Table 2. Locations of the Drilling Site sampling stations. area

A	centre	54^{3}06'15"N 04^{3}45'33"E (fishing)
B	125 m	in the direction of the residual current (= 66^{3})
C	250 m	
D	500 m	
E	1000 m	
F	2000 m	
G	5000 m	(fishing)
R	15000 m	(reference station (fishing)

Deployment of techniques. Many contaminants in seawater that dissolve, or are neutrally buoyant, are diluted and become evenly distributed throughout the water column and thus are diluted to concentrations less likely to have detectable toxic effects. However, there are others which accumulate in higher concentrations at interfaces of the water column: the sea surface and sea bottom. These are likely to be the most vulnerable points in the marine ecosystem to contaminants and therefore the most appropriate to monitor. The Workshop therefore concentrated on deployment of techniques to monitor gradients at the sea surface, the water column and at the sea bottom.

Choice of techniques. The biological measures employed included the first attempt to integrate different indices of stress on fish using the techniques of cellular pathology, gross pathology, and biochemistry; where possible using the same fish collected from the same stations and at the same time. Bioassay techniques were employed using water samples from the sea-surface microlayer, and then relating the resulting data to bioassays conducted on subsurface samples. Another approach, employed for the first time in European waters, is the Triad. This technique involves the simultaneous use of benthic community data, sediment bioassay, and sediment chemistry to provide an overall indication of environmental quality on the sea bottom.

Workshop Groups and Coordinators. The groups of projects were led by coordinators for each disciplinary area. The groups and their coordinators are as follows:

a. Fish - dab (Limanda limanda)
-biochemistry - R.F.Addison
-molecular and cellular pathology - M.N.Moore
-gross and histopathology - A.D.Vethaak

b. Water quality bioassays - L. Karbe

c. Benthos
-community structure - C. Heip
-sediment bioassays - P. M. Chapman & R. C. Swartz
-sediment triad - P.M. Chapman

d. Chemistry - W. Cofino

e. Statistics - M.Carr

f. Vessels - V. Dethlefsen

The techniques tested during the workshop included the four selected by the North Sea Task Force for use in its Master Monitoring Plan. They are the oyster embryo bioassay (for water and sediments), EROD and disease in dab and benthic macrofaunal community structure. The workshop provided an opportunity to evaluate new techniques, as well as the first simultaneous deployment of all these techniques on the same contamination gradient, and so provides a good basis for comparison of the chosen techniques.

2. HYDROGRAPHY OF THE GERMAN BIGHT (abstracted from a paper by G.A. Becker and S. Dick, Federal Maritime and Hydrographic Agency, Hamburg, Germany).

Topography - the bathymetry of the area of the German Bight (Fig. 2) of relevance to the workshop is dominated by the River Elbe's pronounced post-glacial valley which extends from the Elbe estuary to the northwest and passes the Dogger Bank on the eastern side of the Tail End. The Heligoland Deep is the deepest part of the German Bight. The workshop transect coincided with the western flank of the Elbe Valley.

Sedimentology - the superficial benthic sediments contain a high proportion of silt and clay (< 63 microns), with greater than 50% in the Heligoland Deep decreasing to less than 1% at Station 9 on the eastern extremity of the Dogger Bank. The surface sediments are quite mobile and turbulence induced primarily by wave action can resuspend material, which is then transported by tidal or residual currents in a northerly direction.

Hydrography - two main water masses characterise the German Bight: the Continental Coastal Water and Central North Sea Water. The Continental Coastal Water is a mixture of water from the Atlantic and English Channel, as well as the effluent of the Rivers Rhine, Meuse and Ems. The thermohaline stratification in the German Bight is triggered by the run-off from the Rivers Weser and Elbe and the development of a seasonal thermocline in the outer area. Three types of frontal systems occur in the inner part of the German Bight due to estuarine plumes of the Elbe and Weser, to temperature and upwelling. The distribution of frontal systems and mesoscale variability occurs in a zone running northwest from the mouths of the Elbe and Weser, coinciding to some extent with the transect.

The inflow of Atlantic water via the English Channel in 1990/91 had an unusually high salinity, and during the period of the workshop a tongue of Atlantic water with salinities exceeding 35 ppt extended from the Southern Bight into the western German Bight (Fig. 3).

During the workshop R.V. Valdivia made hydrographic measurements with depth along the transect apart from the surface salinity measurements shown in Figure 3. The vertical section for salinity (Fig. 5) shows the transition from the weakly stratified waters of the Elbe/Weser plume in the region of Continental Coastal Water to the homogeneous, more saline Central North Sea Water. The boundary between the two water masses lies between Stations 3 and 5 during the first traverse, moving offshore to between Stations 4 and 6 during the second. The transition between the two water masses is marked by a clear frontal zone reflected by the vertical salinity sections (Fig. 4) and closely related density data.

Residual circulation and modelling. Tides in the German Bight are quite strong and cause turbulent horizontal and vertical exchanges. The residual circulation pattern shows a moderate transport of water from the Southern Bight to the north-east into the German Bight. The northern continuation is the Jutland Current. The mean residual currents have a velocity of approximately 5 cm/s.

The residual current velocity pattern is seen on the output of the BSH's Operational North Sea Model (Fig. 5). This three dimensional circulation model calculates daily the currents and water levels on three nested grids, which have their highest resolution (1 nautical mile) in the German Bight. The model is driven by tidal forcing, wind stress and surface pressure. The wind input is computed using surface pressure forecasts supplied by Deutscher

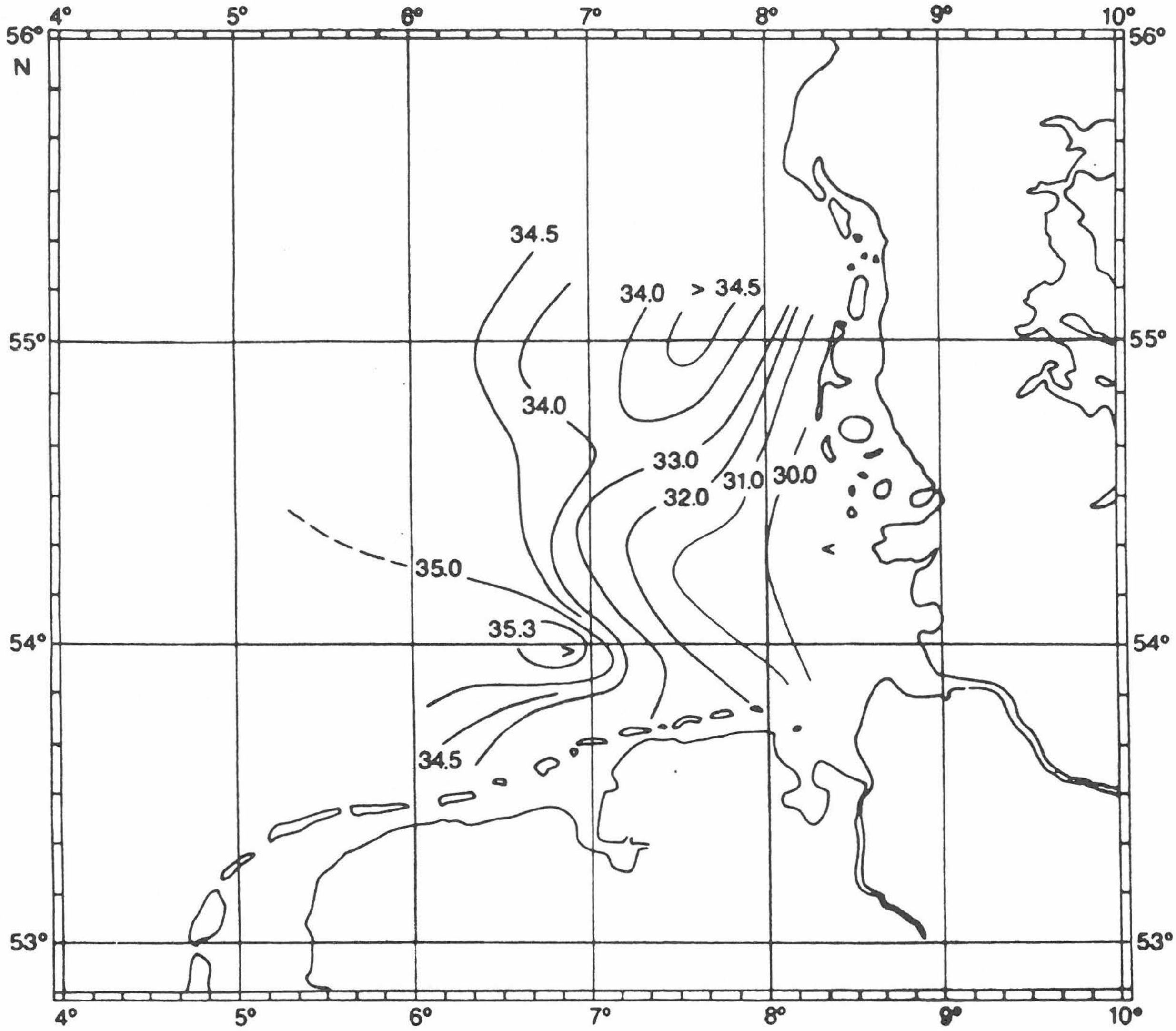
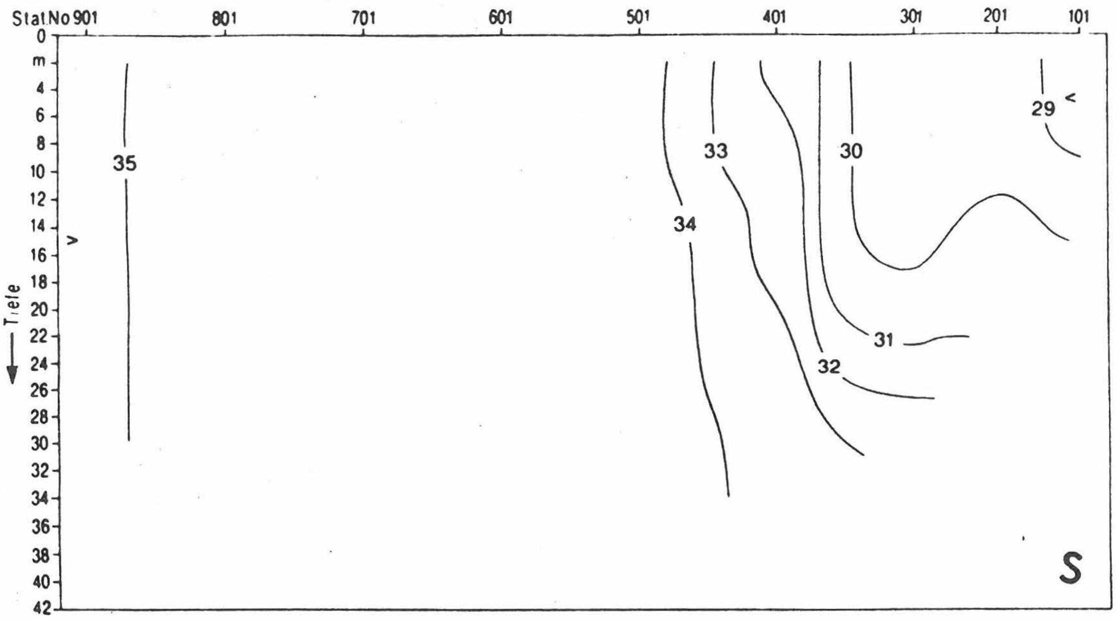


Figure 3. Surface salinity distribution of the German Bight. R.V. Valdivia 5-12 March 1990.



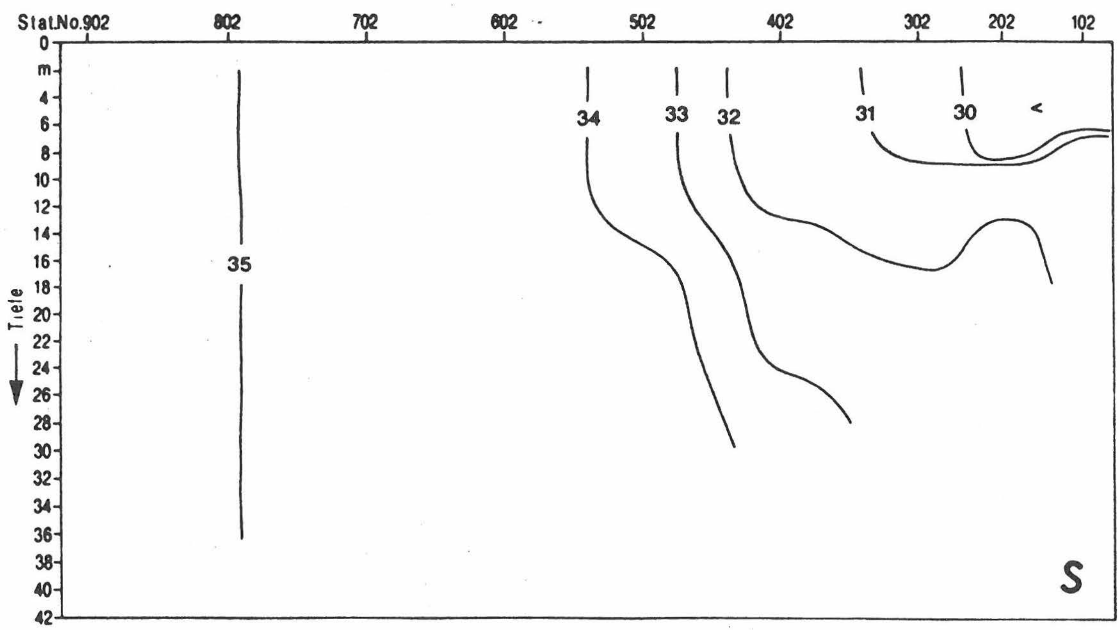


Figure 4. Vertical section for salinity along the German Bight transect. The first digit on the horizontal axis refers to the station number. The designation 01 identifies the first traverse of the transect made by R.V. Valdivia and 02 the second.

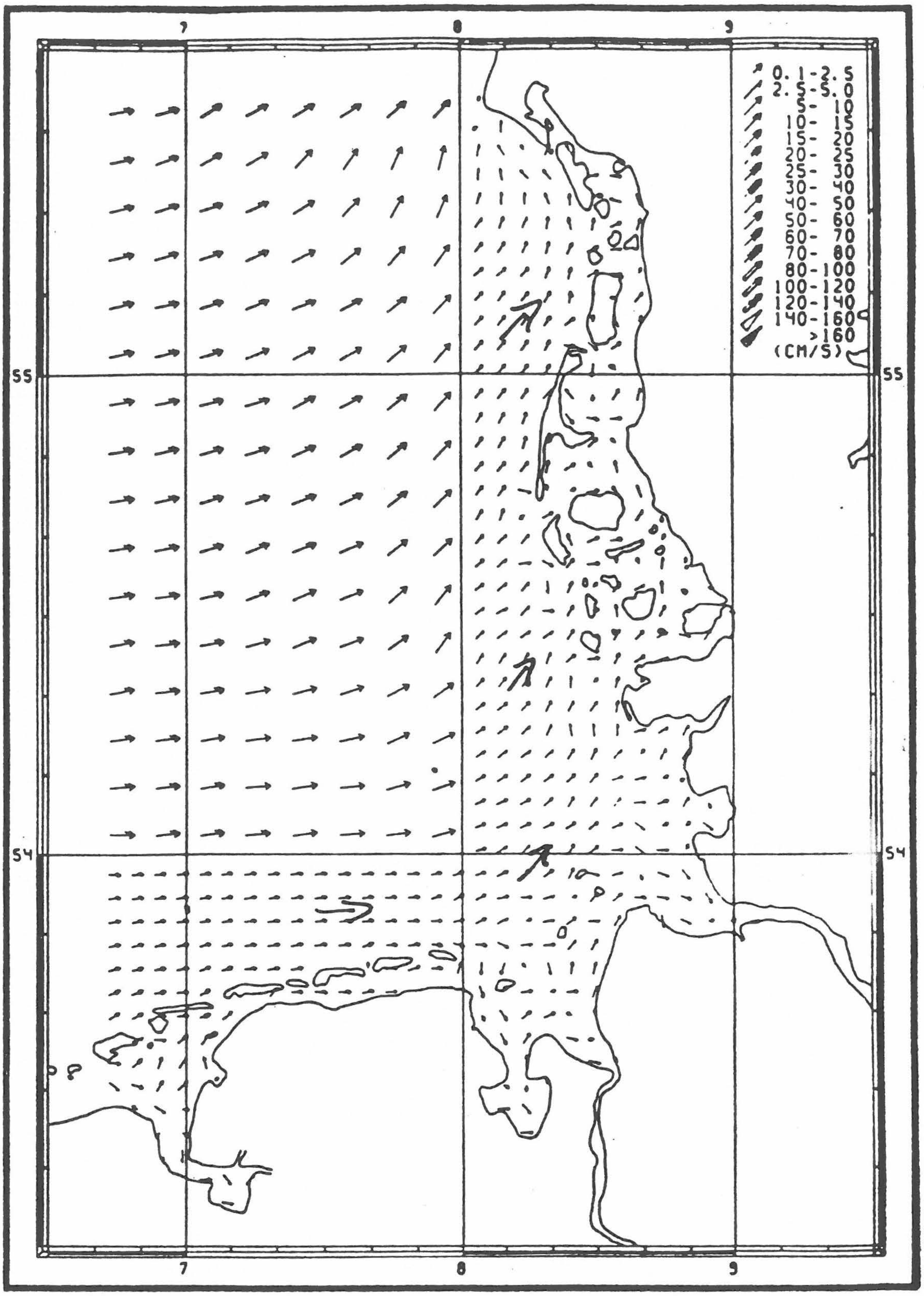


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Figure 5. Surface layer residual current model calculation for 21 March 1990.

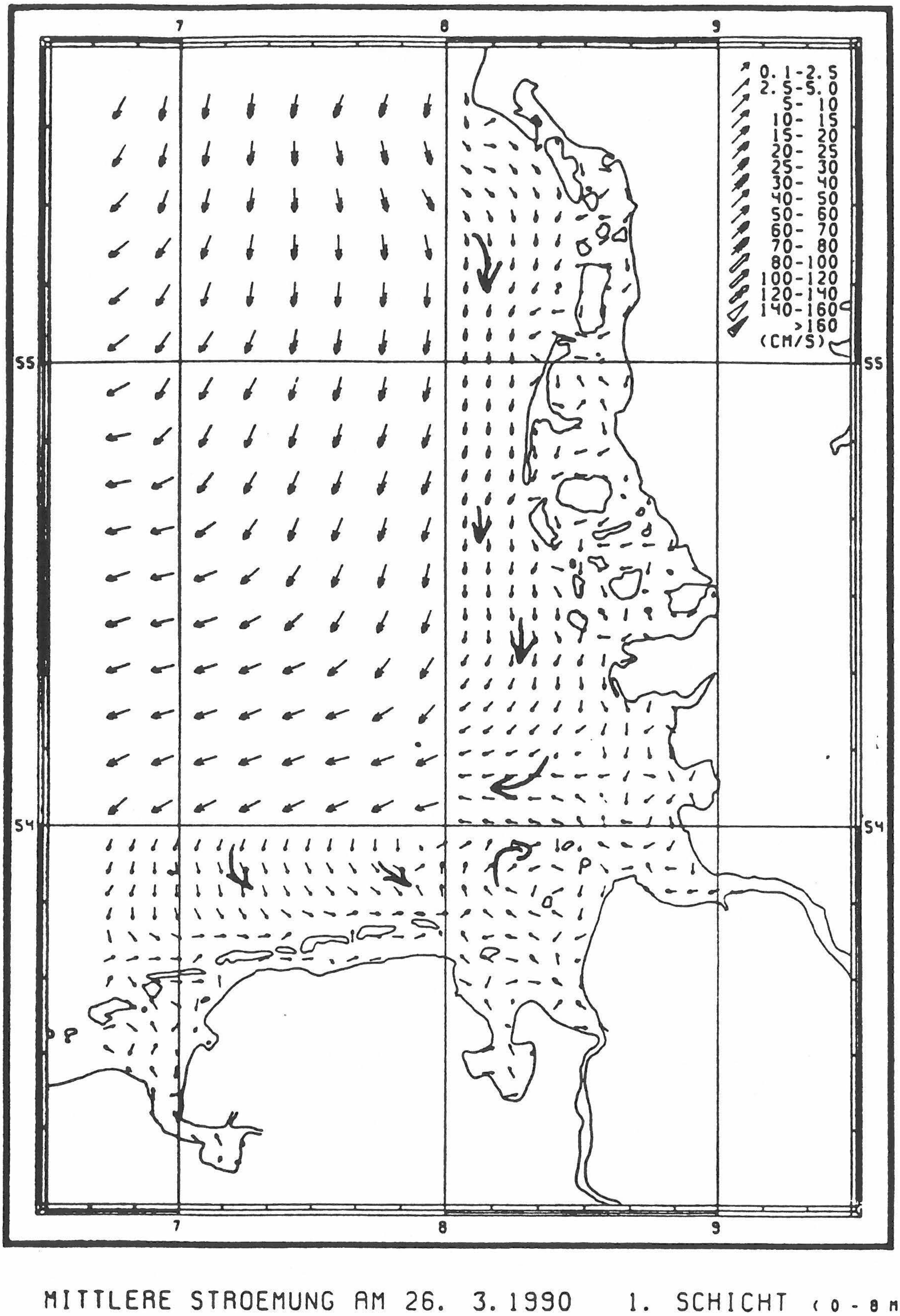


Figure 6. Surface layer residual current model calculation for 26 March 1990.

Wetterdienst (DWD). Actual wind strengths during the workshop were moderate to fresh (southwesterly Beaufort Force 3-4 on 17 March, increasing to Force 7 on 24 March, veering west north westerly on 25 March).

At the beginning of the workshop the residual current velocities calculated by the model (temporal means over two tidal periods) confirmed the usual circulation pattern (Fig. 5). However, in the coastal belt the mean current velocities were relatively weak (< 2.5 cm/s). Southeast of the Dogger Bank velocities in the upper 8 m increase to 10-15 cm/s. Towards the end of the experiment (26 March 1990), the residual current systems in the German Bight reversed direction and velocities increased to 20 cm/s (Fig. 6).

3. THE F-18-9 DRILLING SITE - CHEMICAL AND BIOLOGICAL BACKGROUND.

(Abstracted from a short paper by S.A. de Jong, Dutch Ministry of Transport and Public Works, North Sea Directorate, Rijswijk, The Netherlands; R. Daan, Netherlands Institute of Sea Research, Den Burg, The Netherlands; H. van het Groenewoud, TNO Laboratory for Applied Marine Research, Den Helder, The Netherlands).

Background information. The drilling site F-18-9 off the Dutch coast was used for exploration drilling for oil in 1987, but did not become a production platform. The contaminating input at the site is primarily drilling mud. Although oil-based drilling muds were introduced in the 1970s, at this site both water and oil-based drilling muds were used. Since the use of diesel oil-based muds was banned because of the toxicity of diesel oil, low aromatic oil compounds are used. Separation procedures recover some oil from the drilling cuttings, but this does not prevent the release of considerable quantities to the sea bottom in the vicinity of the drilling site.

The residual current direction at the site is approximately west-northwest (66^3), which was used as the orientation of the transect of stations used during the workshop. With no rig or platform at the site "dynamic positioning", together with sidescan sonar and video cameras, were used to relocate the exact site. (Remots data and sediment chemistry indicate that the region of highest contamination on the bottom may be at Station B, 125 m WNW of the site).

Previous Study of the Site. The Dutch Ministry of Transport (RWS, North Sea Directorate) initiated a study carried out by the Netherlands Institute of Sea Research (NIOZ), and the TNO Laboratory of Applied Marine Research, at the F-18-9 site a year after exploratory drilling ceased in June 1988. Grab samples for chemical analysis and for macrofauna were taken on four transects at 90^3 to each other with drilling site at their centre. One transect was aligned with the direction of the residual current, and so was the same as that used for the workshop.

Chemical data for this transect showed a clear gradient of decreasing barium and oil in the sediment samples (Fig. 7). The vertical distribution of oil in the upper 10 cm revealed that in June 1988 76% of the total oil concentration was found in the upper 2 cm, while the remaining 24% was found in the 2-10 cm section of the cores.

A study of the benthic community along the transect demonstrated that not only did the abundance of benthos increase with distance from the drilling site (Fig. 8), but species richness was also significantly reduced. These effects were considered to be the net result of drilling cuttings contaminated with oil-based drilling mud, effects occurring at oil concentrations of 20-400 mg per kg DW. Below 20 mg per kg effects apparently decline gradually in the first year after drilling.

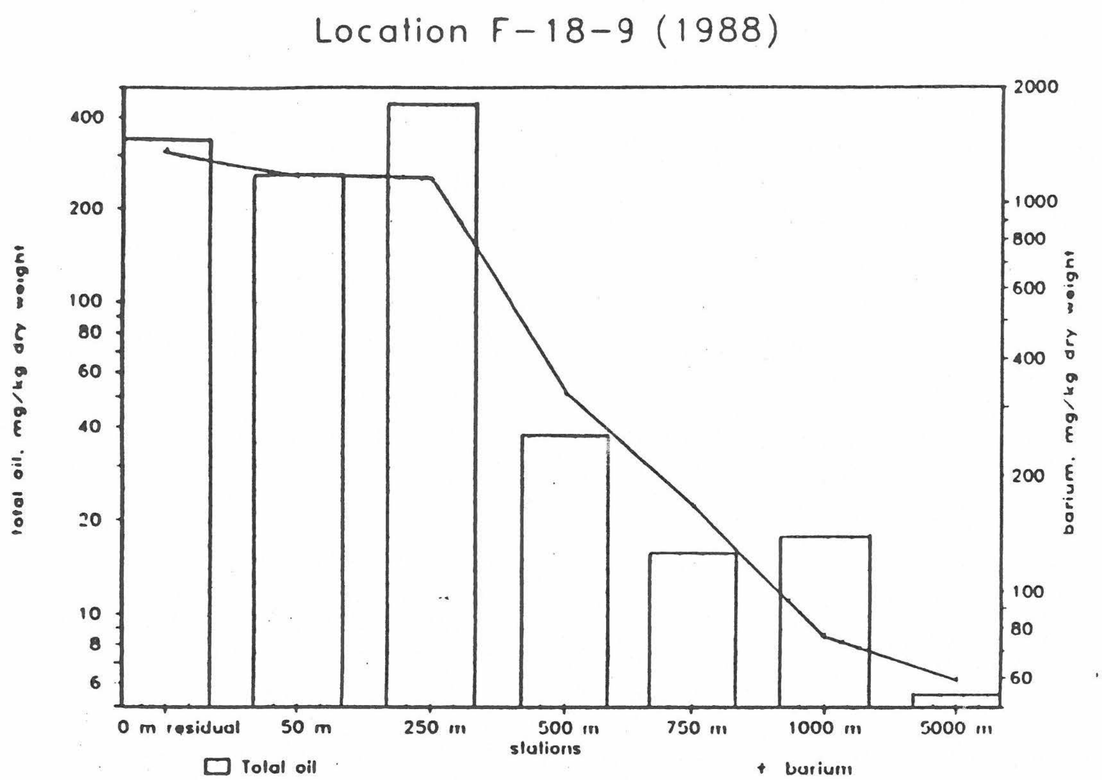


Figure 7. Concentrations of oil (histogram) and barium (line) in sediment taken from the drilling site transect.

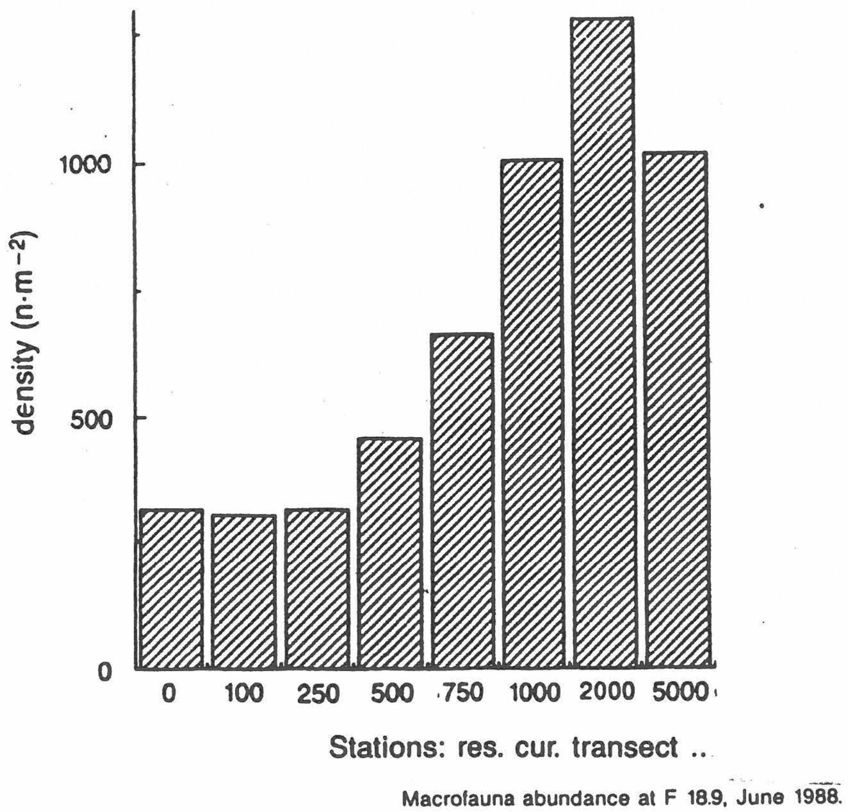


Figure 8. Abundance of benthic organisms along the drilling site transect.

4. POPULATION BIOLOGY OF THE DAB (*Limanda limanda*). (Abstracted from the paper by A.D. Rijnsdorp, Netherlands Institute for Fishery Investigation, Ijmuiden, The Netherlands; A.D. Vethaak, Rijkswaterstaat, Tidal Waters Division, The Hague, The Netherlands; P.I. van Leeuwen, Netherlands Institute for Fishery Investigation, Ijmuiden, The Netherlands.

Importance of mobility for interpreting workshop results. The use of the dab as an organism for monitoring purposes depends critically on its mobility. The clearest indication of environmental quality at any point will be given by a species that does not move, and the greater the distance and speed of its migrations the more its movement will obscure any response to spatial or temporal contamination gradients. Evidence relevant to these questions is considered from existing data on the maturation and spawning of the dab, their distribution and migrations, as well as that from the workshop itself.

The distibution of eggs (Fig. 9) indicates spawning areas for the dab in the southeast North Sea, the major concentrations of eggs occuring in the German Bight, north of the Frisian Islands and along the southern edge of the Dogger Bank. The German Bight transect as well as the Drilling site gradient coincide with major spawning areas. Spawning takes place from January to September, reaching a peak from February to April. The period of the workshop therefore coincides with peak spawning activity. O-group dab occur in coastal and estuarine waters typically 3-10 m deep (Fig. 10) and the smallest size groups decrease in density offshore. 1-group fish occur in highest numbers in coastal regions of the southern North Sea. Larger dab are found further offshore (Fig. 10). The spatial separation of eggs and young dab indicates they must move inshore from the open sea spawning areas to the coastal nursery grounds; then as adults the fish move offshore, the large fish preceding the smaller and the males preceding the females.

Size distributions. The size group of dab used by most of the workshop participants was the female dabs with a length of 20-24 cm, typically representing fish of 4-6 years of age. However such fish have a lower mean age at stations 3 and 9 than at stations 5-8. Differences in age structure and abundance at different stations during the workshop, and between cruises, suggests movement during this period.

Tagging experiments. No clear pattern of dab migration emerges from the tagging experiments to date, except that all the fish migrate significant distances. The dab population at a sampling site is therefore a transient aggregation of fish originating from a large area. In the German Bight, between February and March, an east or southeastward migration predominates, resulting in a net emigration of dab from the offshore sites to the inner German Bight sites. While this may be unimportant for those responses that are rapidly induced (eg biochemical indices), it is of paramount importance for the correct interpretation of "slow response techniques" such as gross pathology (the epidemiology of liver tumours) and tissue residue data.

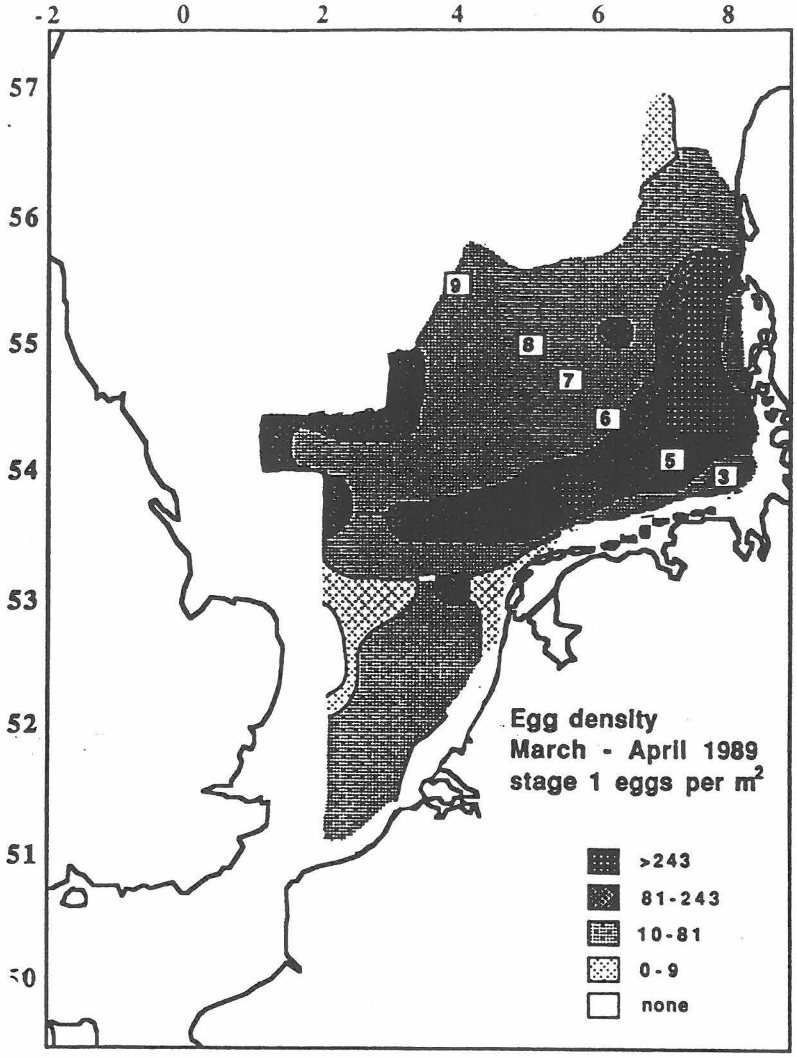


Figure 9. Dab egg density in the North Sea.

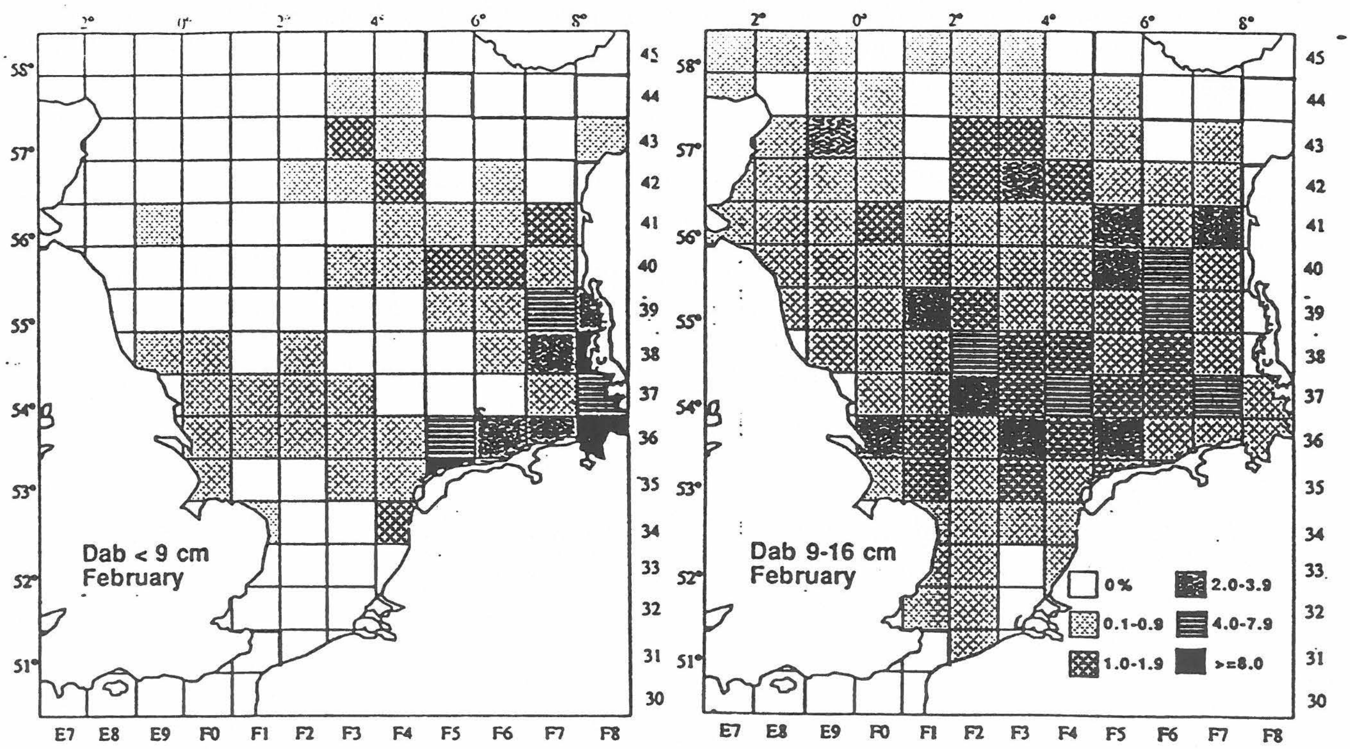


Figure 10. Distribution of dab in the North Sea < 9 cm and 9-16 cm in length.

5. CHEMISTRY OF THE WORKSHOP SAMPLES (Coordinator: W.Cofino)

Workshop tissue, water and sediment samples appropriate to interpreting results from the biological techniques tested during the workshop were analysed by A. Abarnou (IFREMER, France), J. Boon (NISR, Netherlands), I. Davies (SOAFD, UK), A.de Jong (TWD, Netherlands, J Klungsoyr (IMRN, Norway), K Booij (NISR, Netherlands), R.Law (MAFF, UK), F.Smedes (TWD, Netherlands) and W.Cofino (TWD & IvM, Netherlands). Analyses of metals in water surface microlayer and subsurface samples were analysed during the workshop by J. Cleary (PML, UK) (see Section 9, Water Quality Bioassays). Metals in water samples from the German Bight transect were analysed by D. Schmidt (BSH, Germany).

Objectives The objectives of the chemistry programme were to assess the level of contamination and the spatial distribution of contaminants along the transects in relation to the biological techniques deployed. The compartments analysed were water samples, sediments (unfractionated and < 63 microns), dab liver and five benthic species (Aphrodite aculeata, Pagurus bernhardus, Ophiura texturata, Nephtys hombergii, Nucula nitidosa). Trace metals were measured in all compartments, organochlorines in sediments and tissues, and aromatic hydrocarbons in sediments and benthic biota only.

Sampling Water samples were taken using teflon water samplers. Samples for analysis were deep frozen, apart from those for mercury determination which were stored at ambient temperature. Filtration was with 0.4 micron nuclepore membrane filters, while those unfiltered were acidified as quickly as possible.

For other compartments 3-6 samples were taken at each station. Samples of dab livers and individual benthic organisms were pooled for analysis. The sampling scheme and protocols were was not always adhered to. Sample administration in the field was not wholly effective, due in part to a lack of personnel on the vessels whose main responsibility was the samples for analysis. There were also problems with the preservation of dab liver samples. Nevertheless a substantial set of chemical data has been brought together which characterise the contamination gradients on both transects.

Conclusions -German Bight. Parameters describing unfractionated sediment characteristics correlate highly, suggesting that the trends seen in whole sediments are due to dilution of contaminants by sand with increasing distance offshore. The fine fraction (< 63 microns) reveal more detail and suggestive trends, but interpretation is difficult due to the decline in the proportion of the fine fraction and changing organic carbon content at the offshore stations.

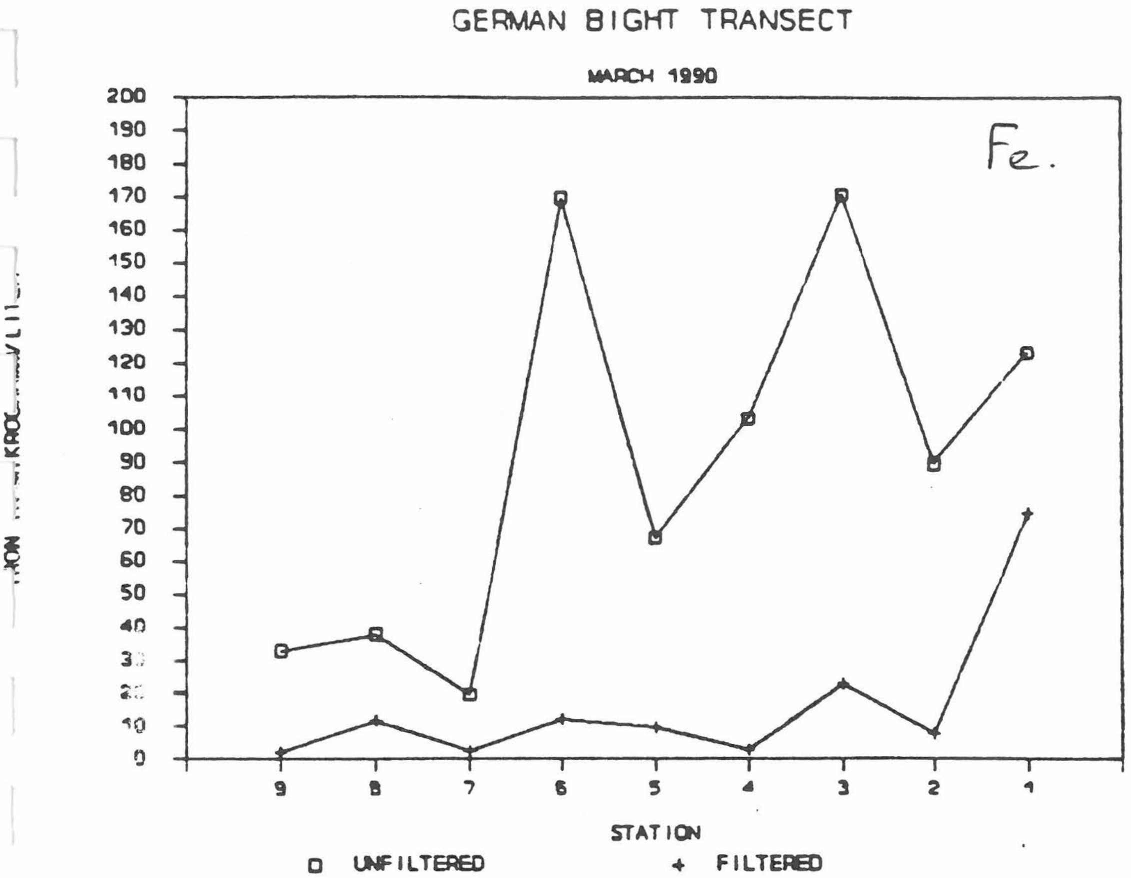
a. The dominant trend in the metal data for water samples is a decline in concentrations offshore, with highest concentrations for all metals analysed at Station 3.

b. For all compartments CBs decrease in concentration from Station 1 to 9. Unexpected haul differences for CBs in dab liver may corroborate the suggestion that migration has brought together at spawning fish with different histories of exposure.

c. Zinc in the fine sediment fraction decreases in concentration with distance offshore.

d.PAHs decrease offshore in Pagurus, Aphrodite, and total hydrocarbons (UV) in sediments.

e. On the drilling site gradient fluoranthene, chrysene, pyrene, benz[a]pyrene and benz[a]anthracene decrease over the gradient in Aphrodite.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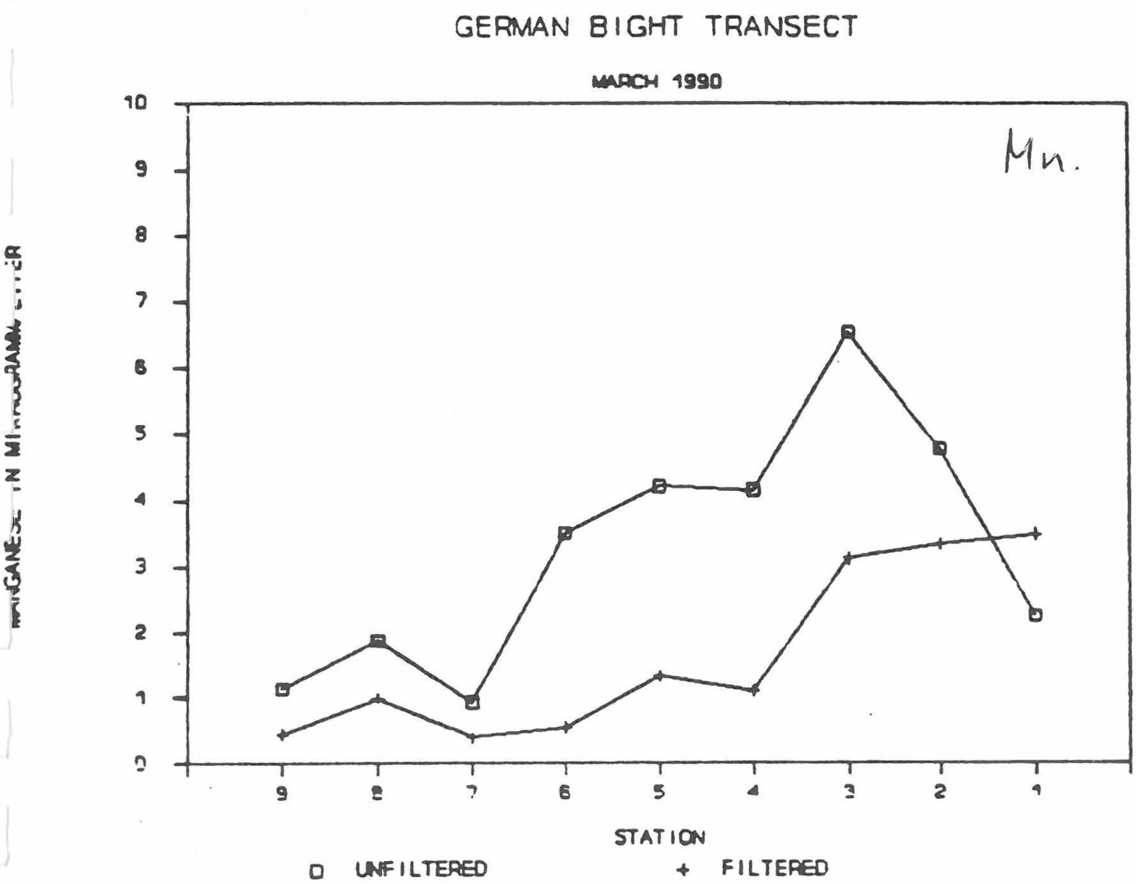
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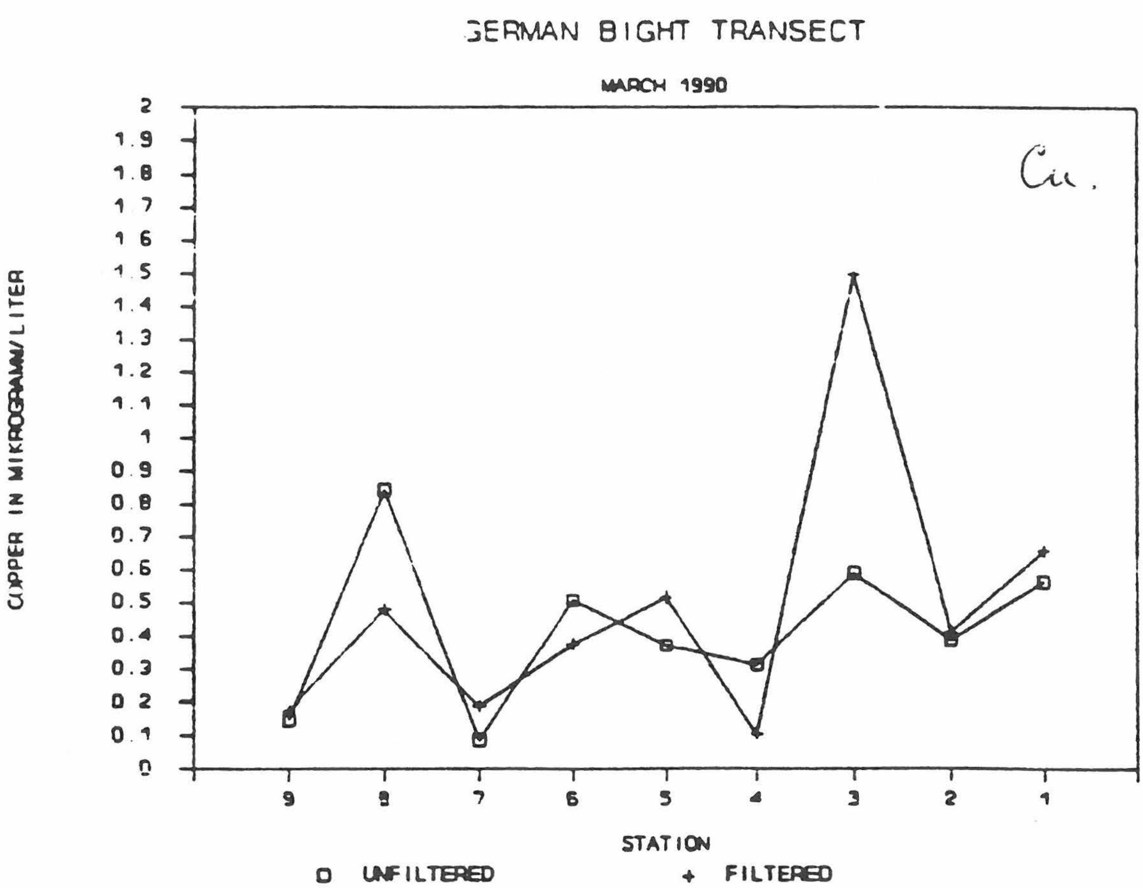
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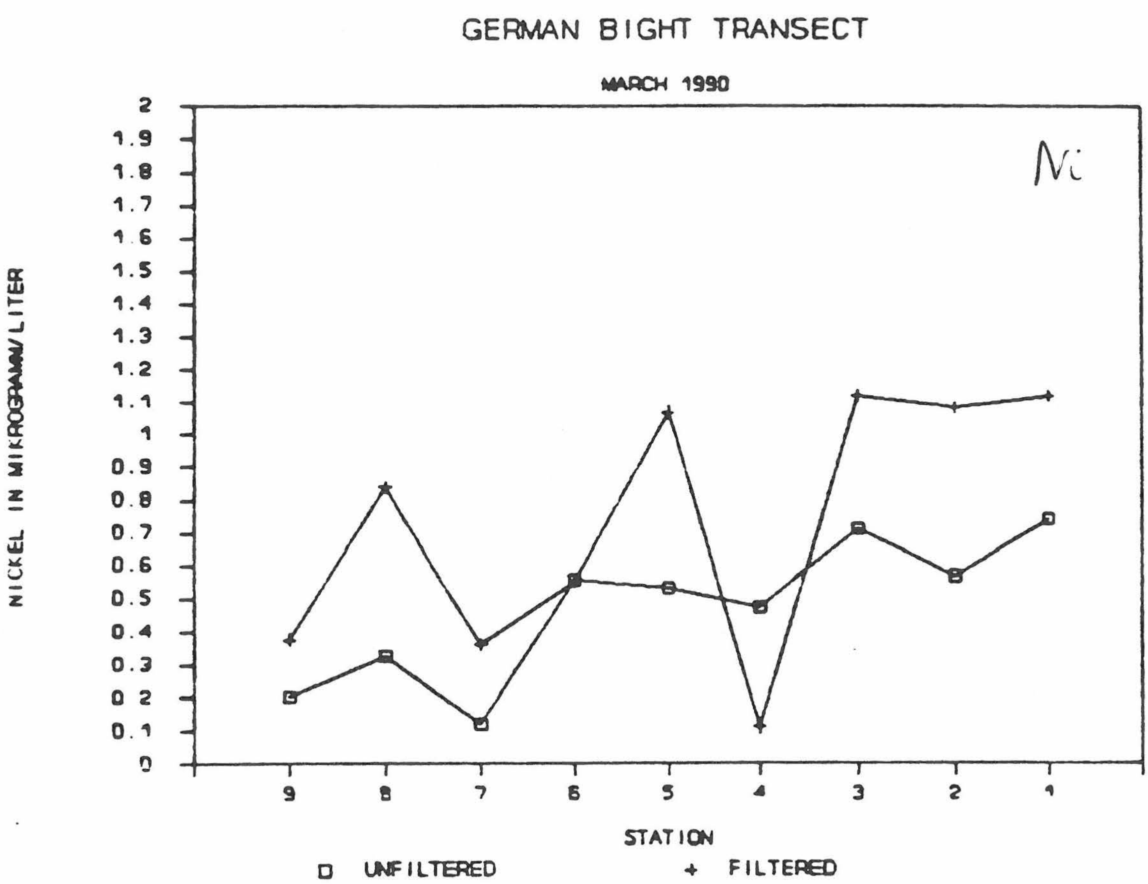
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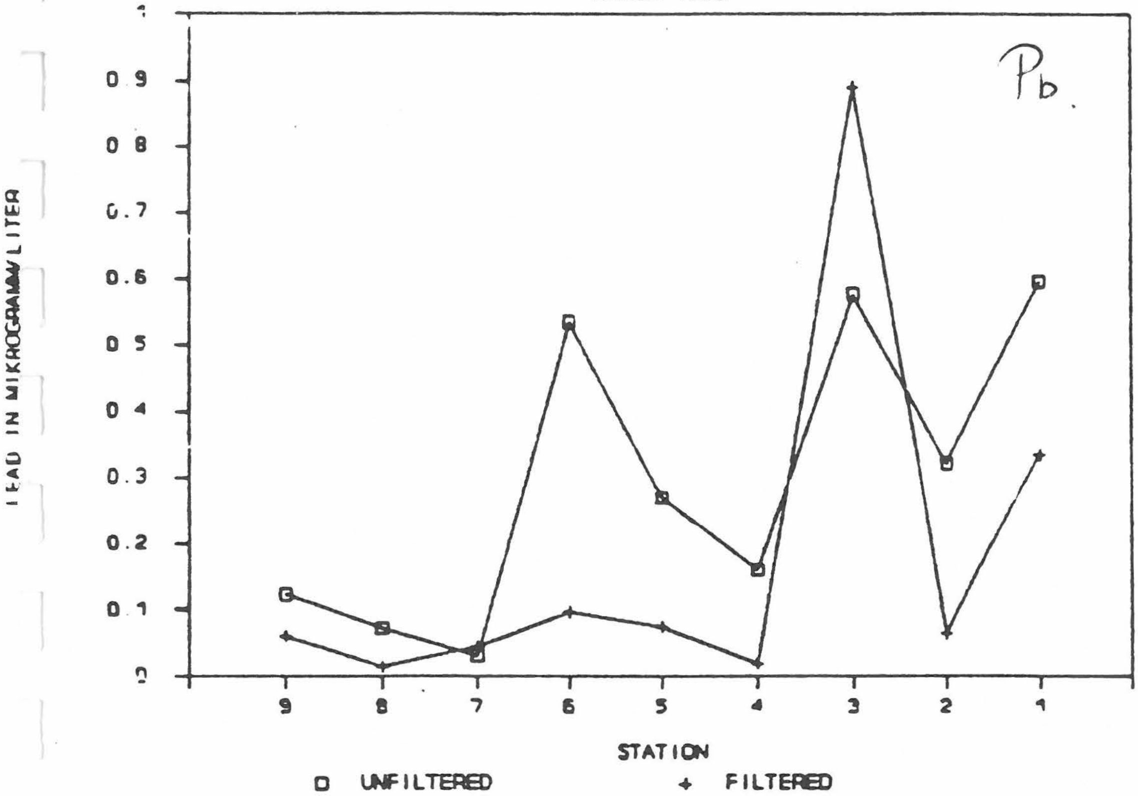
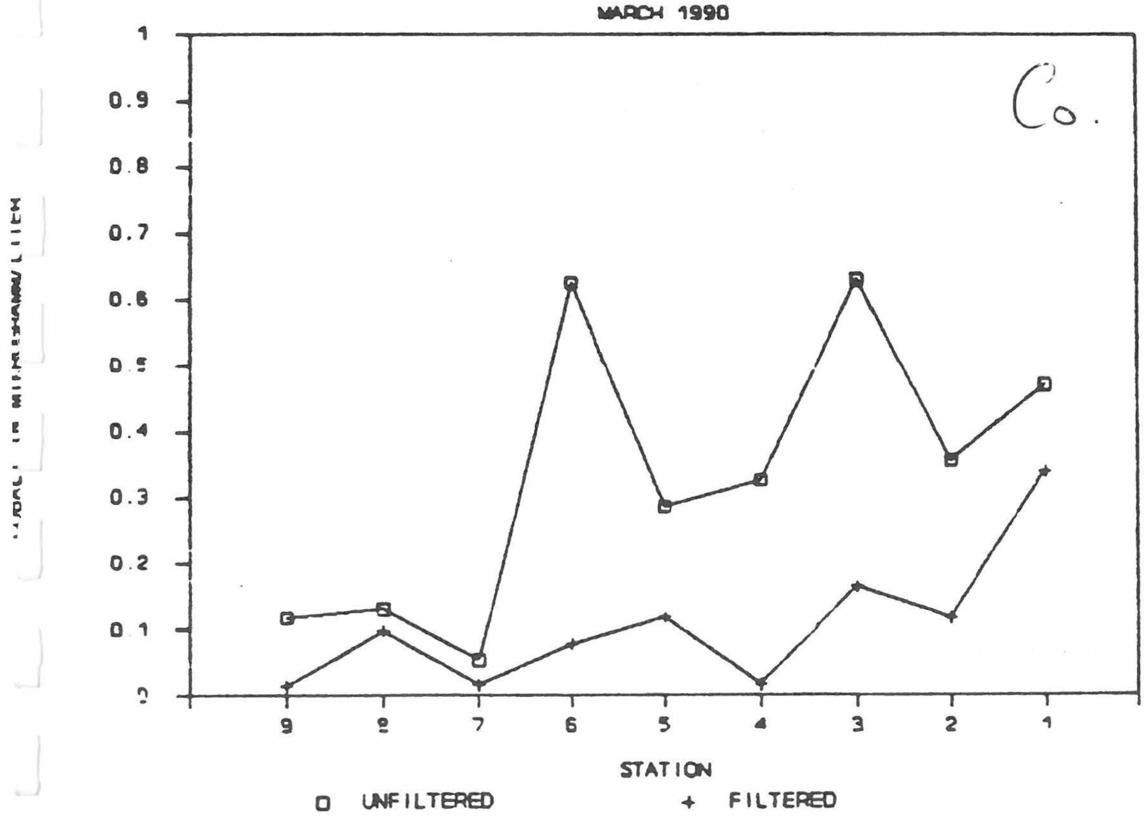
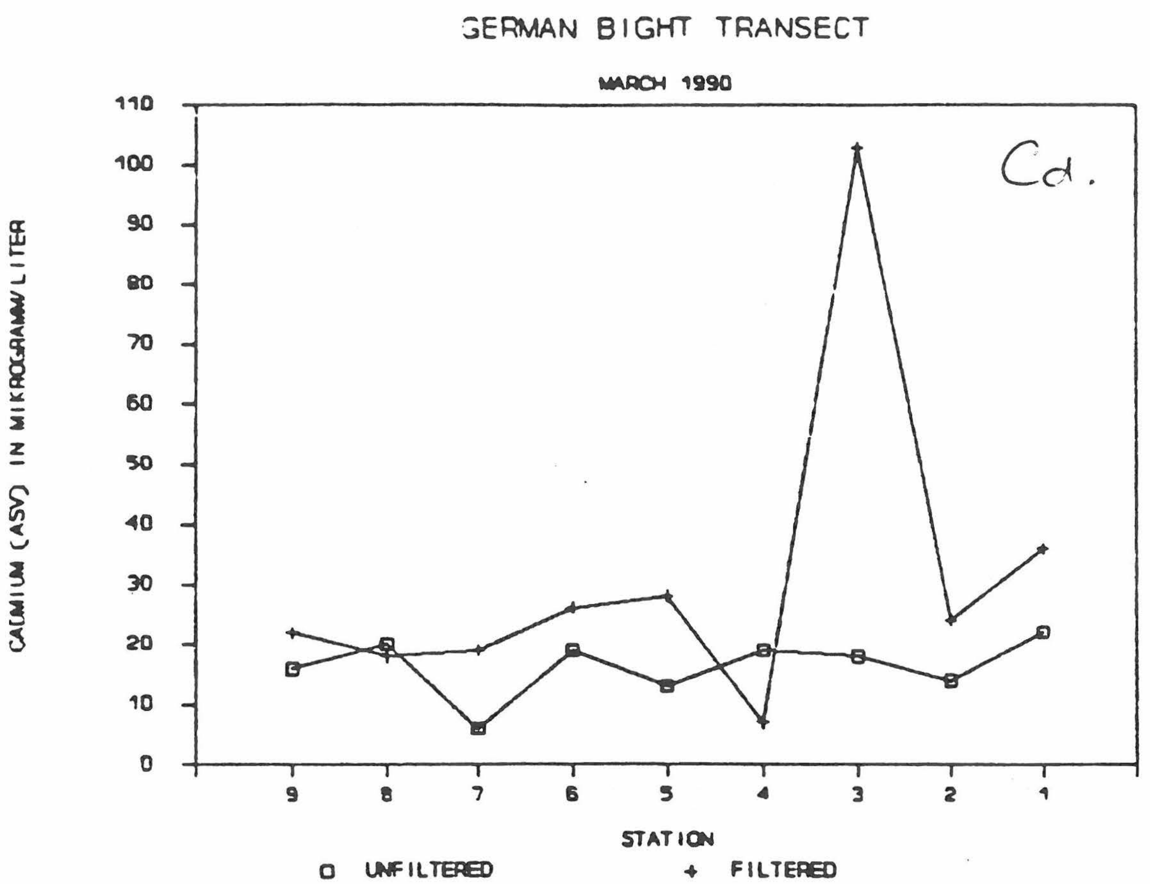


GERMAN BIGHT TRANSECT

MARCH 1990

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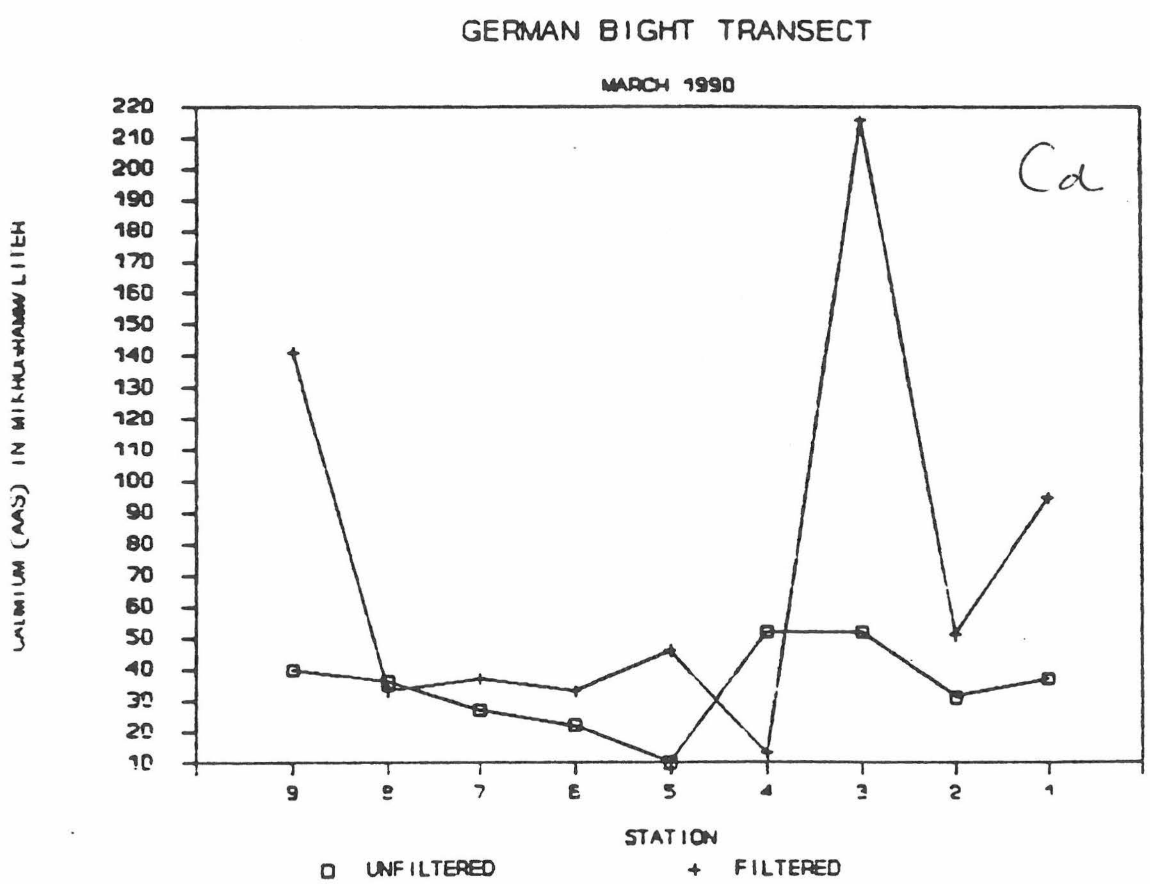
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GERMAN BIGHT TRANSECT

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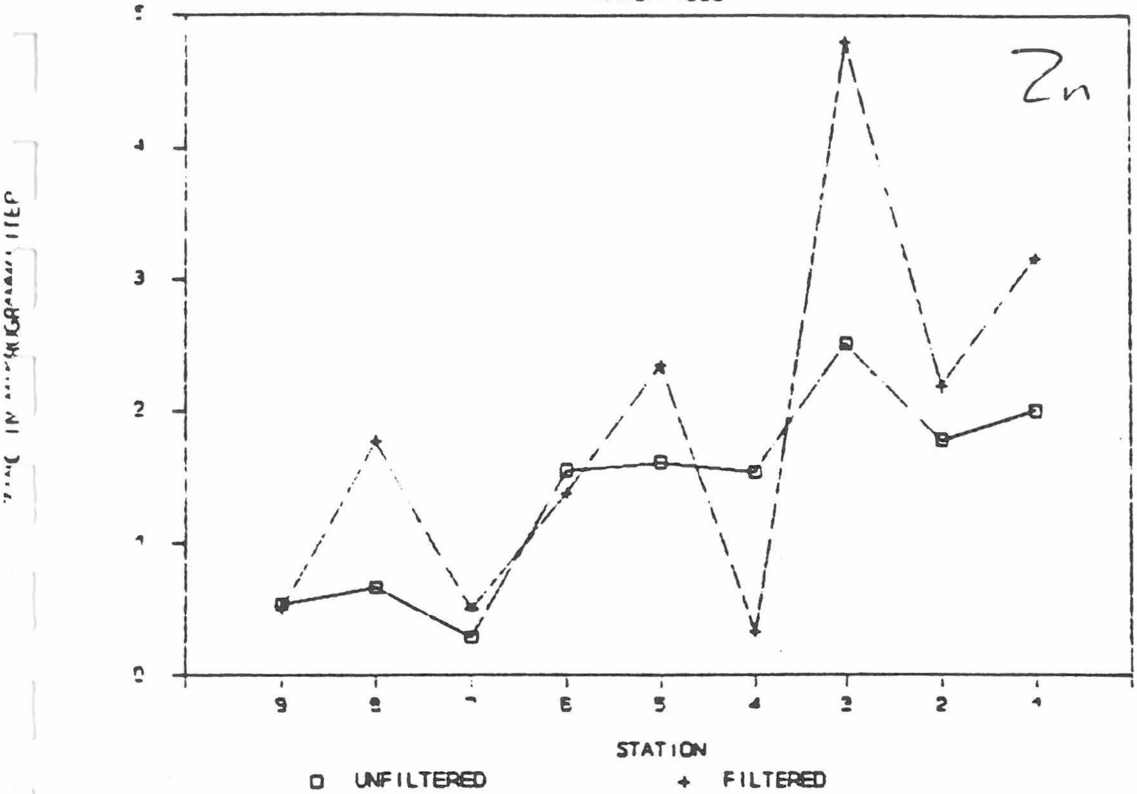
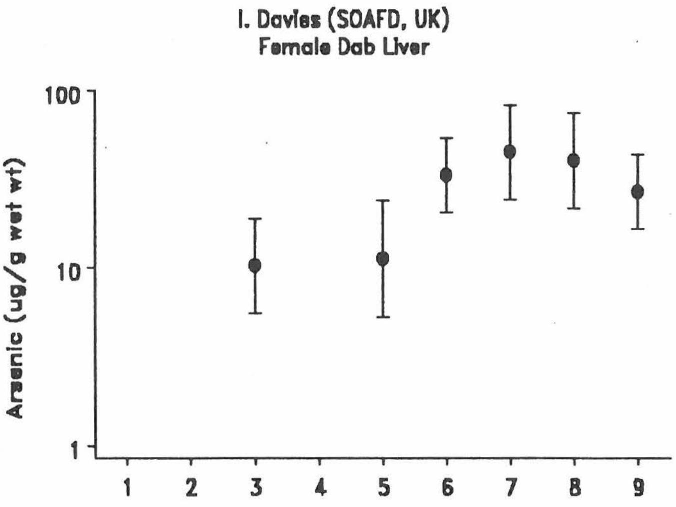
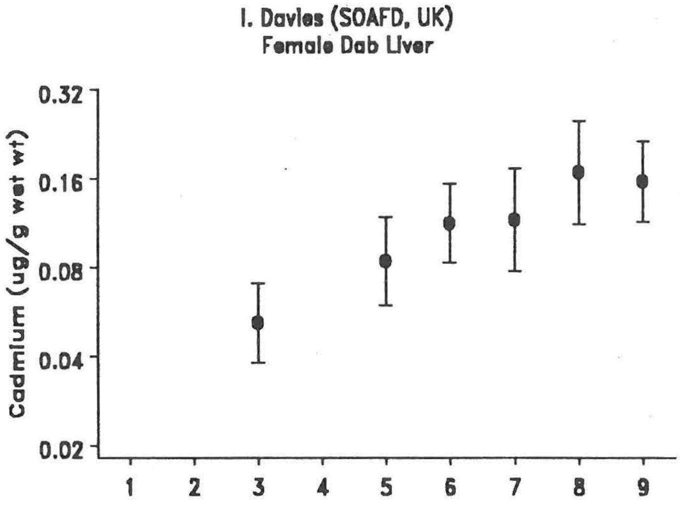
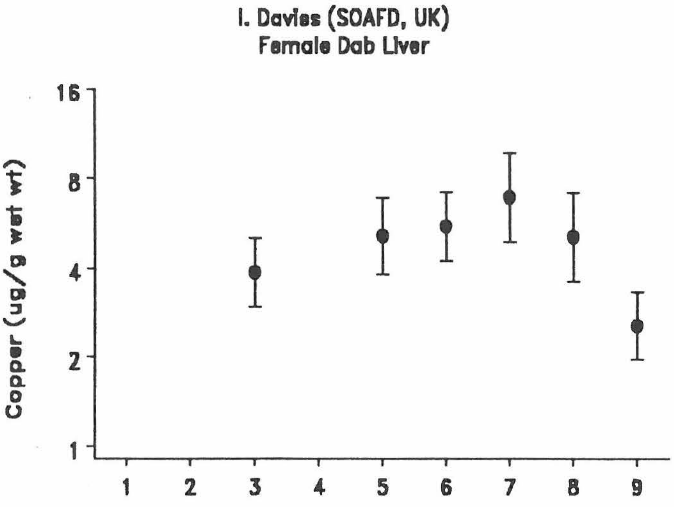
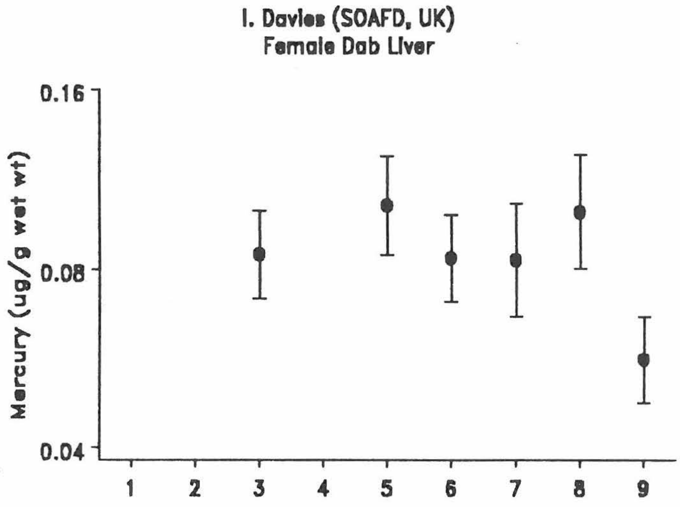


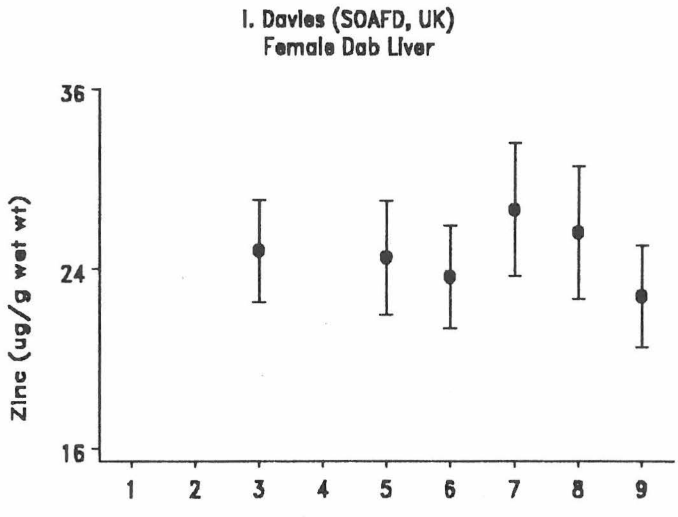
GERMAN BIGHT CHEMISTRY
FEMALE DAB LIVER



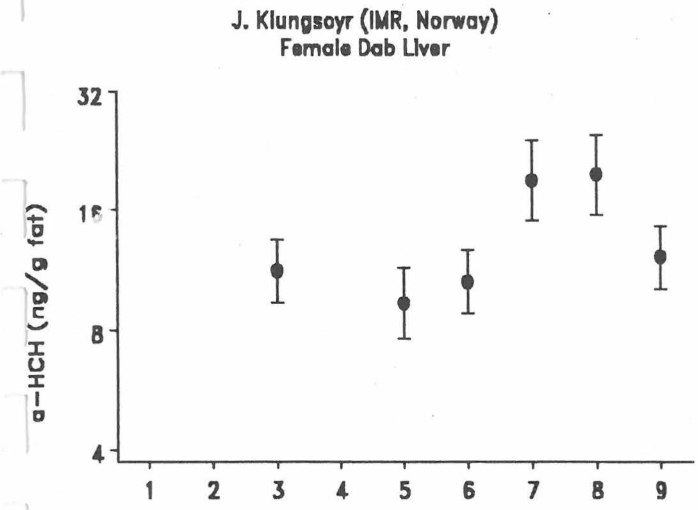


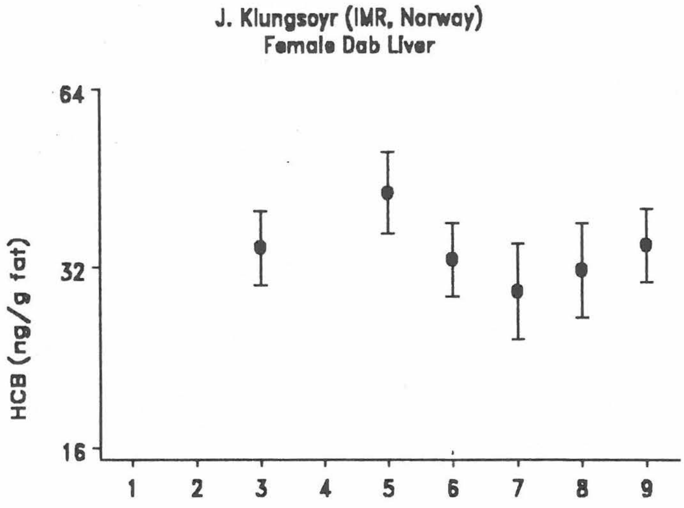


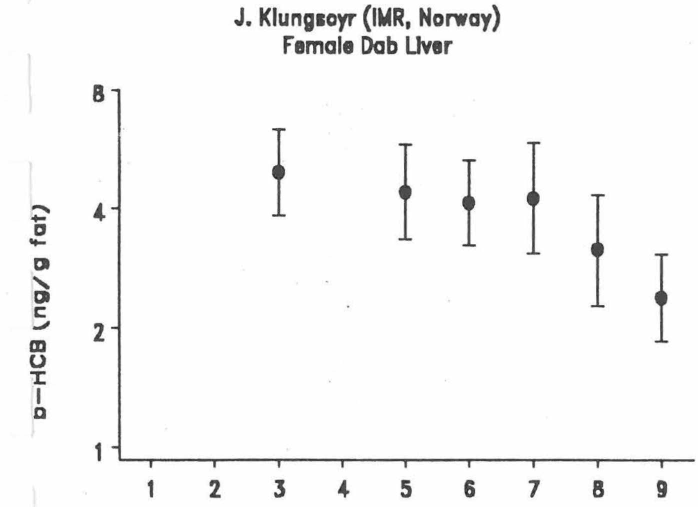


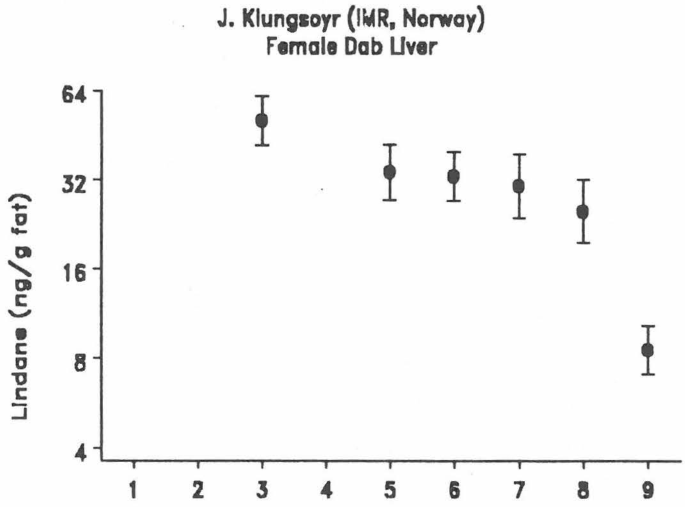


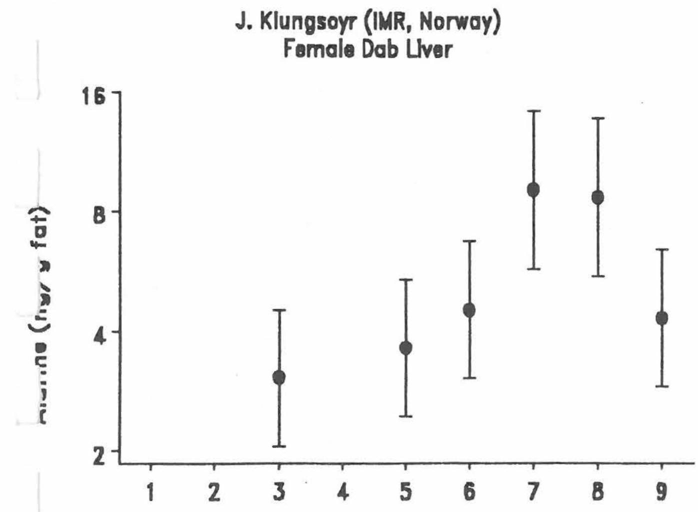
GERMAN BIGHT CHEMISTRY
FEMALE DAB LIVER

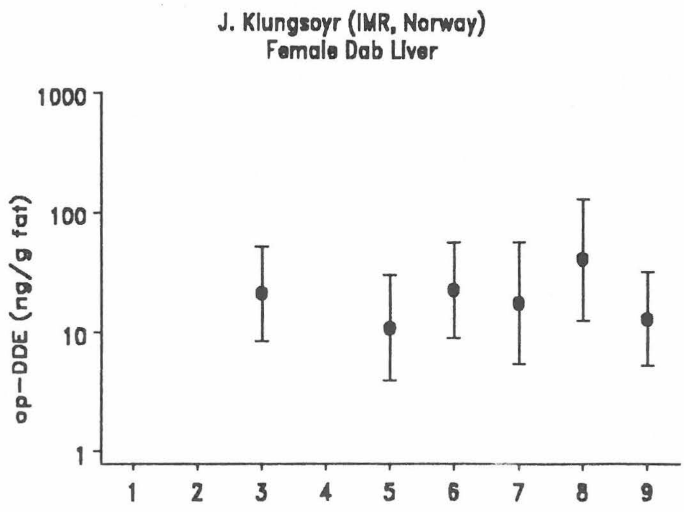




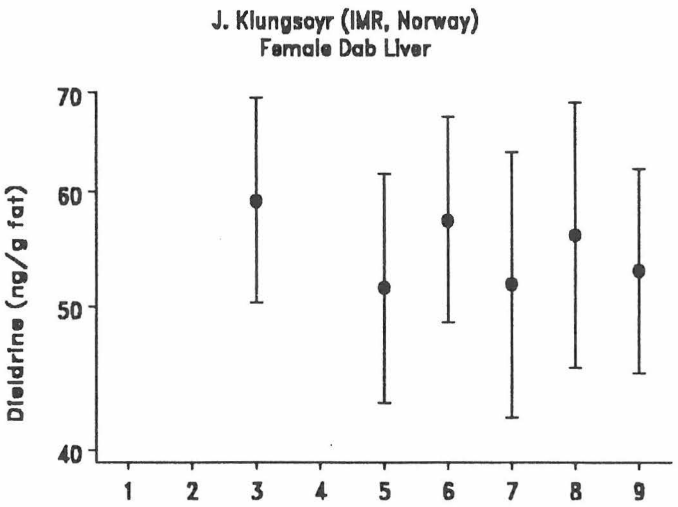


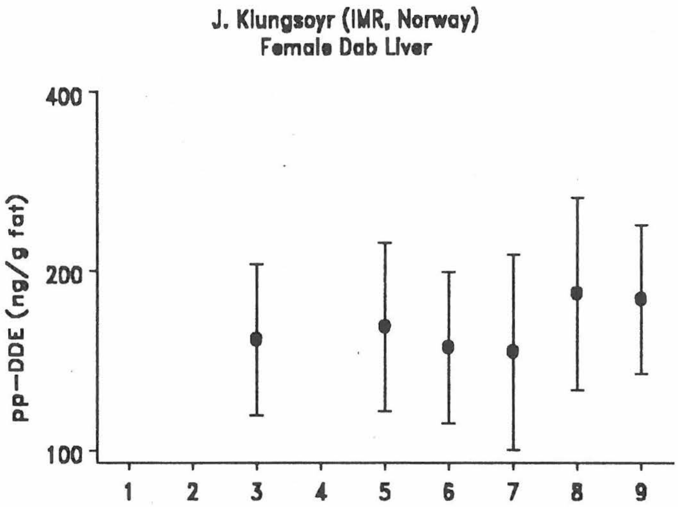


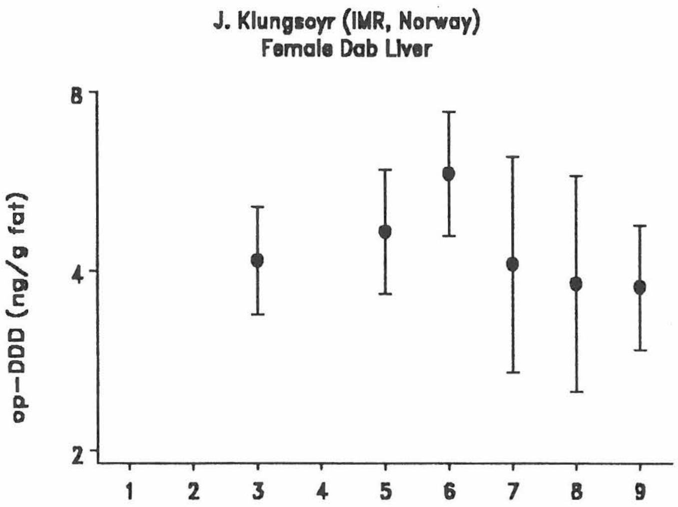


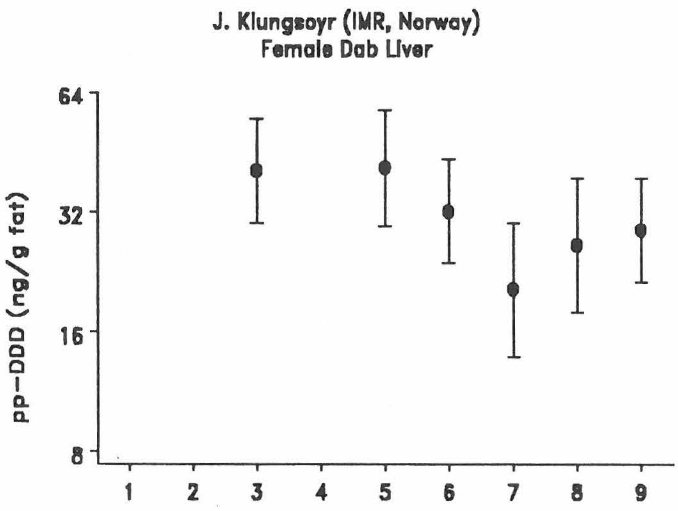


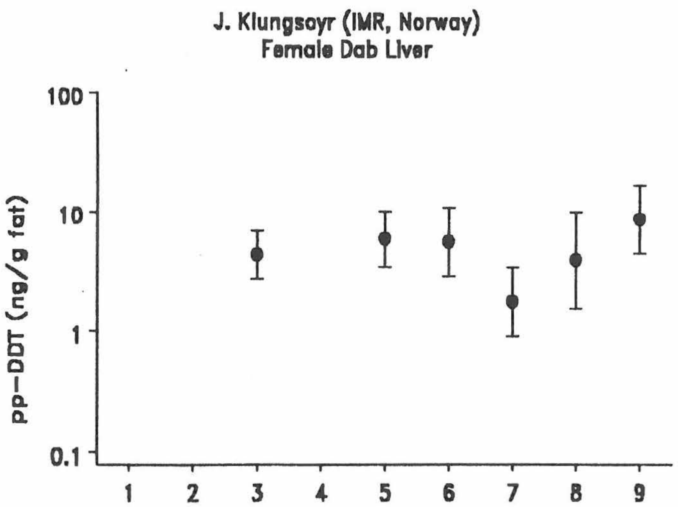
GERMAN BIGHT CHEMISTRY
FEMALE DAB LIVER





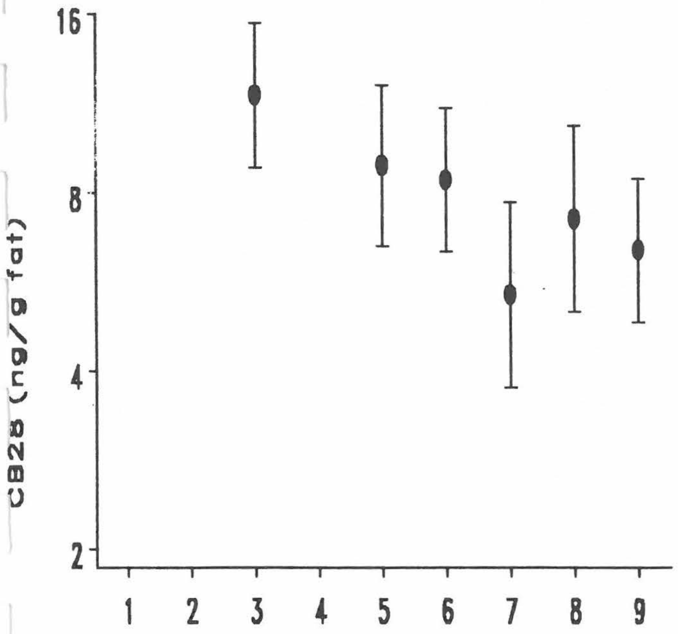




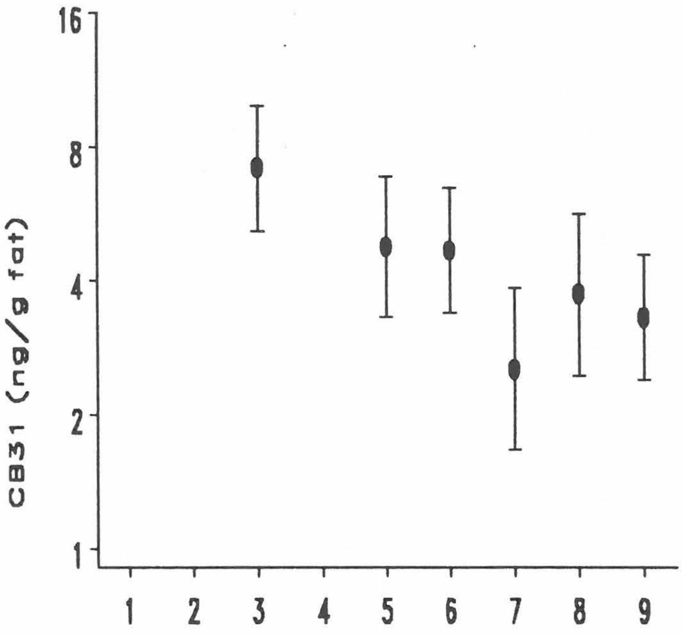


GERMAN BIGHT CHEMISTRY
FEMALE DAB LIVER

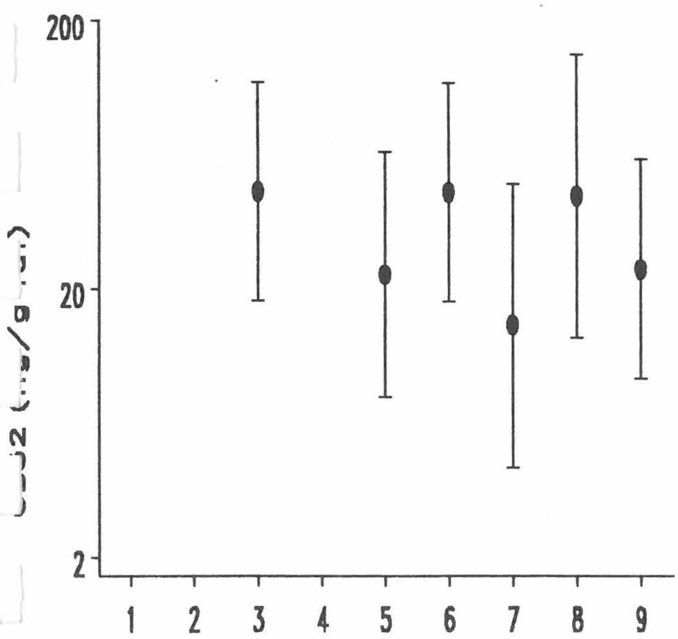
J. Klungsoyr (IMR, Norway)
Female Dab Liver



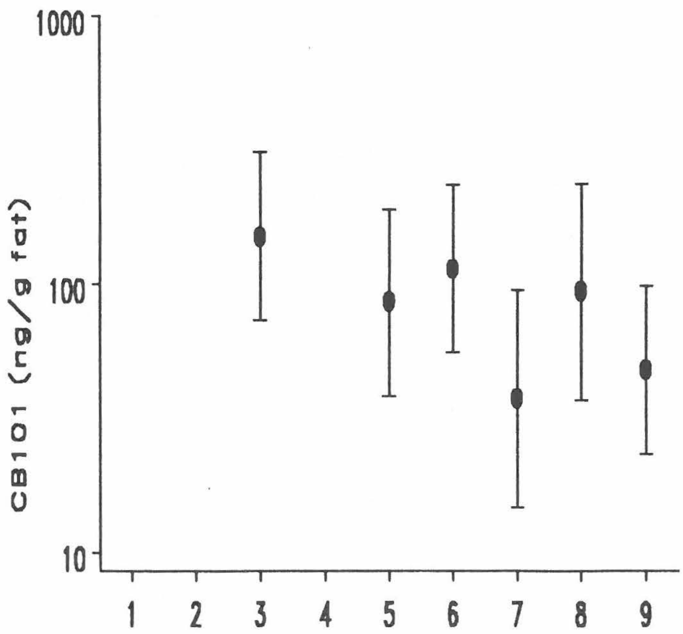
J. Klungsoyr (IMR, Norway)
Female Dab Liver



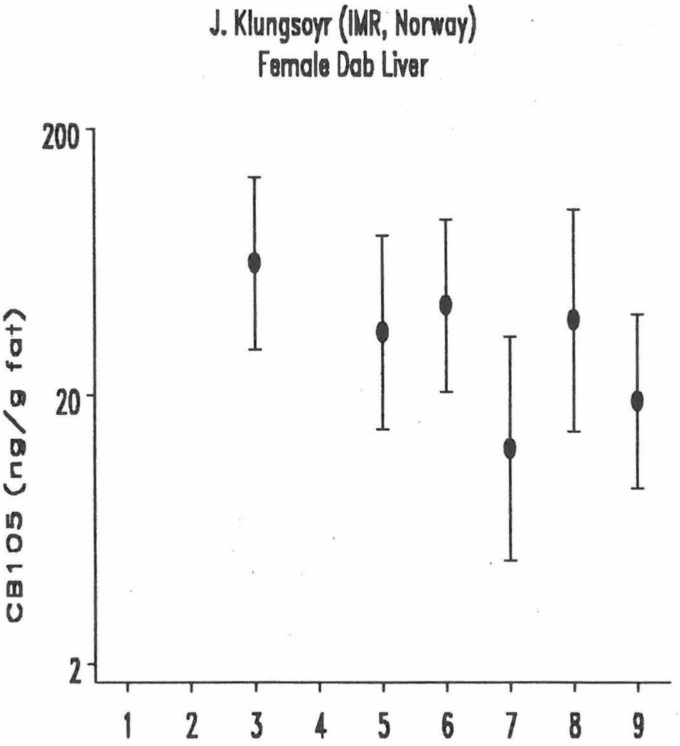
J. Klungsoyr (IMR, Norway)
Female Dab Liver

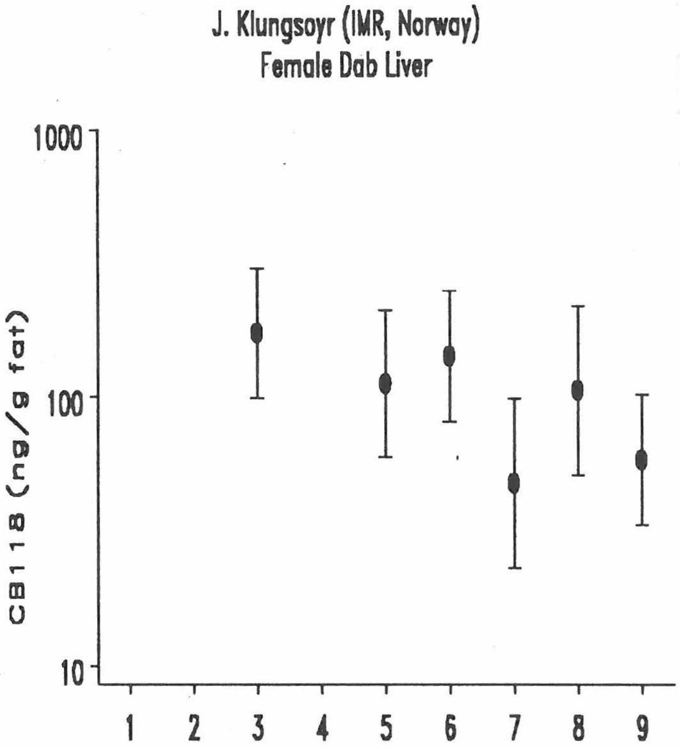


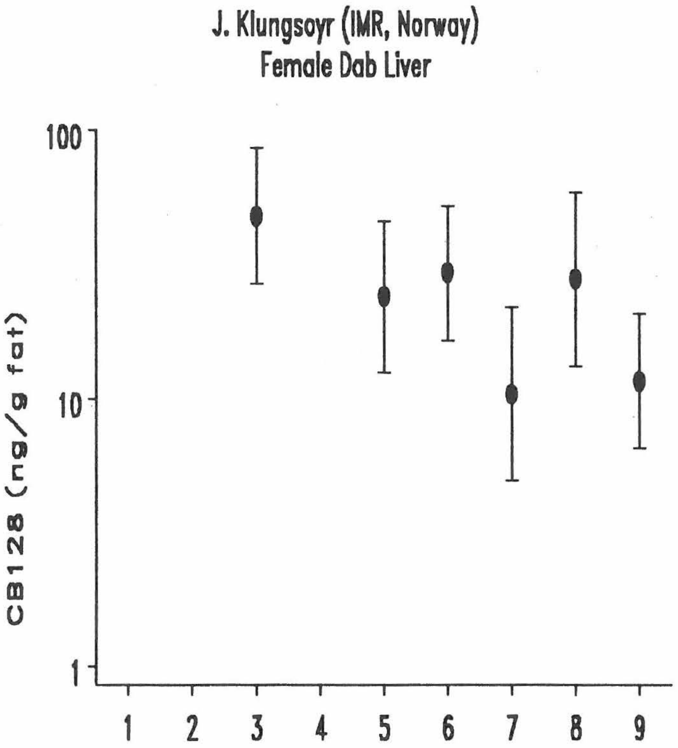
J. Klungsoyr (IMR, Norway)
Female Dab Liver

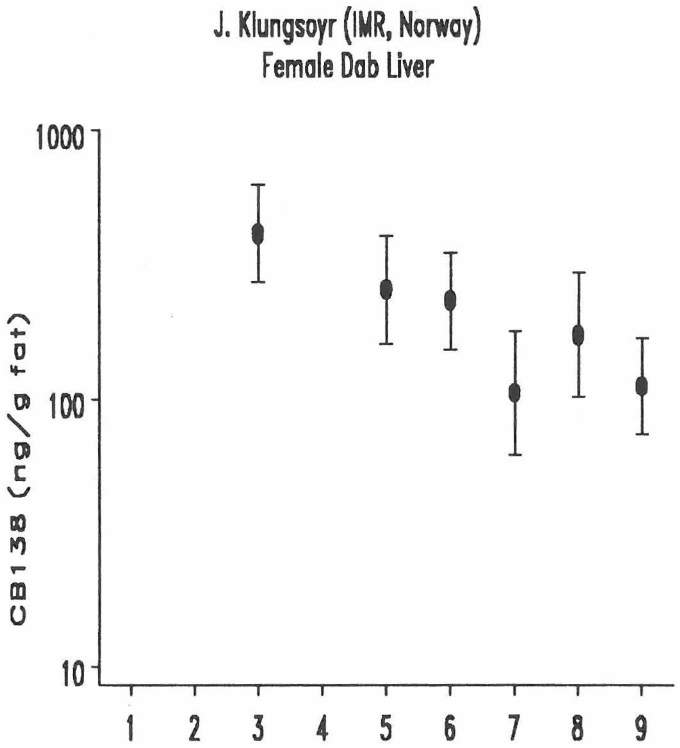


GERMAN BIGHT CHEMISTRY
FEMALE DAB LIVER

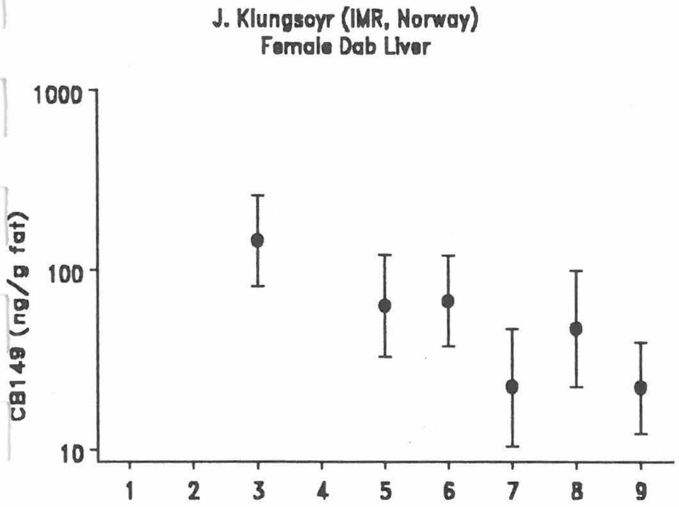


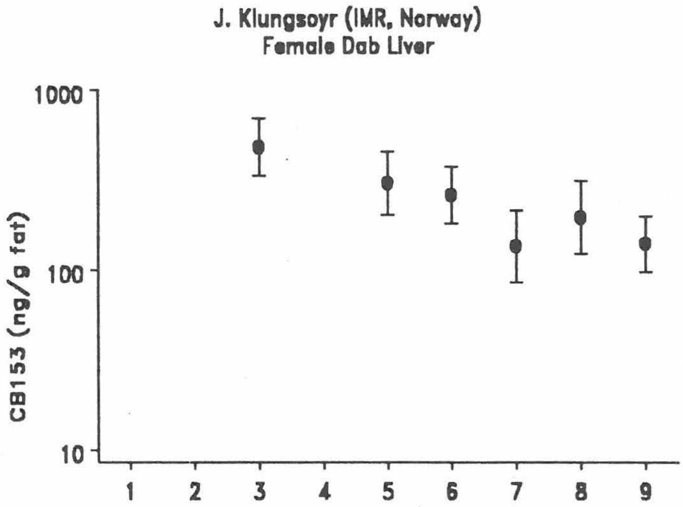


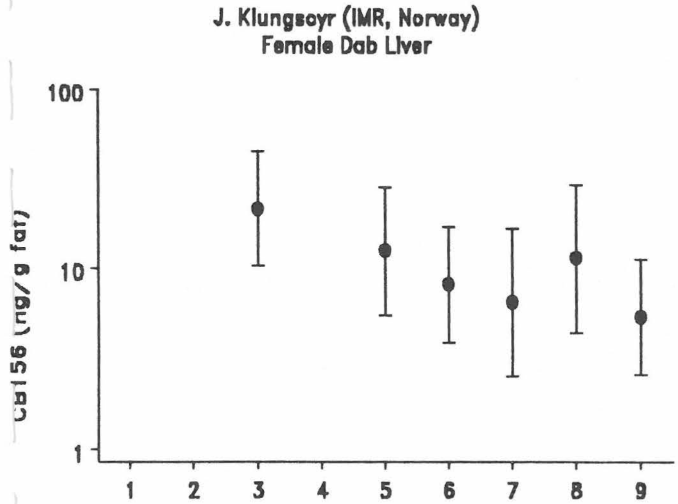


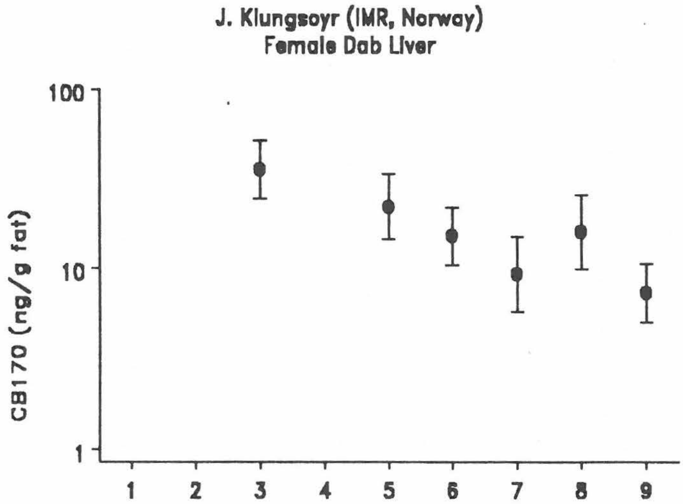


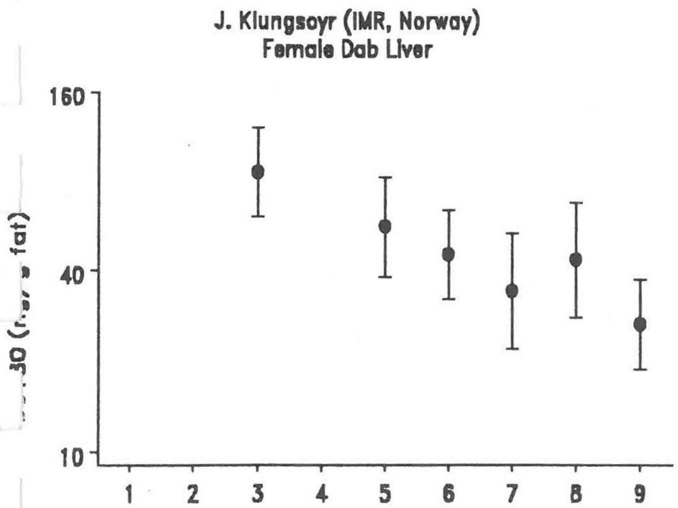
GERMAN BIGHT CHEMISTRY
FEMALE DAB LIVER



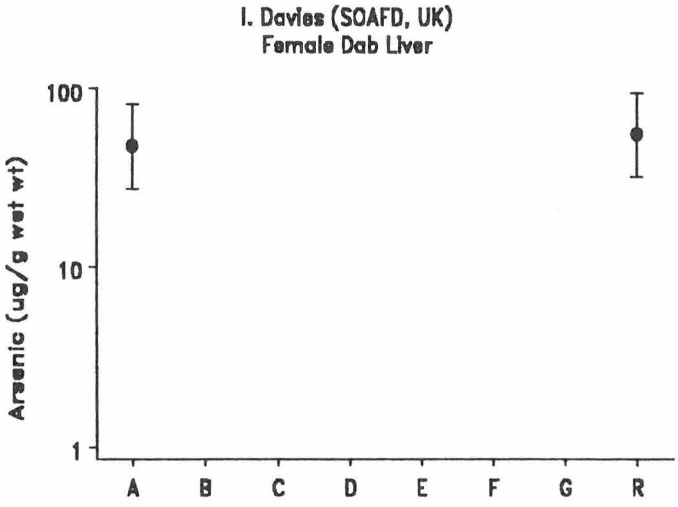


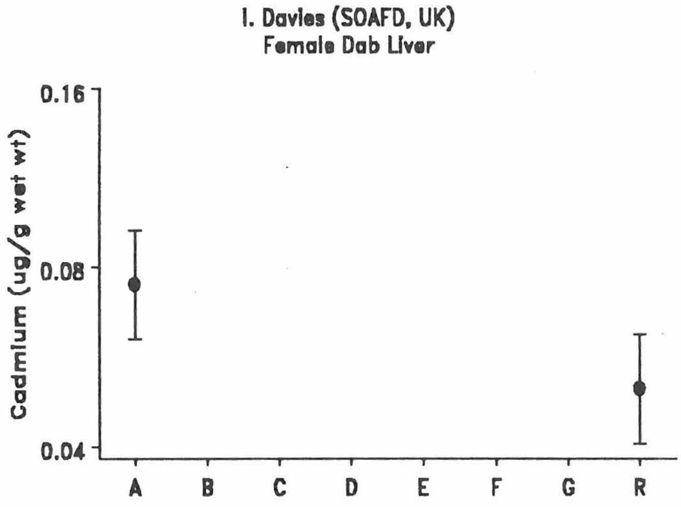


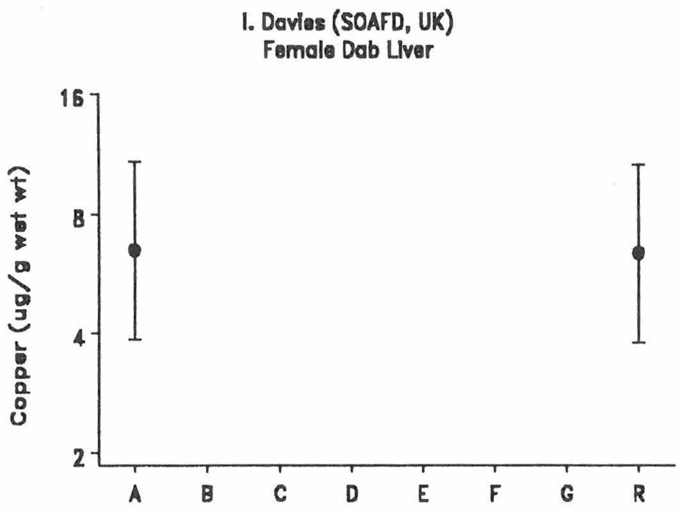


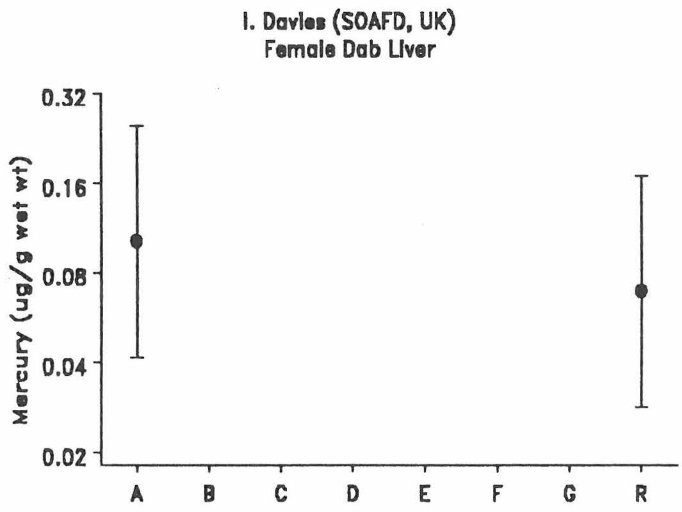


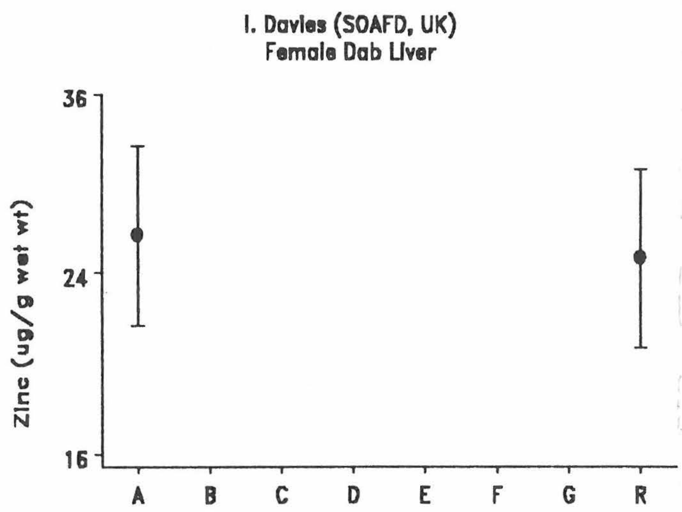
OIL PLATFORM CHEMISTRY
FEMALE DAB LIVER



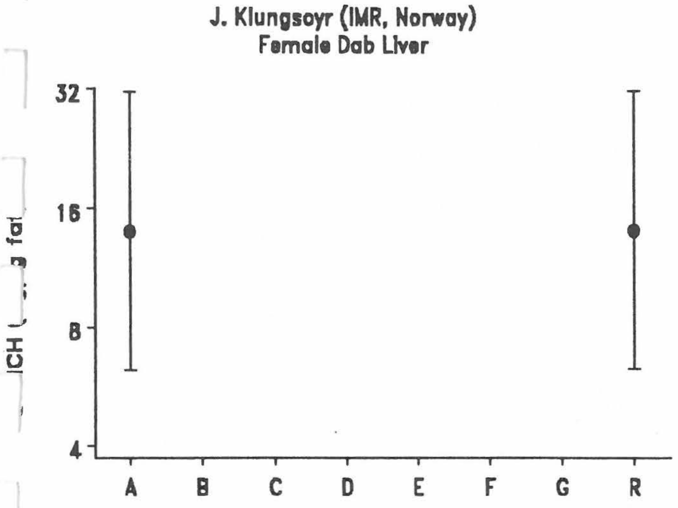


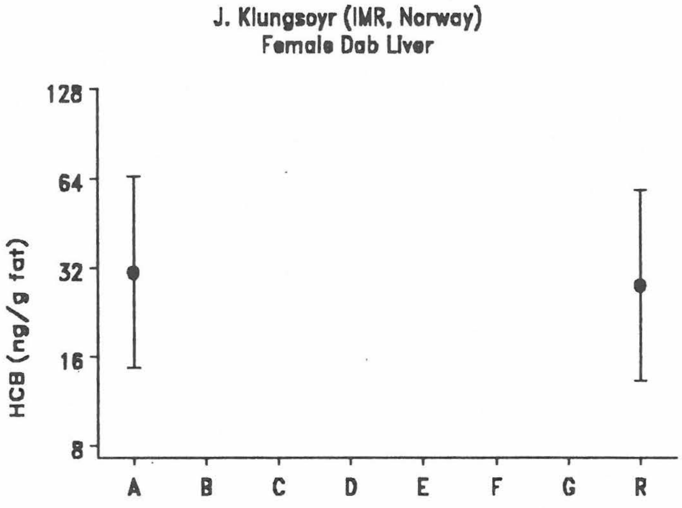


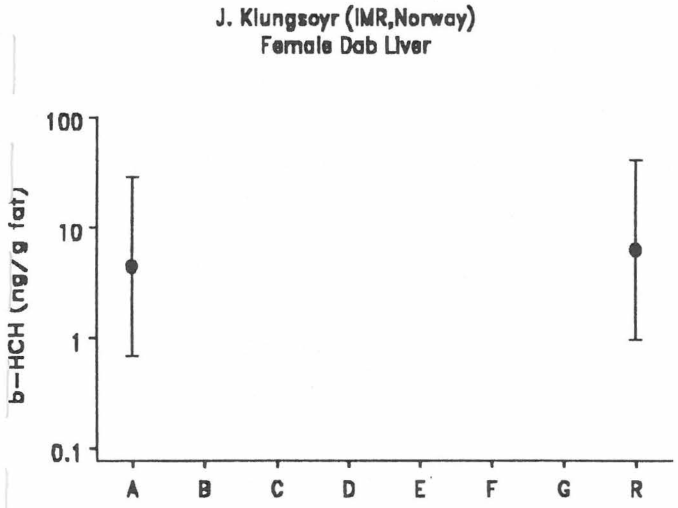


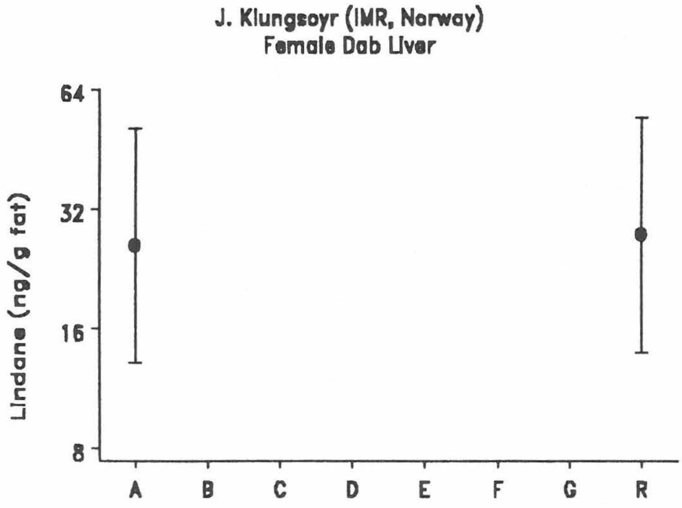


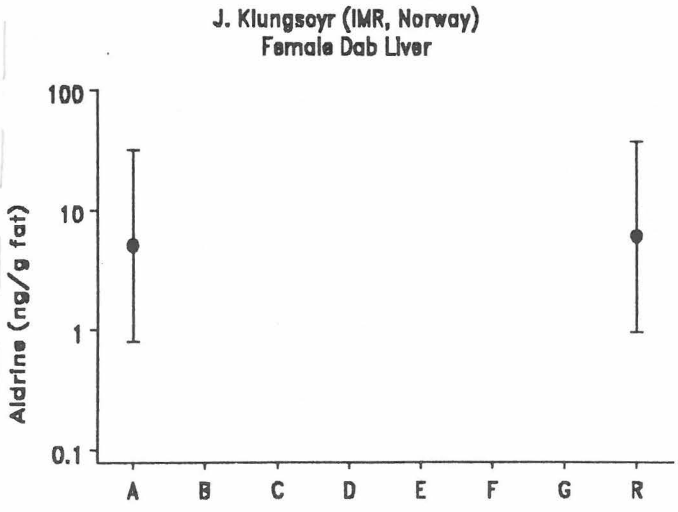
OIL PLATFORM CHEMISTRY
FEMALE DAB LIVER

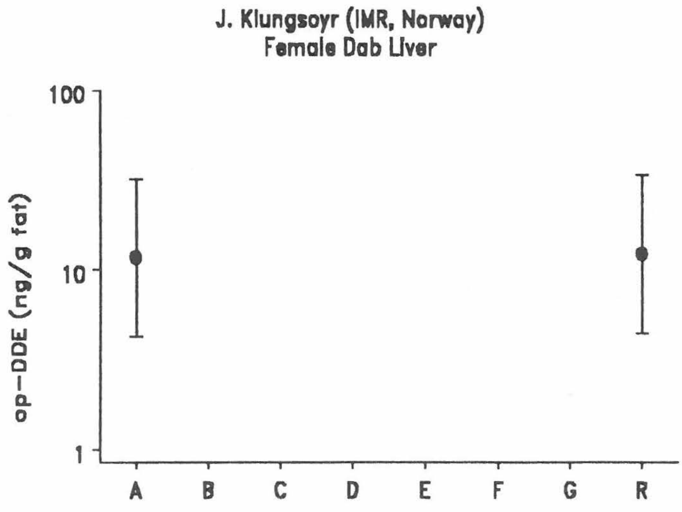




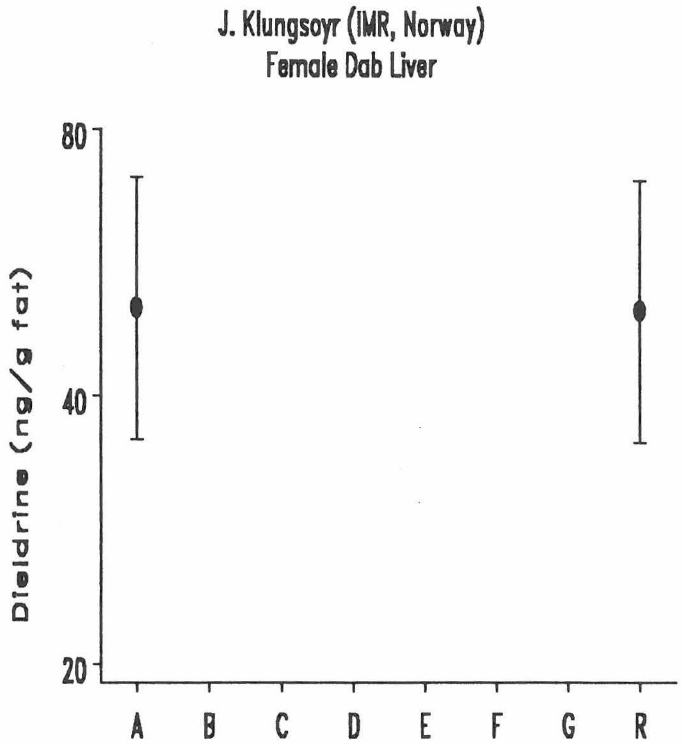


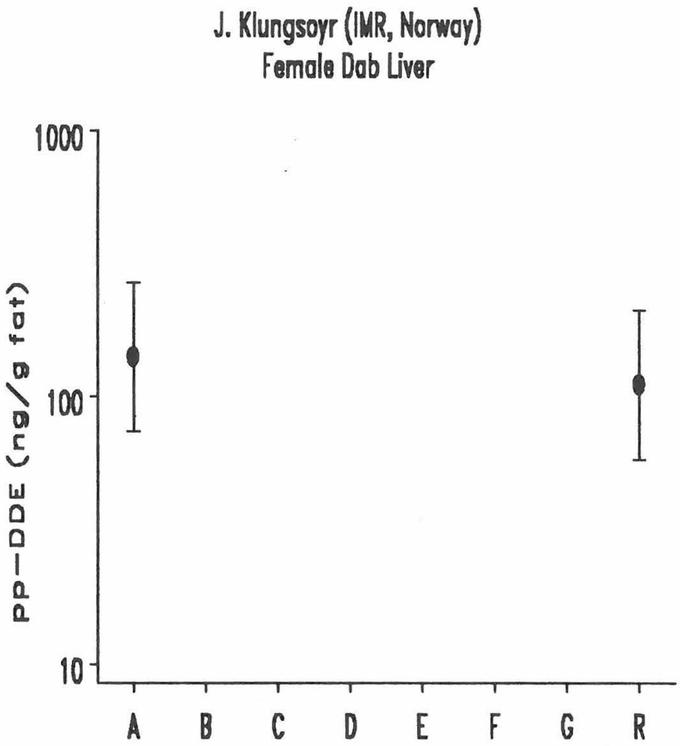


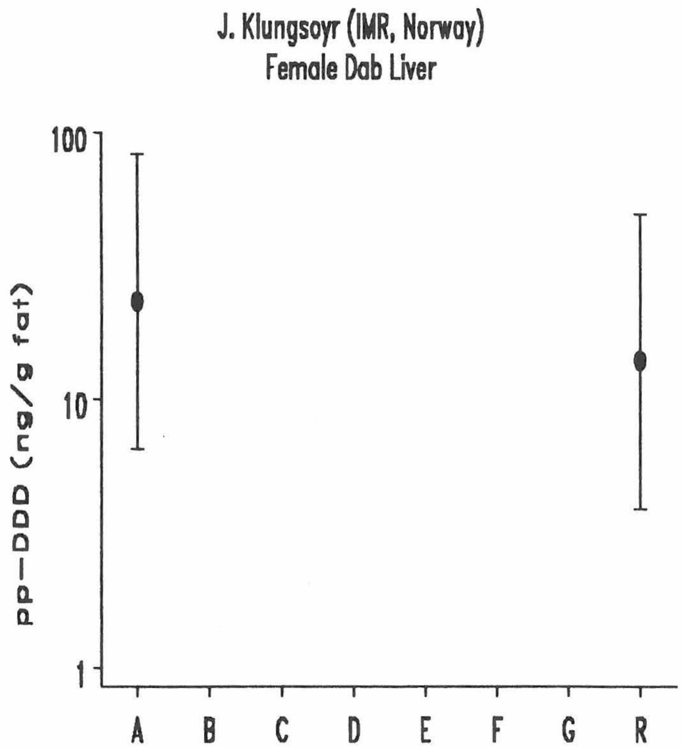


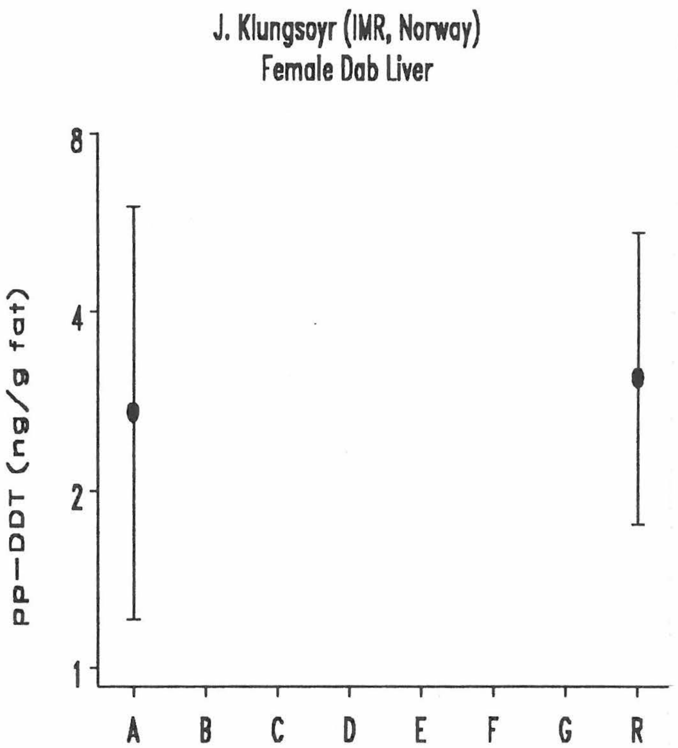


OIL PLATFORM CHEMISTRY
FEMALE DAB LIVER



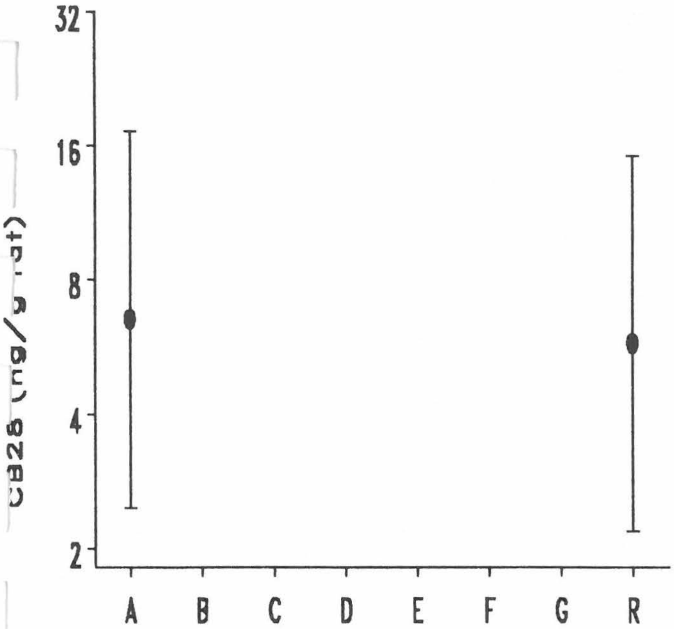




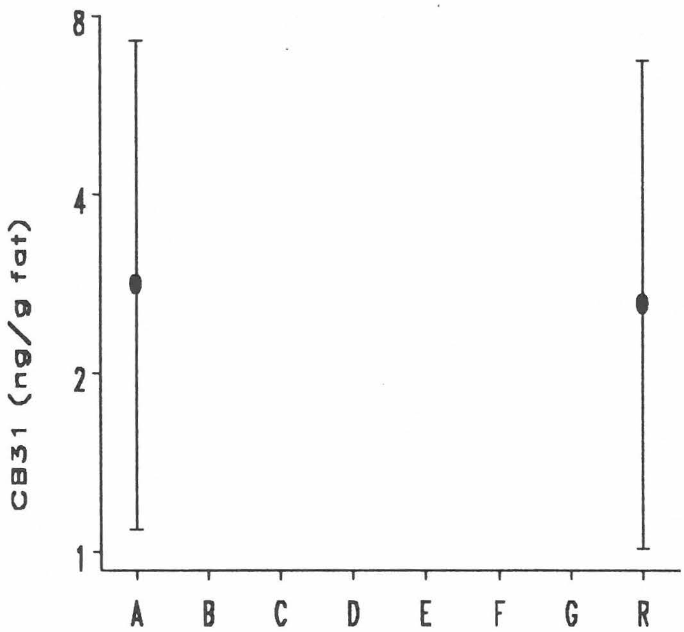


OIL PLATFORM CHEMISTRY
FEMALE DAB LIVER

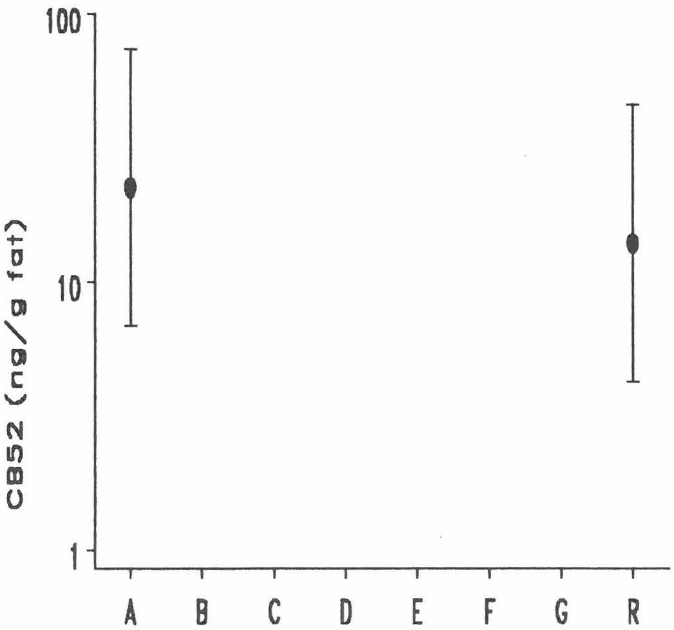
J. Klungsoyr (IMR, Norway)
Female Dab Liver



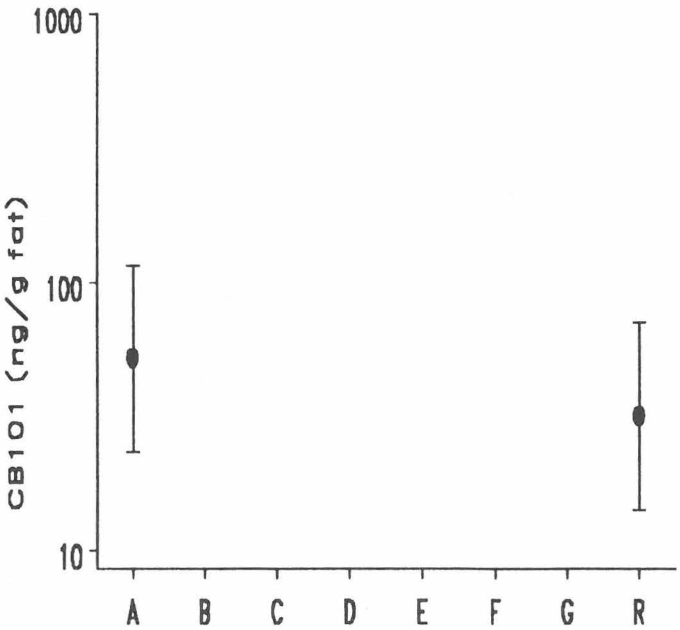
J. Klungsoyr (IMR, Norway)
Female Dab Liver



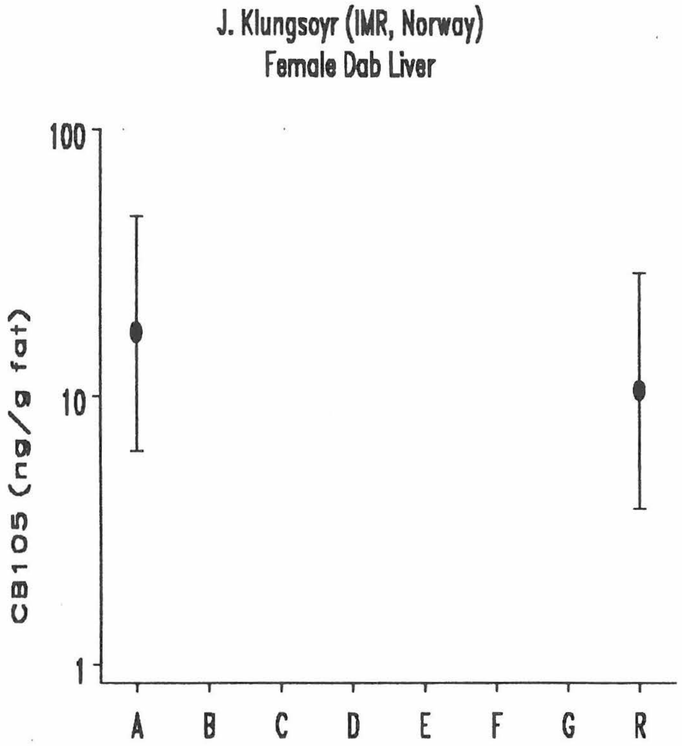
J. Klungsoyr (IMR, Norway)
Female Dab Liver

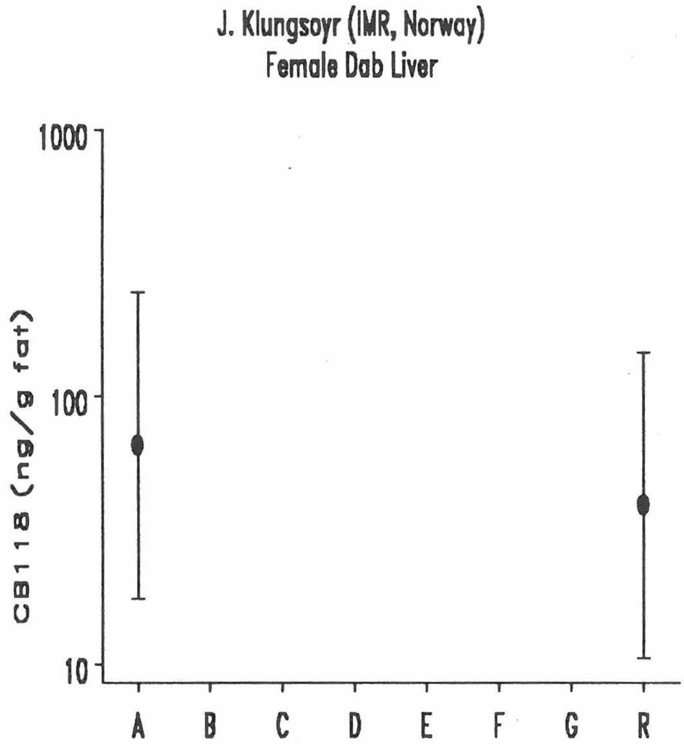


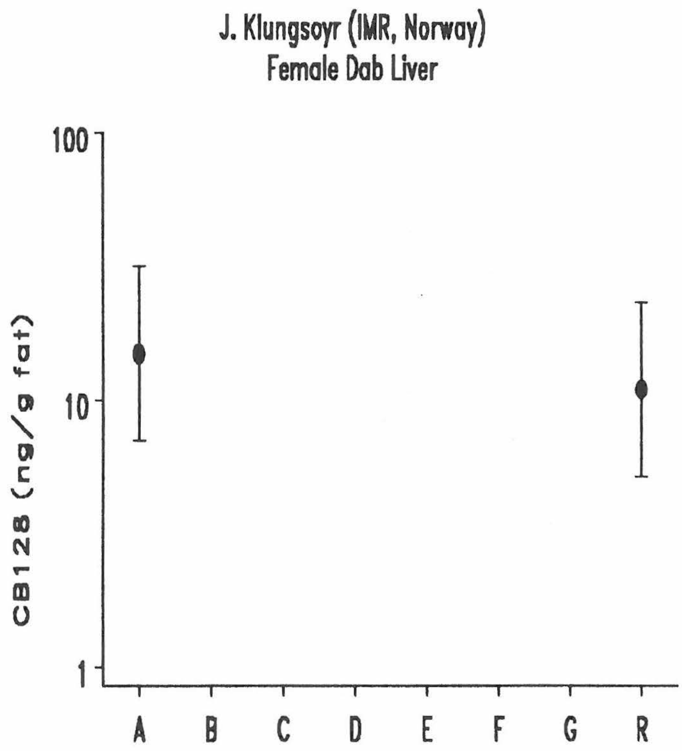
J. Klungsoyr (IMR, Norway)
Female Dab Liver

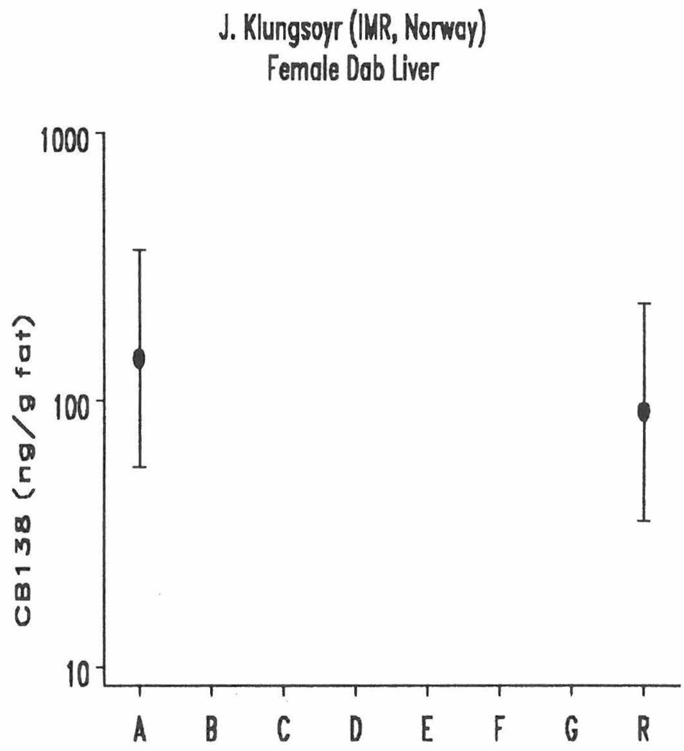


OIL PLATFORM CHEMISTRY
FEMALE DAB LIVER

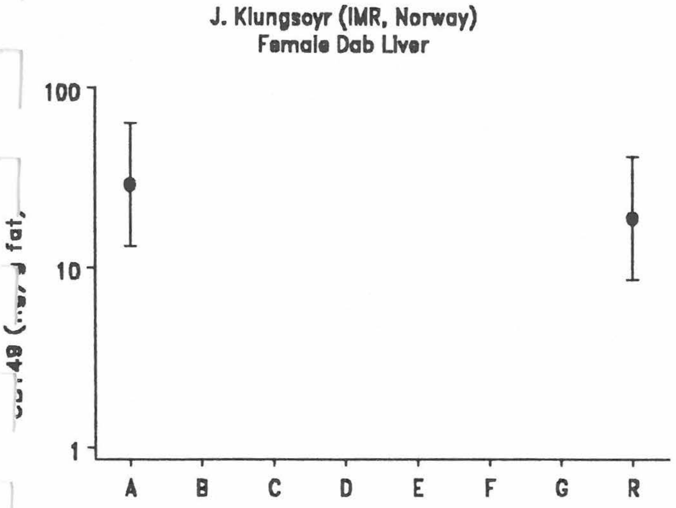


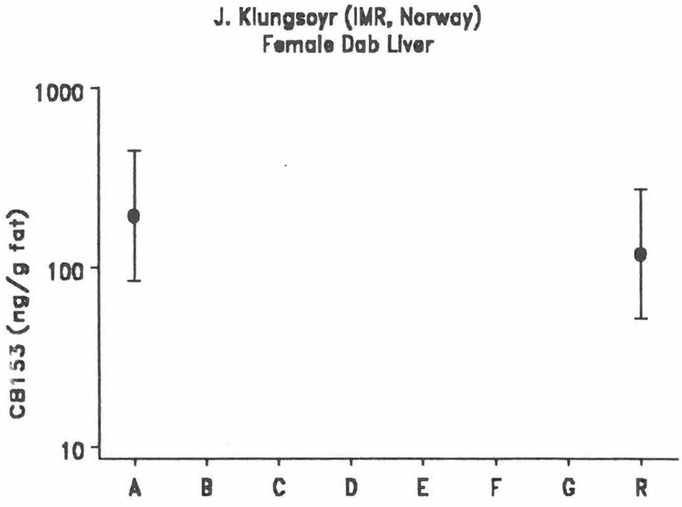


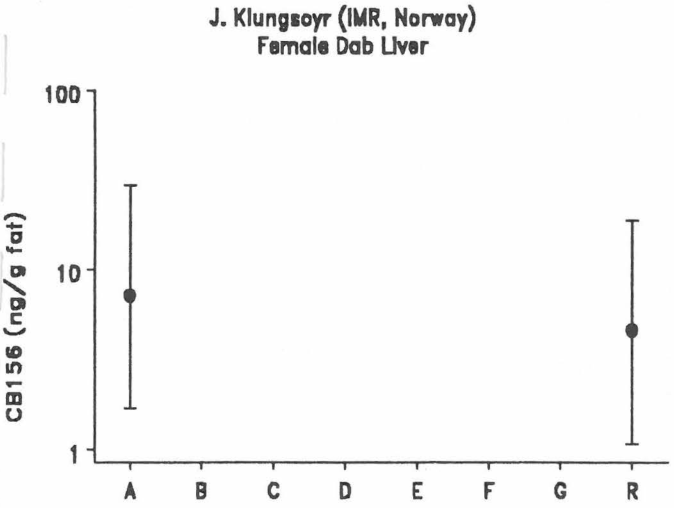


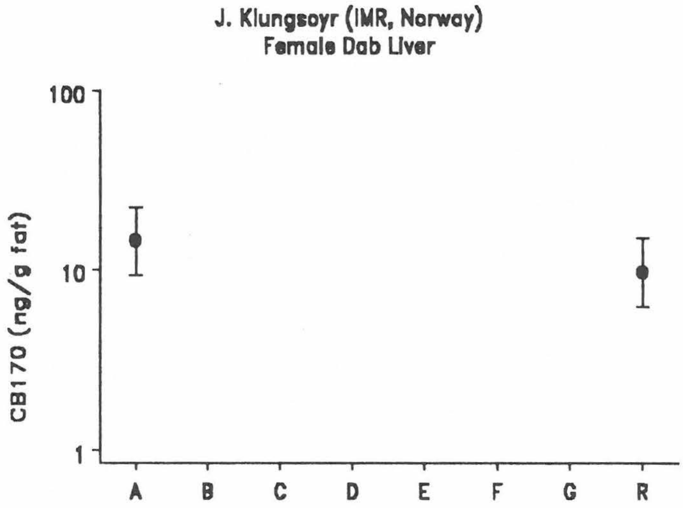


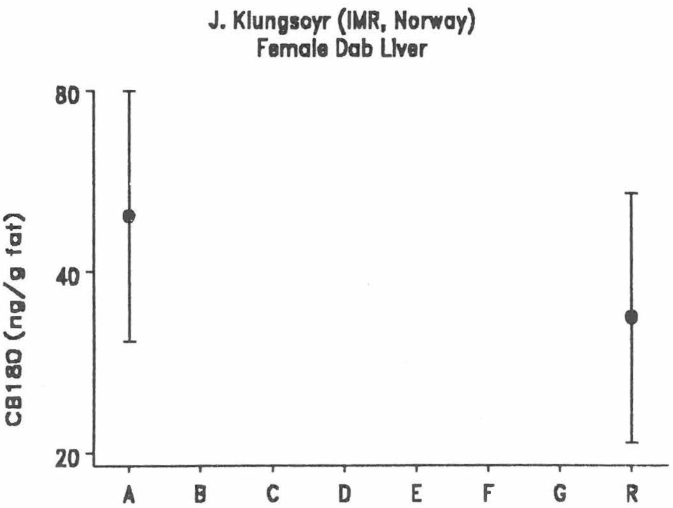
OIL PLATFORM CHEMISTRY
FEMALE DAB LIVER





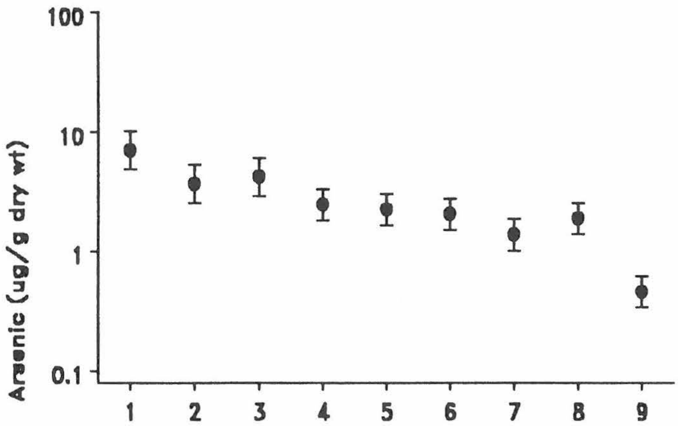




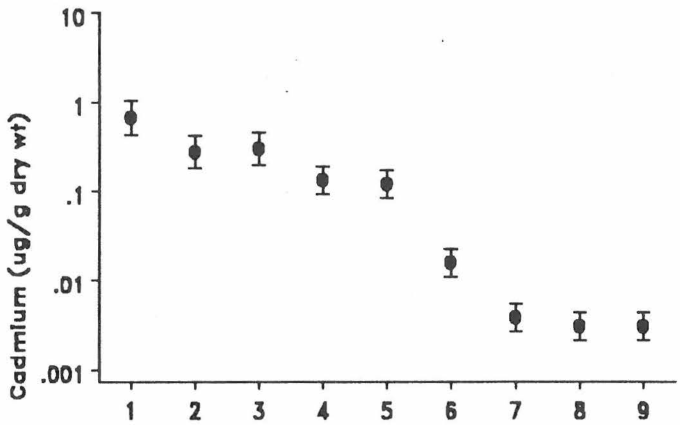


GERMAN BIGHT CHEMISTRY
TOTAL SEDIMENT

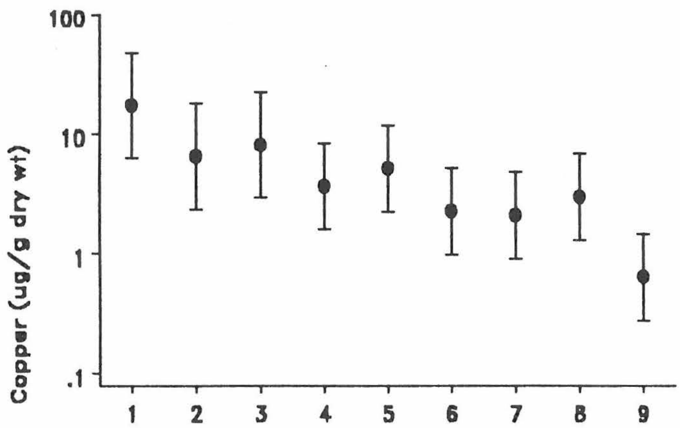
I Davies (SOAFD,UK)
Total Sediment



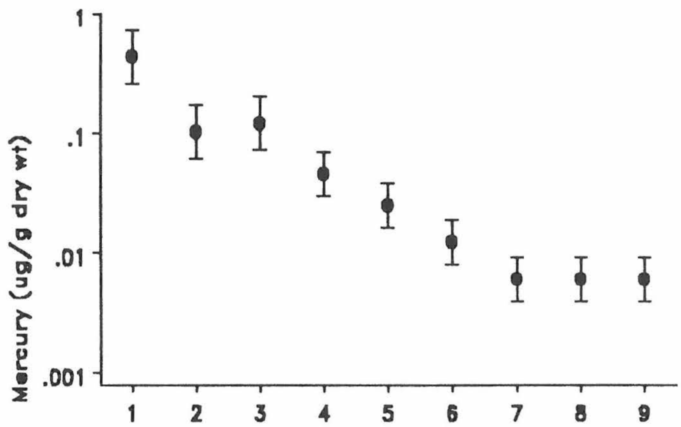
I Davies (SOAFD,UK)
Total Sediment



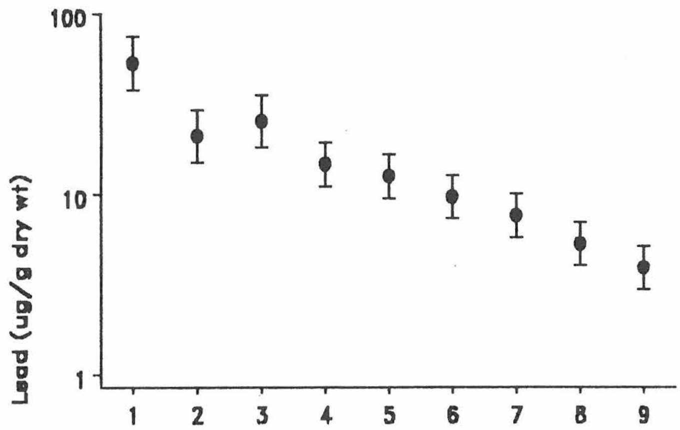
I Davies (SOAFD,UK)
Total Sediment



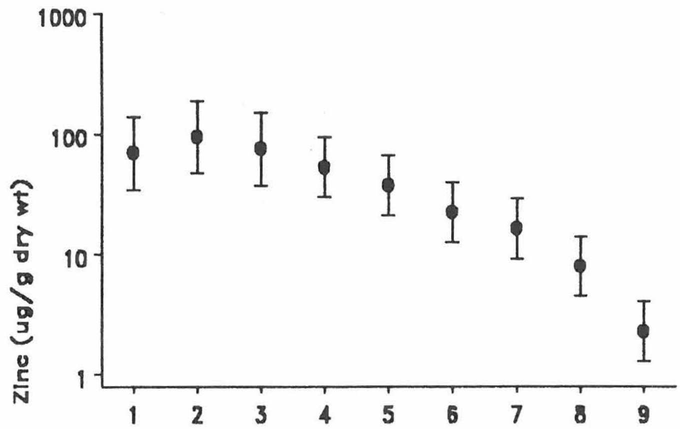
I Davies (SOAFD,UK)
Total Sediment



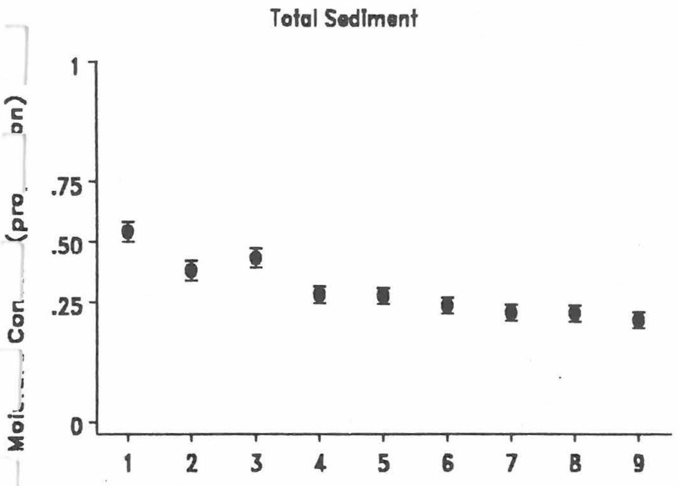
I Davies (SOAFD,UK)
Total Sediment

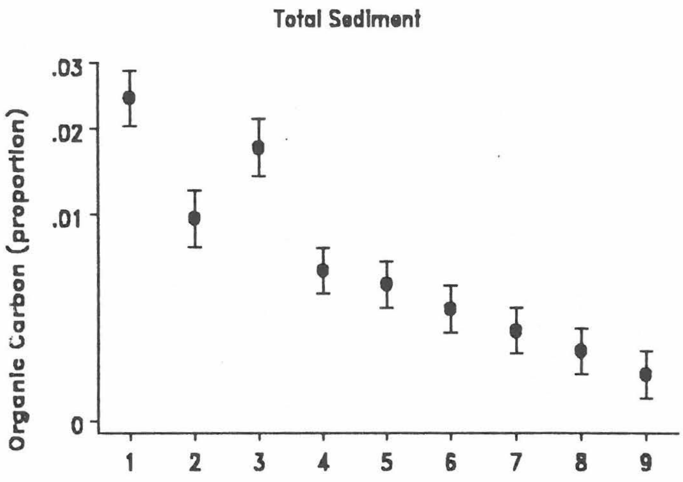


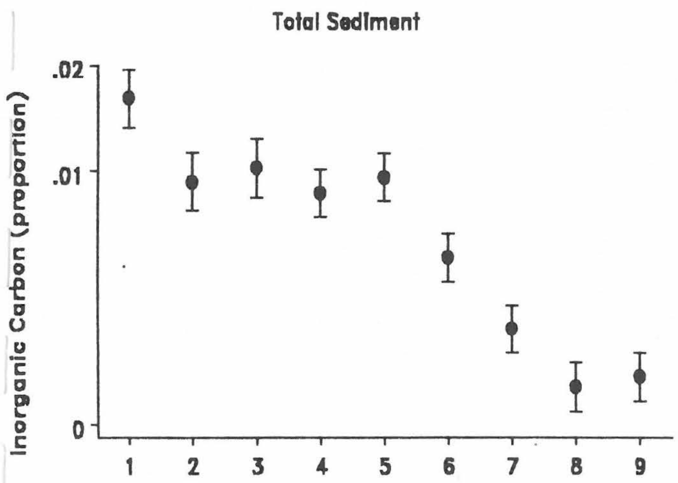
I Davies (SOAFD,UK)
Total Sediment

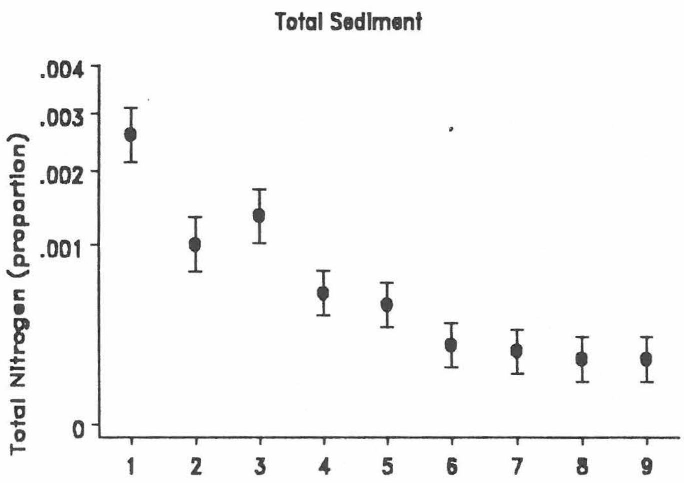


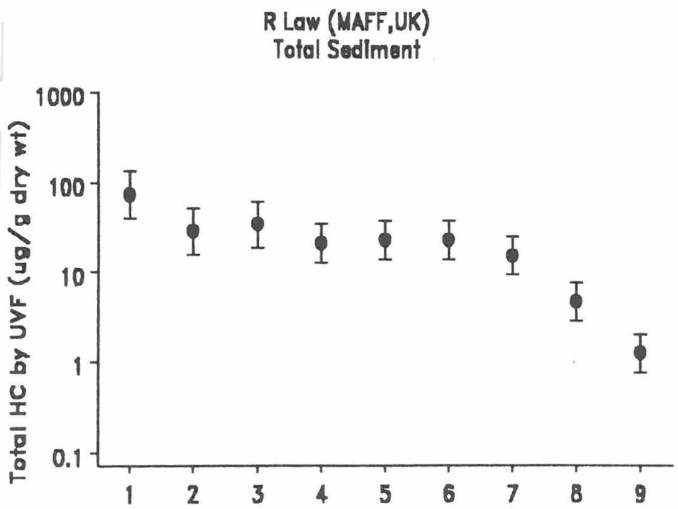
GERMAN BIGHT CHEMISTRY
TOTAL SEDIMENT

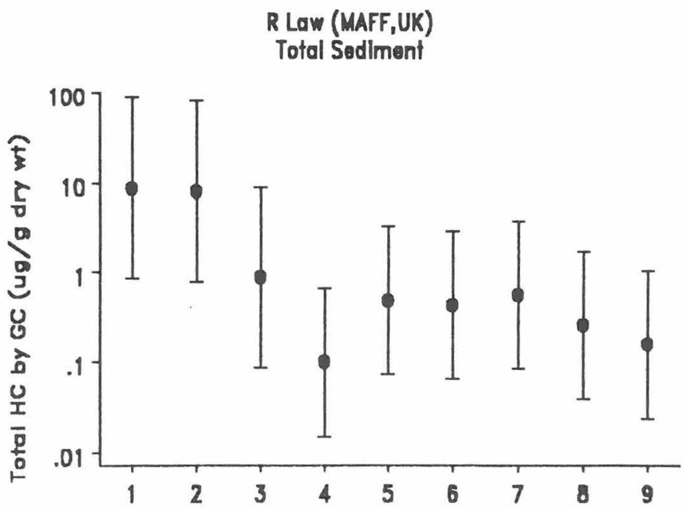




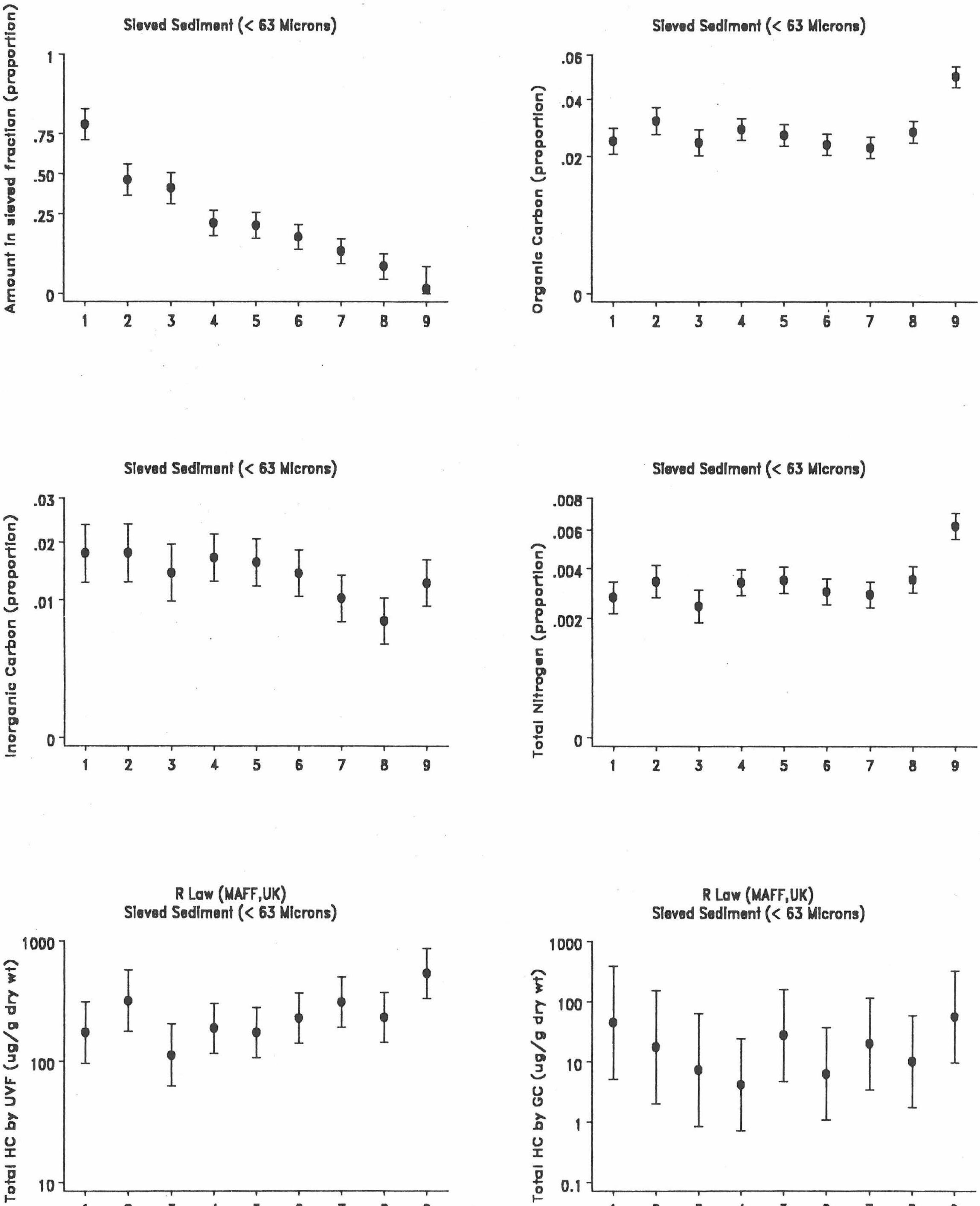




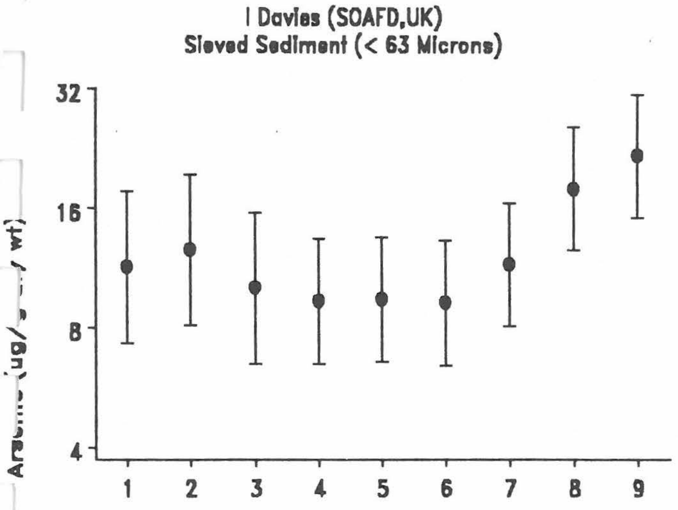


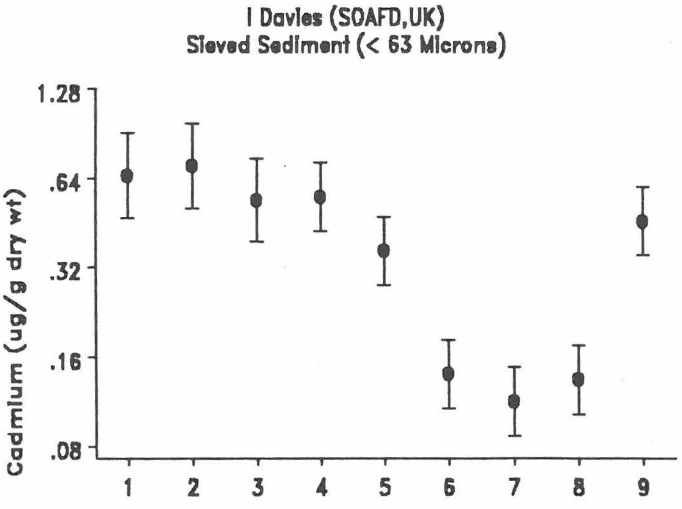


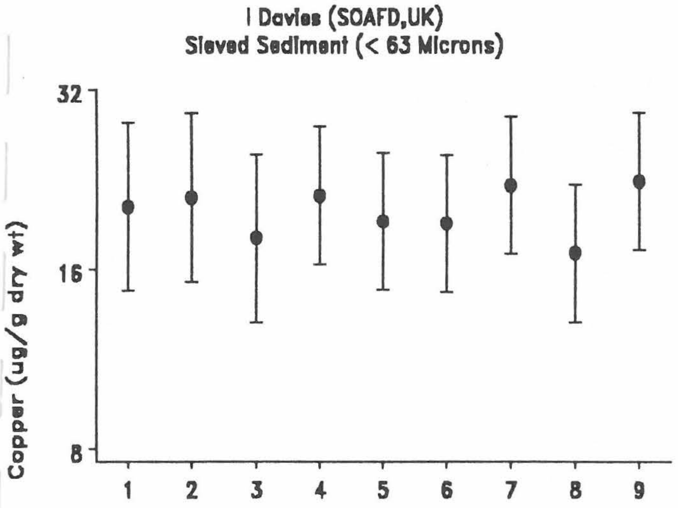
GERMAN BIGHT CHEMISTRY
SEDIMENT (<63 MICRONS)

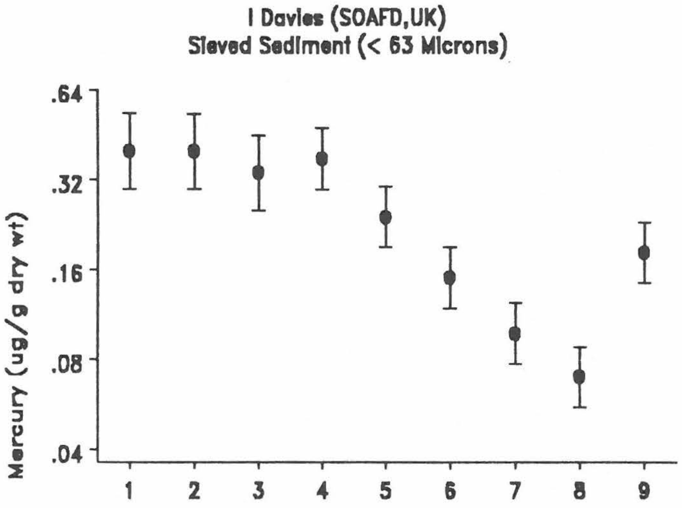


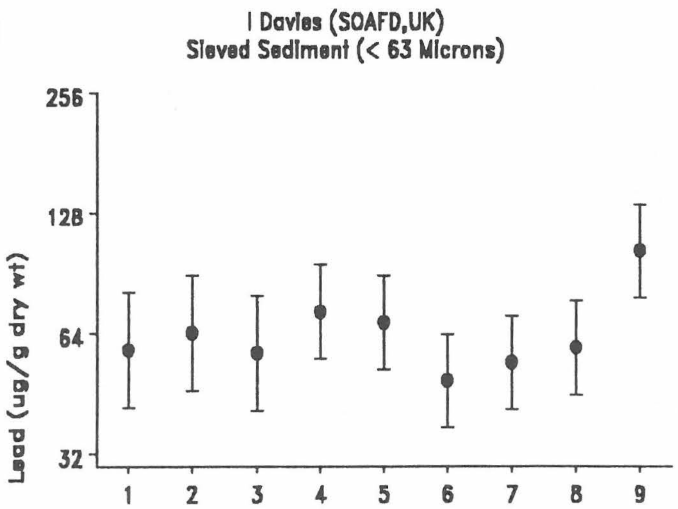
GERMAN BIGHT CHEMISTRY
SEDIMENT (<63 MICRONS)

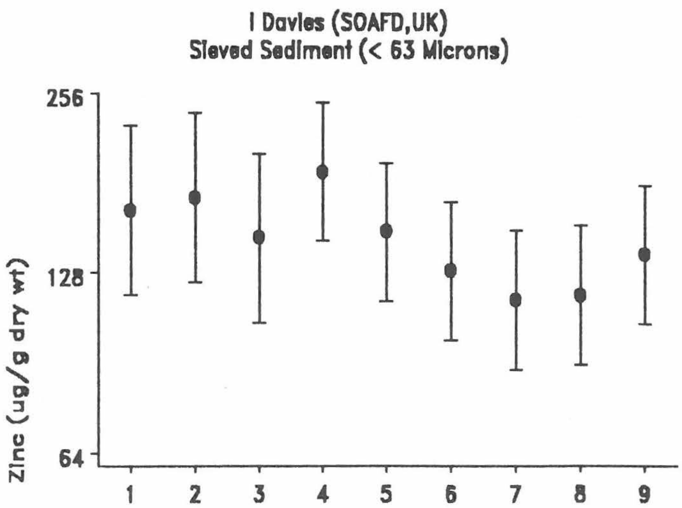




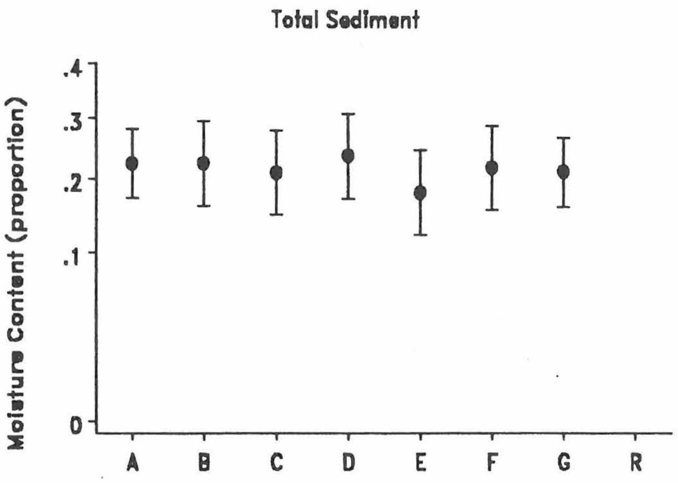


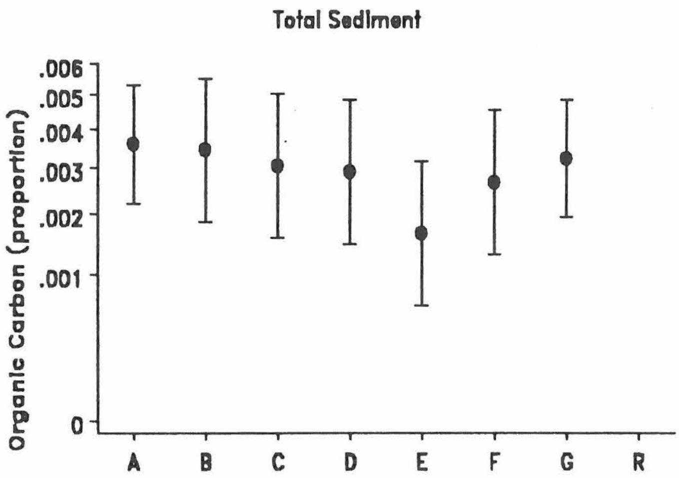


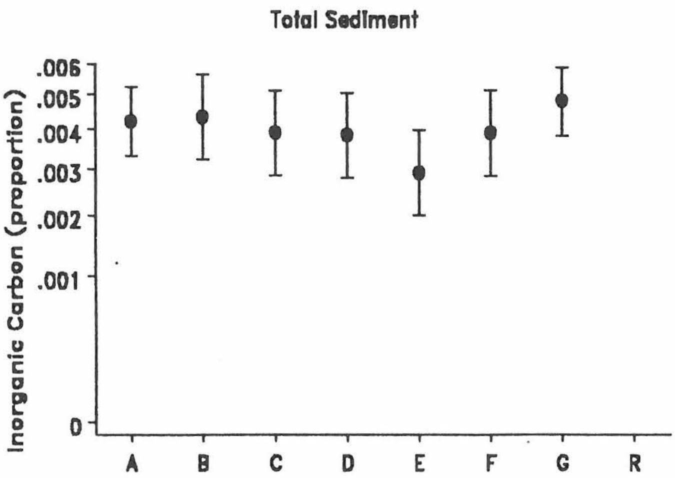


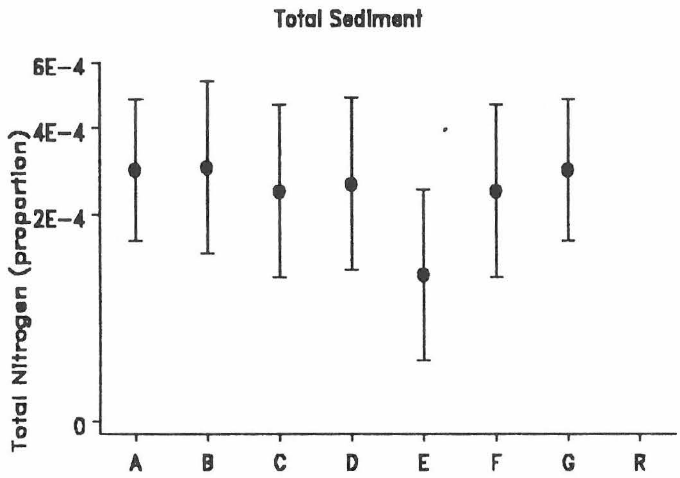


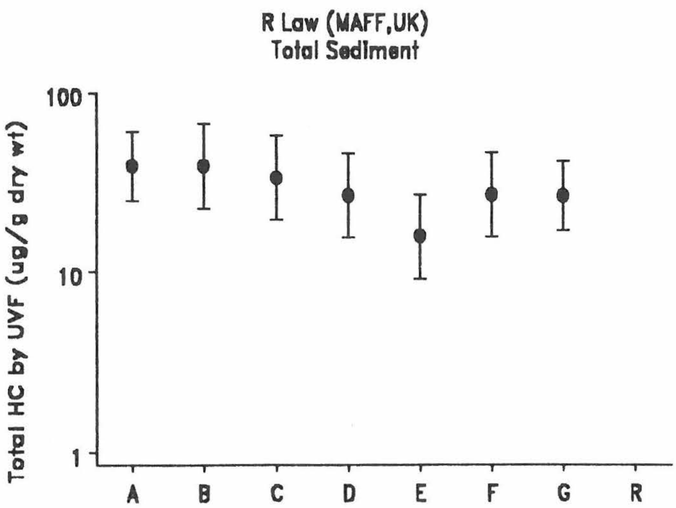
OIL PLATFORM CHEMISTRY
TOTAL SEDIMENT

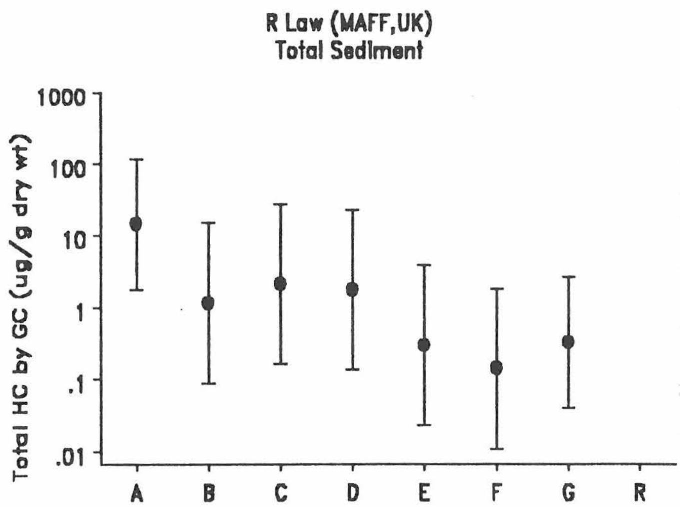




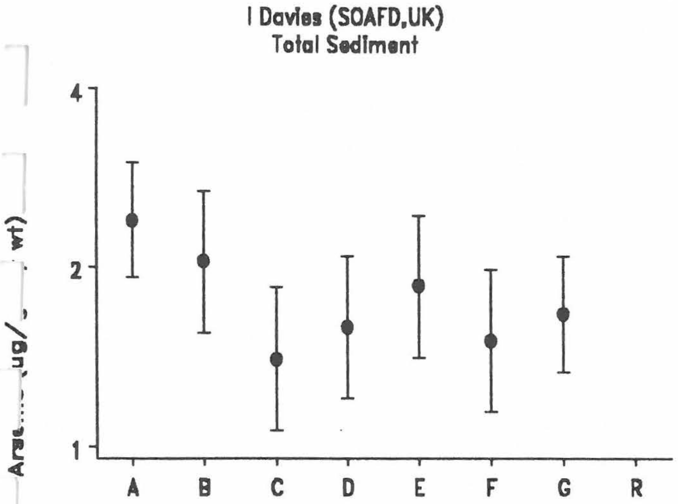


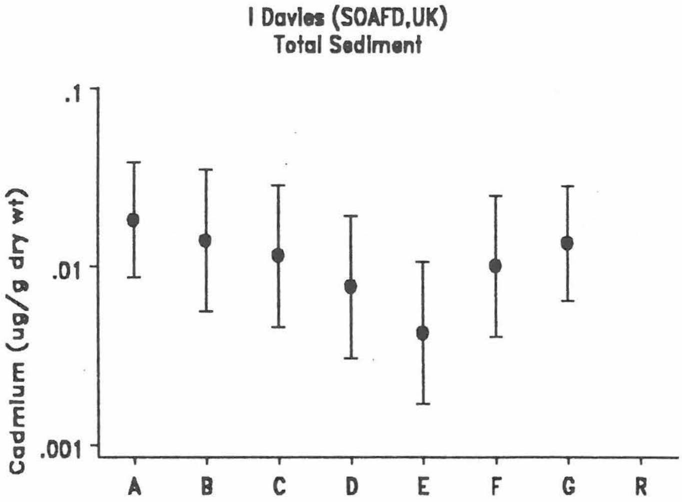


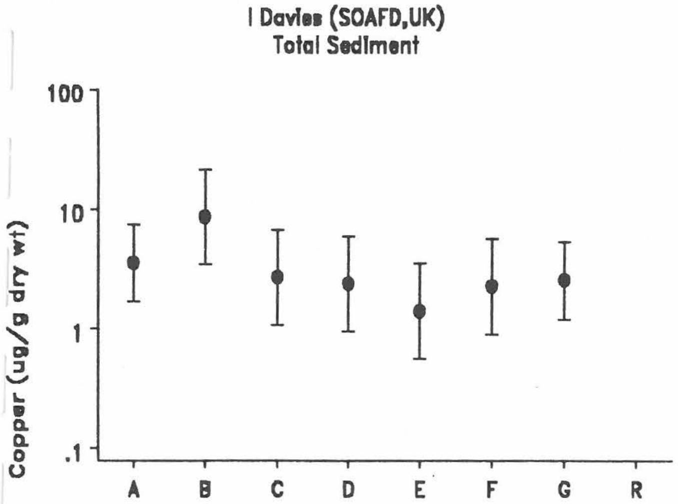


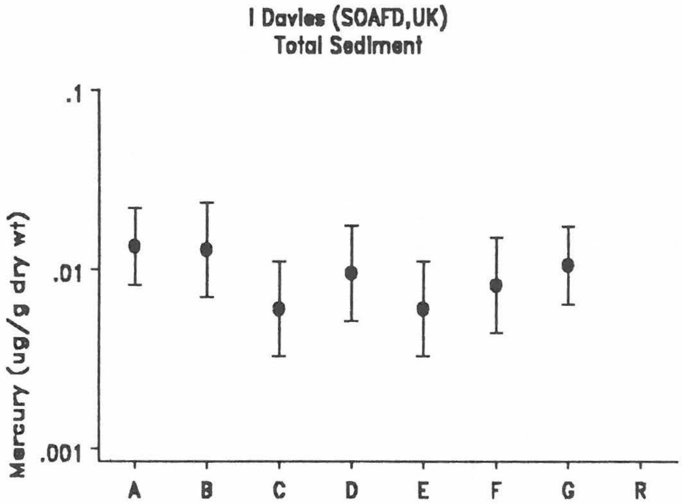


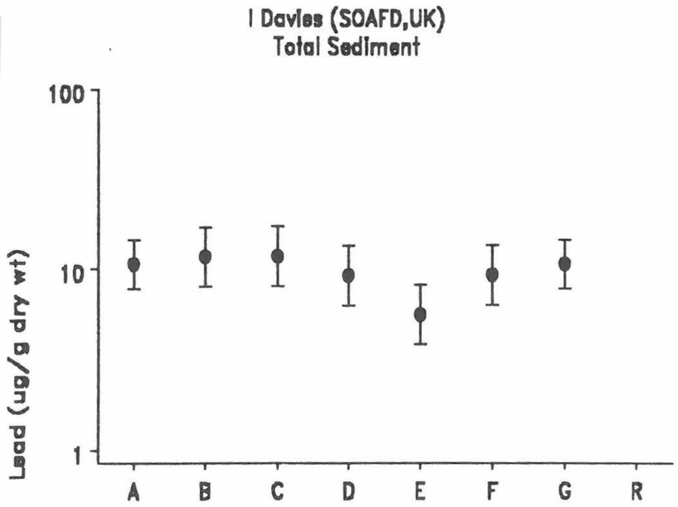
OIL PLATFORM CHEMISTRY
TOTAL SEDIMENT

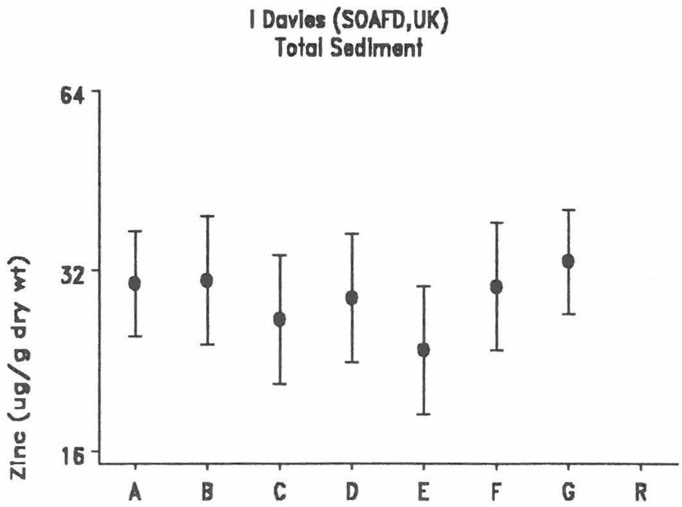




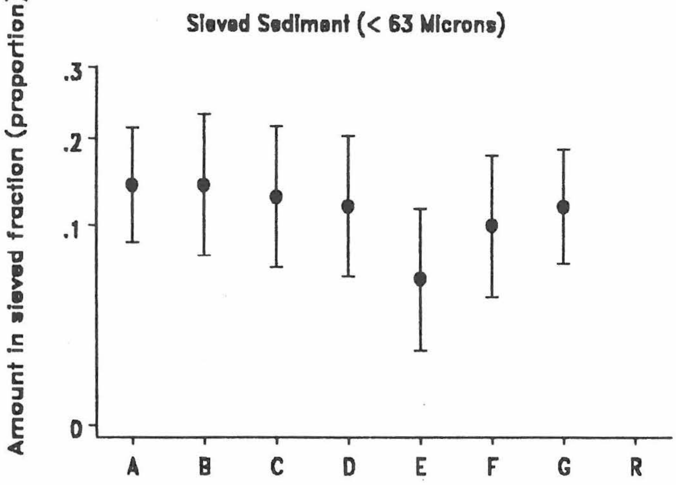


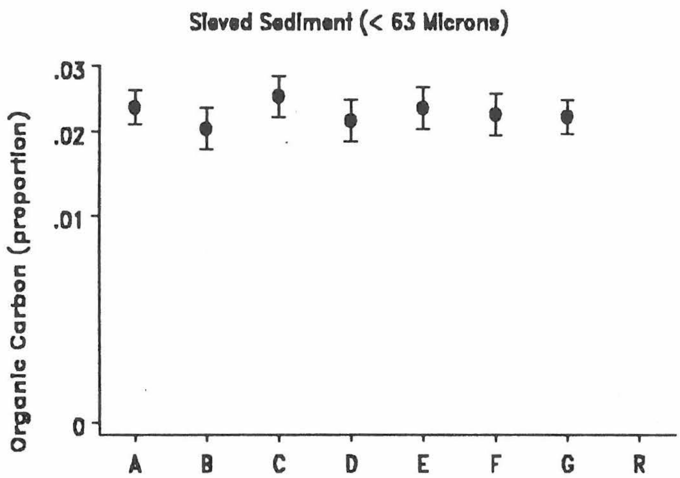


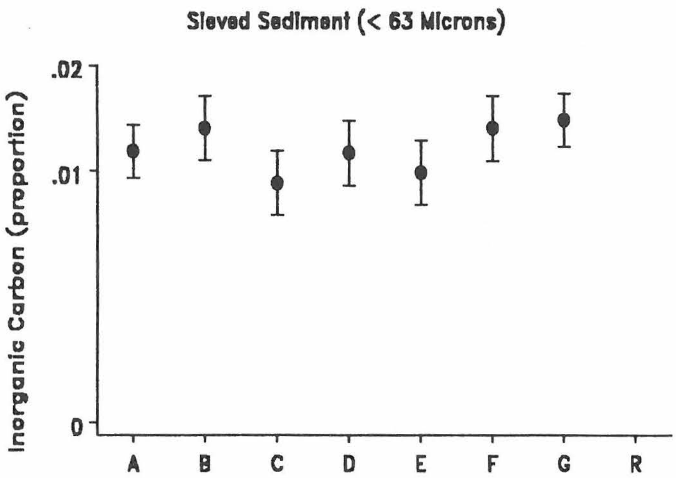


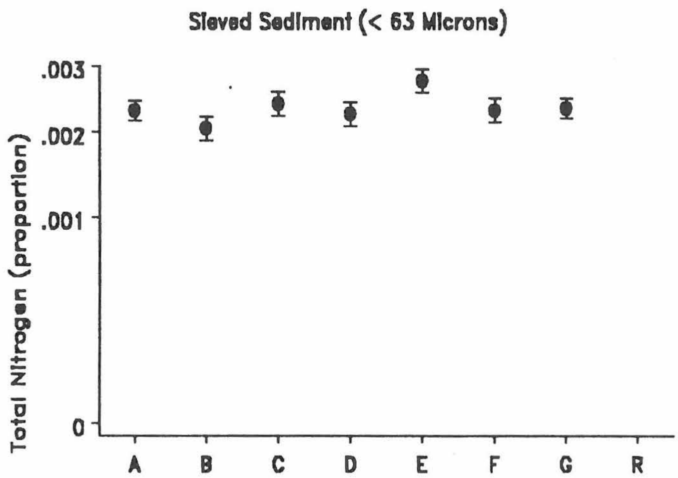


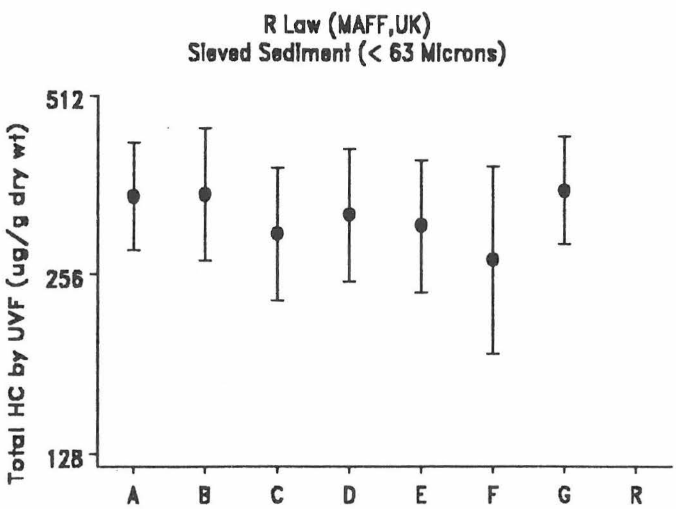
OIL PLATFORM CHEMISTRY
SEDIMENT (< 63 MICRONS)

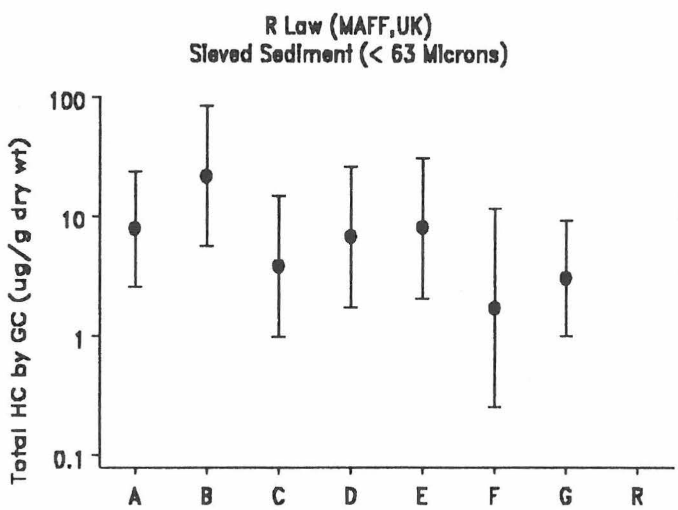




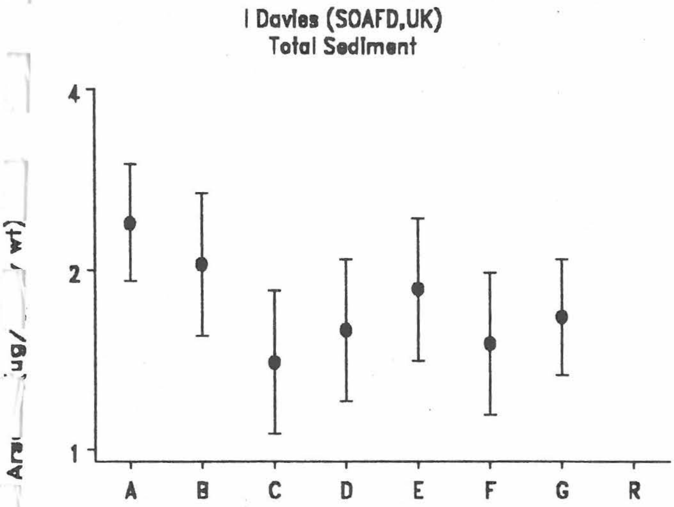


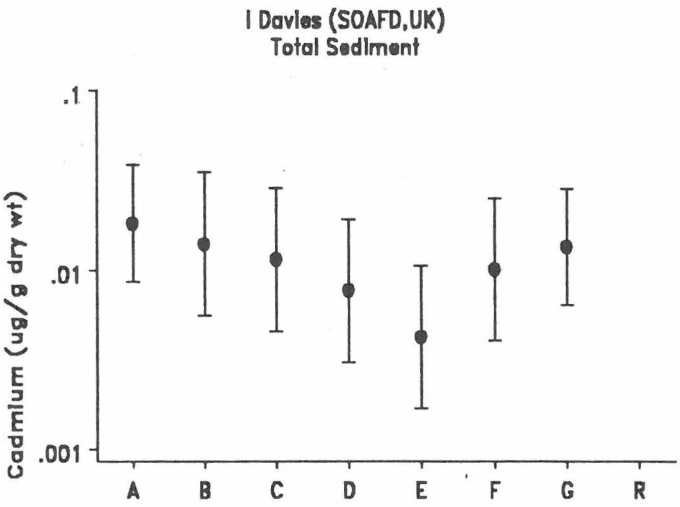


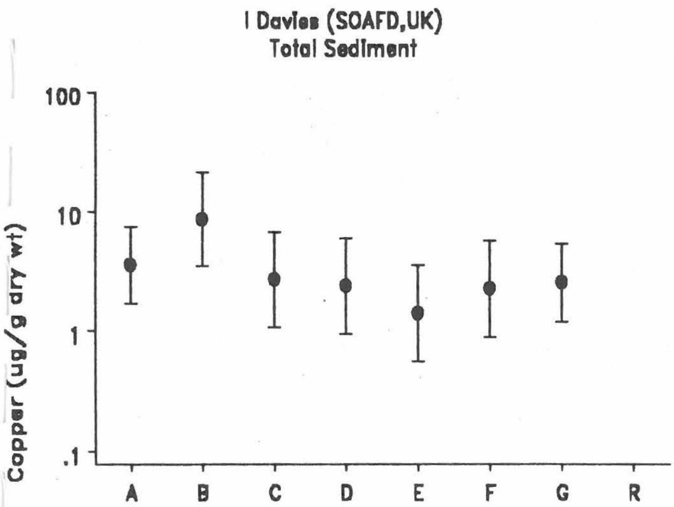


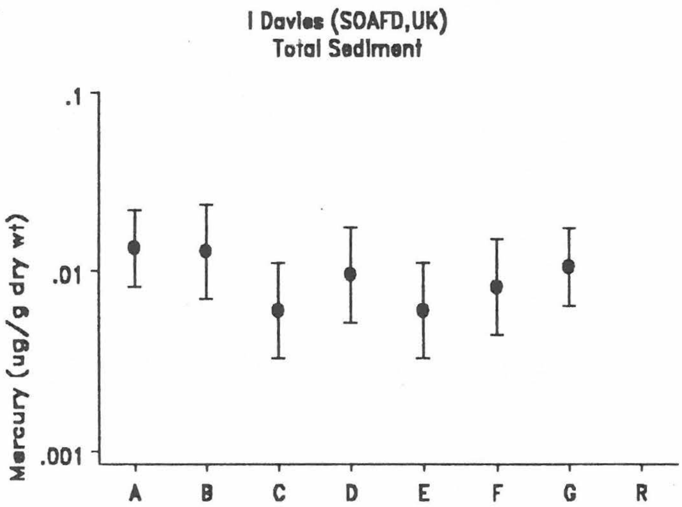


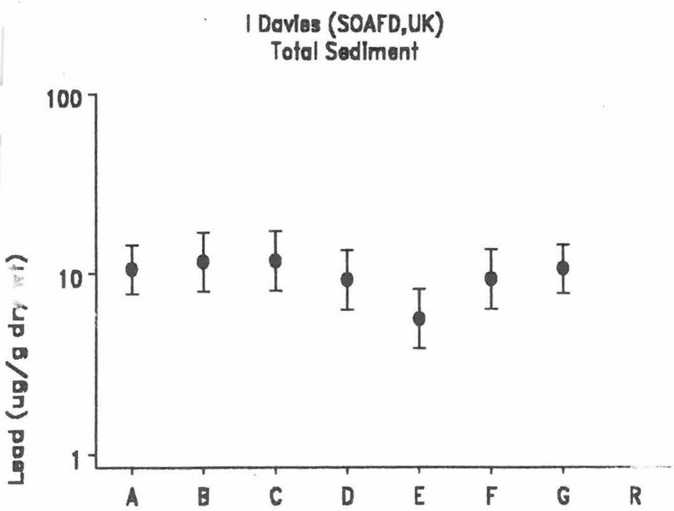
OIL PLATFORM CHEMISTRY
SEDIMENT (< 63 MICRONS)

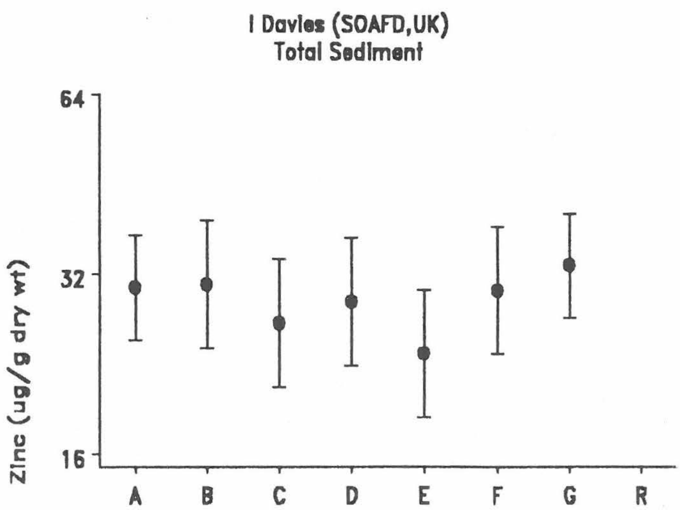


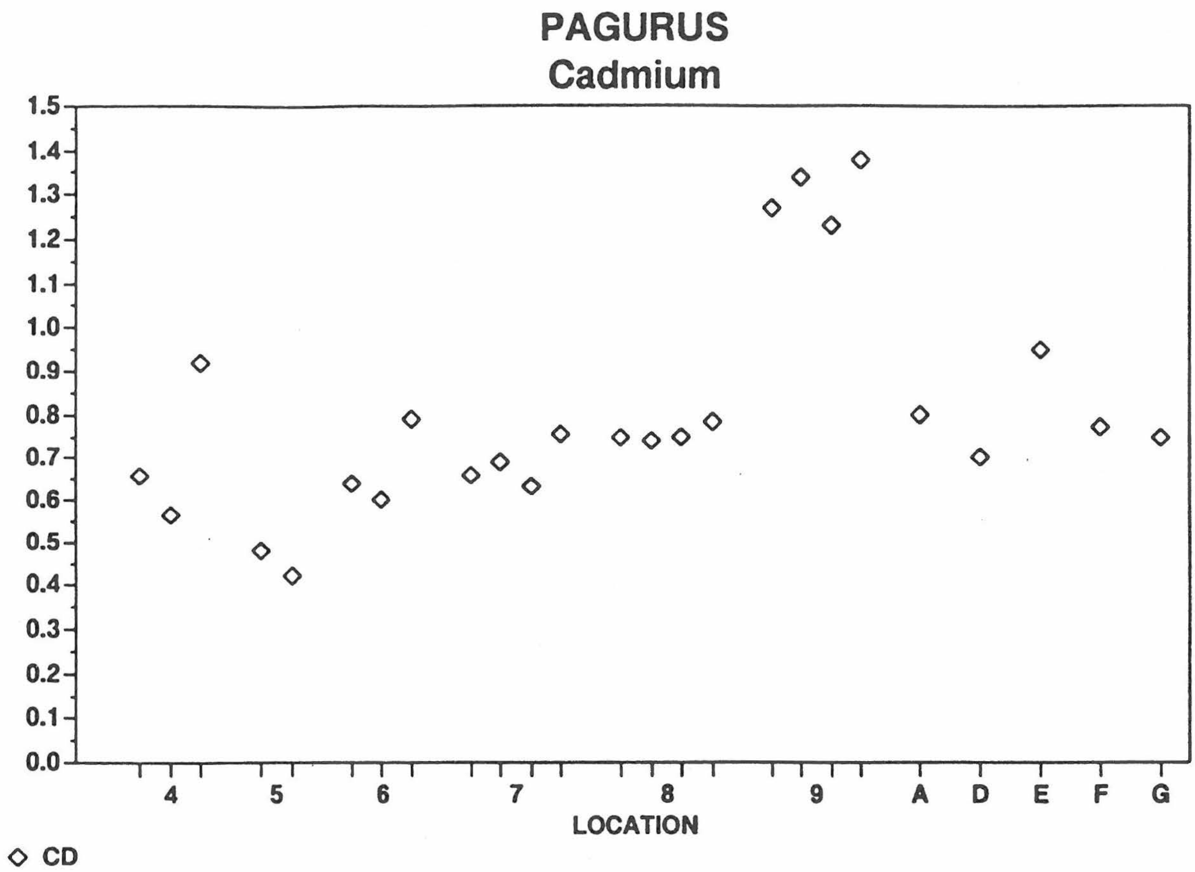


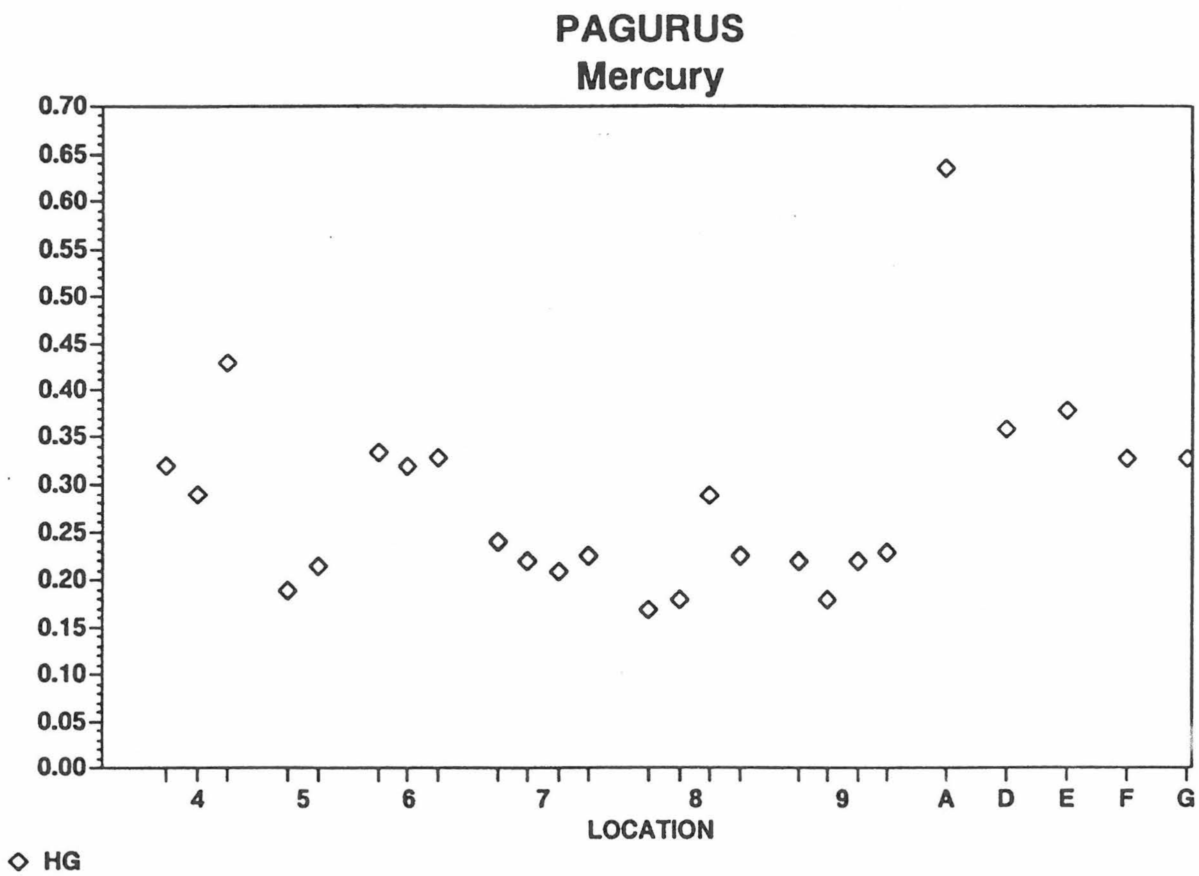


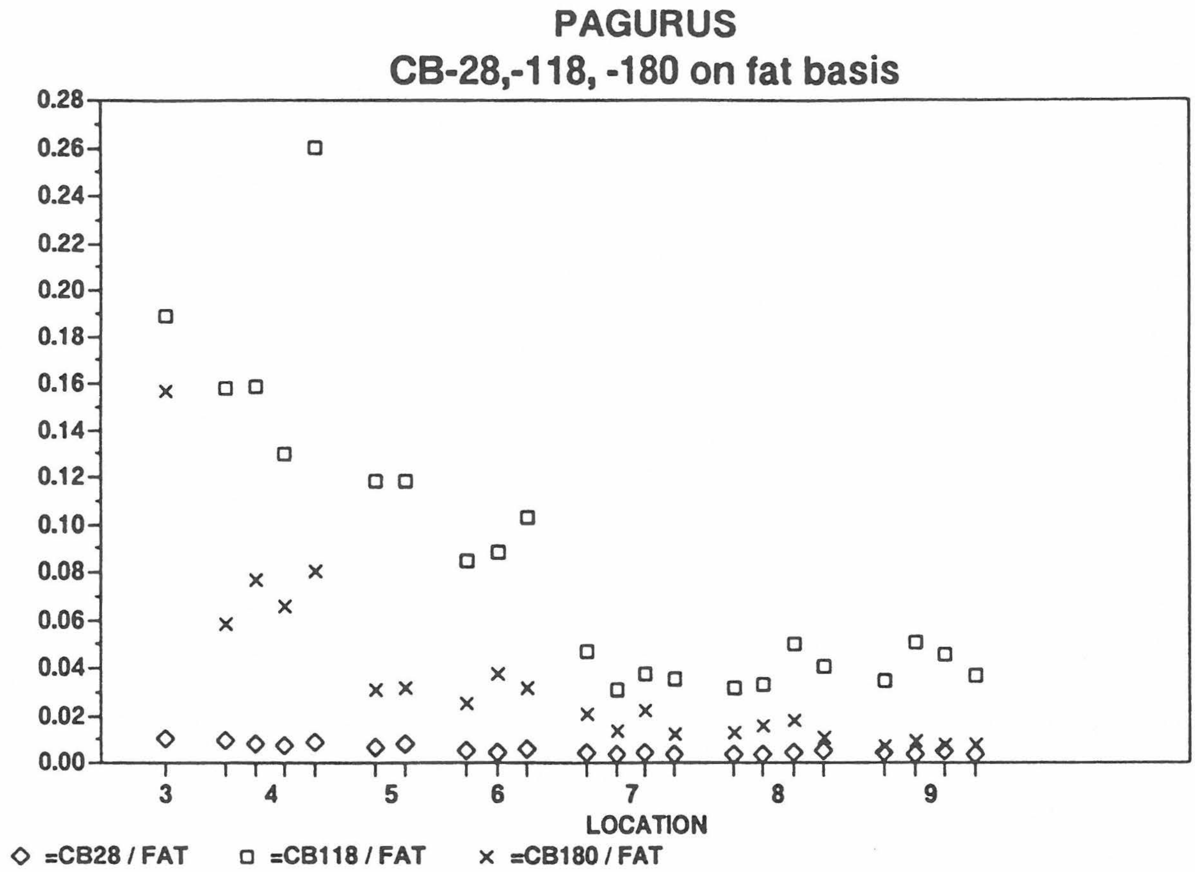


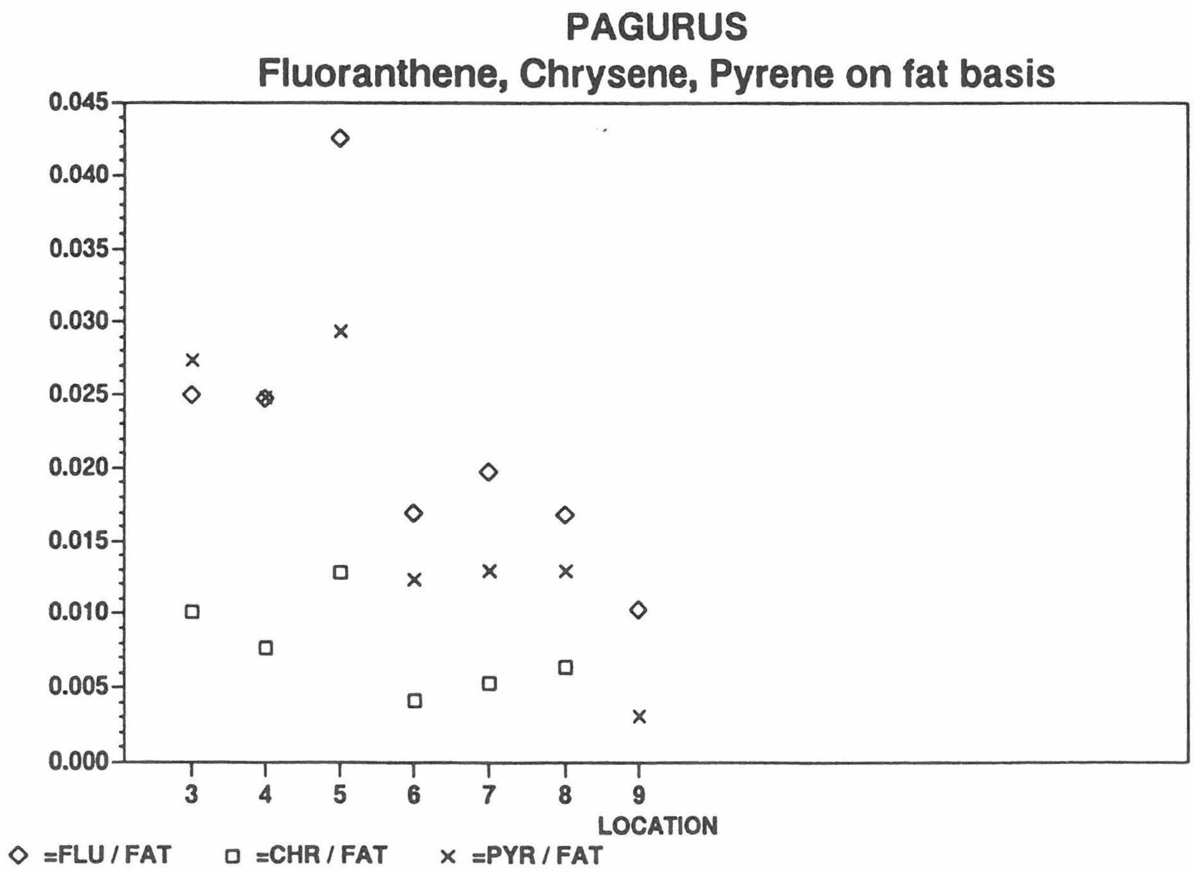





PAGURUS
Cadmium
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
4
5
6
7
8
9
A
D
E
F
G
LOCATION
CD

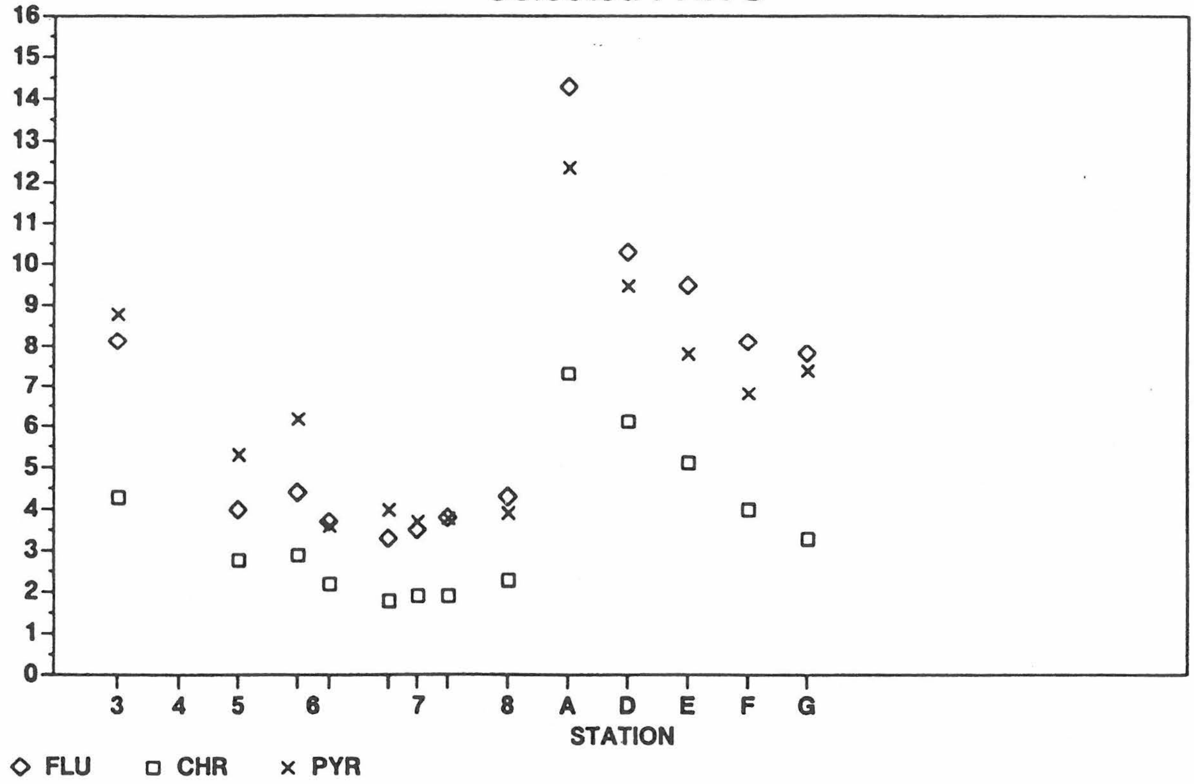

PAGURUS
Mercury
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
4
5
6
7
8
9
A
D
E
F
G
LOCATION
HG


PAGURUS
CB-28,-118, -180 on fat basis
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
3
4
5
6
7
8
9
LOCATION
◇ =CB28 / FAT
□ =CB118 / FAT
× =CB180 / FAT

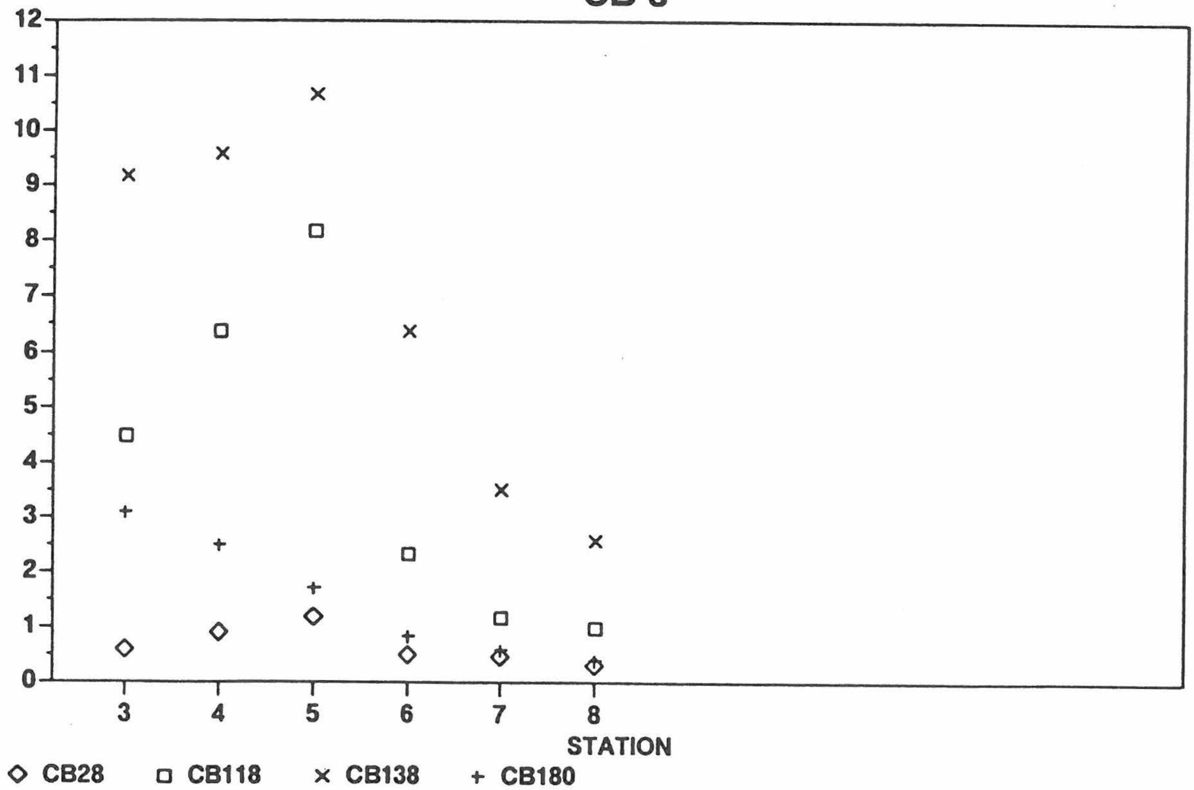

PAGURUS
Fluoranthene, Chrysene, Pyrene on fat basis
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
3
4
5
6
7
8
9
LOCATION
◇ =FLU / FAT
□ =CHR / FAT
× =PYR / FAT

@AVG

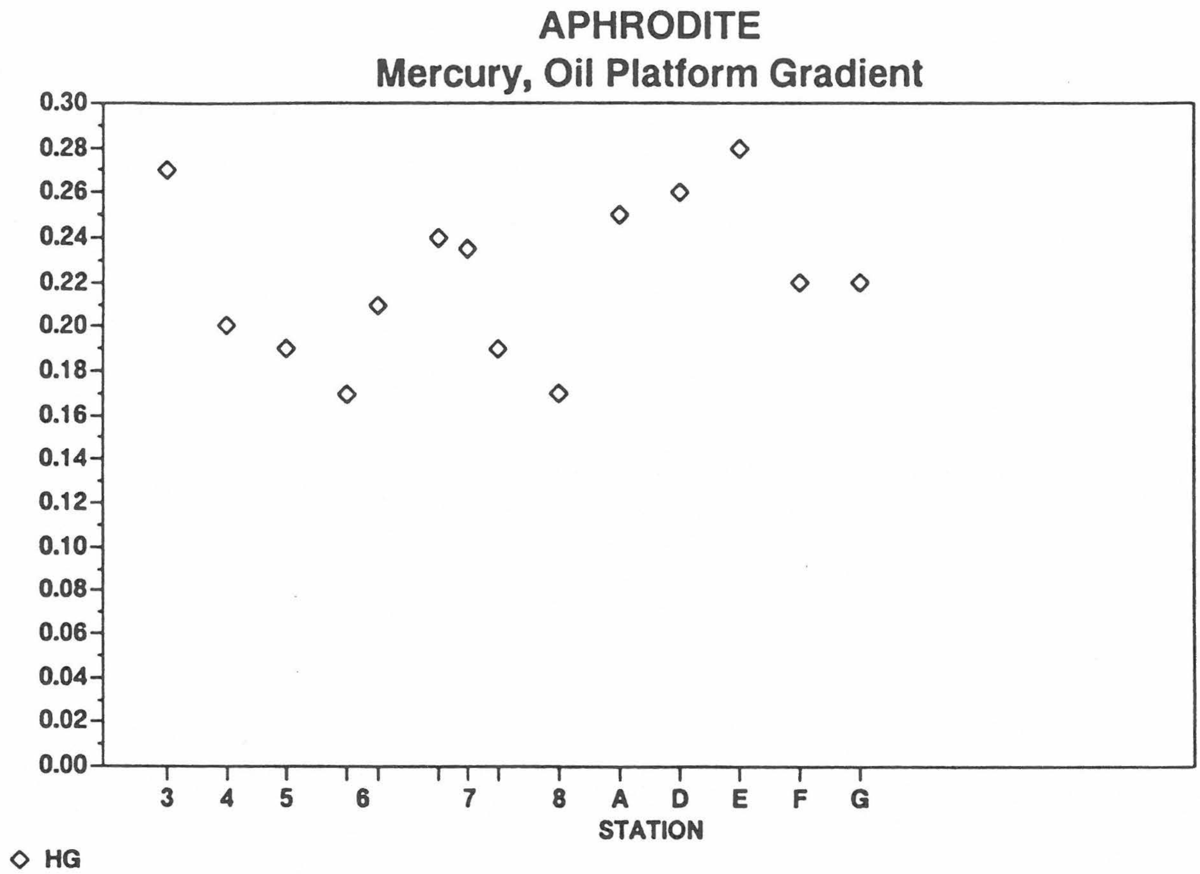
APHRODITE
Selected PAH's



APHRODITE
CB's



@AVG


APHRODITE
Mercury, Oil Platform Gradient
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
3
4
5
6
7
8
A
D
E
F
G
STATION
HG

6. BIOCHEMISTRY (Coordinator: R.F. Addison)

Techniques tested. The following biochemical indices of the effects of contamination were measured:

a. Mixed function oxidase (MFO) induction in dab tissues which is resposive to to presence of PAH, coplanar PCB, CDD and CDF;
b. Metallothionein (MT) induction in dab liver which responds to the presence of group IIb metals, especially Zn and Cd;
c. Cholinesterase (AChE and BChE) inhibition in dab tissues, which is only known to respond to organophosphate compounds at concentrations of contaminants known to occur in the environment.
d. ATPase inhibition in dab gills, which responds to various organic compounds;
e. Lysozyme activity in dab tissues, which indicates immunocompetence;
f. Host defence factors in *Eupagurus* which indicates immune status;
g. Liver enzymatic activities in dab indicating detoxification activity;
h. Lipid composition of dab indicating energy partitioning and nutritional status;

Results Most effort was directed to MFO measurements (eight investigators). Measurements included the enzyme activities EROD and CN-ECOD, total Cytochrome P450 and P450IA1 by ELISA methods, and P450IA1 mRNA. Dab were sampled from stations 3, 5, 7, 8 and 9 and brought to the lab. alive for analysis, and another suite of samples from stations 1, 3, 5, 6, 7, 8 and 9 were frozen for subsequent analysis. Spatial trends in MFO measurements tended to be fairly consistent among the suites of samples and among indices of MFO activity. The German Bight inshore stations (notably stations 1 and 3) showed the highest MFO activity and offshore stations the lowest activity, whether expressed in terms of catalytic function (EROD or CN-ECOD), P450IA1 levels, or P450IA1 mRNA levels. Samples from the oil platform gradient showed MFO activities similar to those from the least contaminated German Bight stations. The activities of enzymes related to the MFO system and involved in oxidative processes appeared to be less sensitive and less informative indices of biochemical effects.

MT measurements showed the opposite trend to that of MFO systems. MT levels tended to increase towards the offshore stations; although this was opposite to the expected trend in the spatial distribution of metals, liver concentrations of Cu and Zn appeared to follow a similar trend to that of MT.

Cholinesterase inhibition was highest inshore, the zone expected to be most contaminated, and decreased offshore. As with MFO measurements, the site least contaminated appeared to be station 8, while the outermost station (9) showed some increase in contamination (perhaps due to a weak gyre concentrating some contaminants).

ATPase inhibition showed relatively little spatial change, although there was a hint of lower activity inshore and at station 9, with intermediate offshore stations showing less inhibition. Lysosozyme activity showed only very slight changes, being slightly elevated at Stations 3 and 8. The immune status of Eupagurus showed variable trends depending on the tissue analysed. Lipid composition of dab showed no clear trend along the transect.

General observations.

a. In the German Bight, spatial trends in dab liver MFO activities were generally consistent with trends in PCB congeners in dab liver analysed chemically. (This does not mean that these PCB congeners caused MFO induction but rather that they co-varied with more probably inducing compounds).

b. Trends in MT activities were consistent with trends in metal concentrations, determined in two independent analyses.

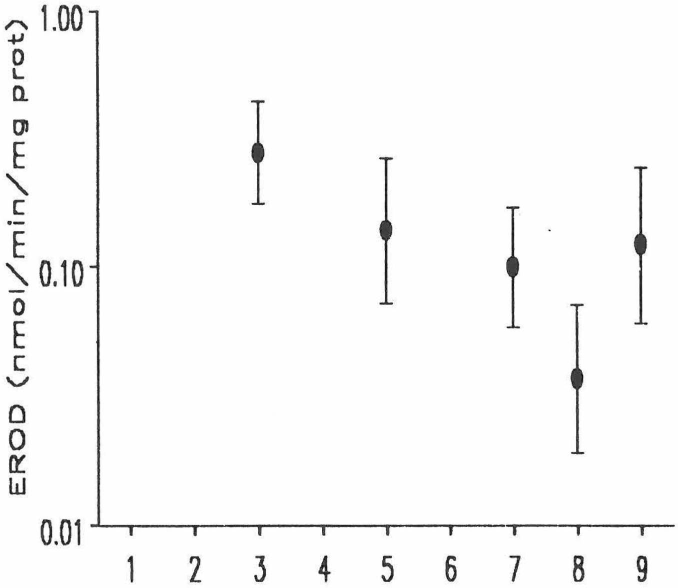
c. AChE trends were consistent with the general organic contaminant distribution, but AChE inhibitors were not analysed specifically.

d. The clearest spatial trends emerged from analysis of male samples; this is probably because sampling occurred (deliberately) at the spawning season and the "noise" associated with disruptions in normal biochemistry caused by reproduction almost certainly obscured any "signal" due to contaminants, particularly in females.

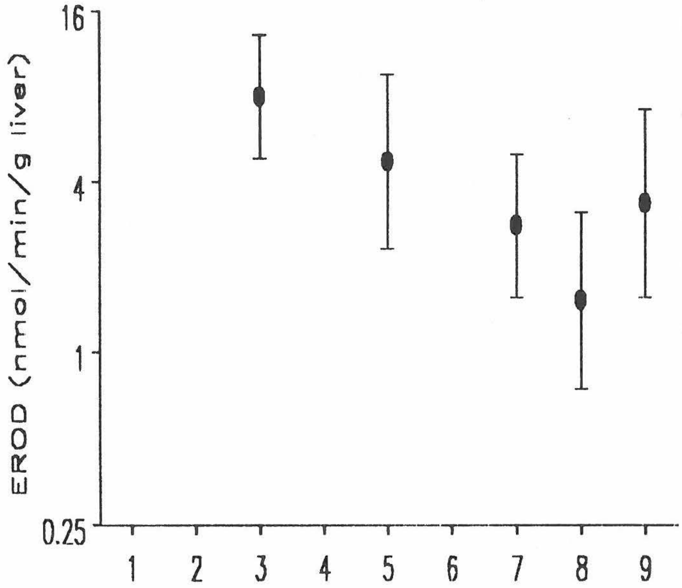
e. In general the biochemical trends reflected trends in organic contaminants determined by analytical chemistry. The exception to this correlation was station 9 in the German Bight: from biochemical evidence, this appeared to be more contaminated than station 8, but the chemical evidence available did not support this.

GERMAN BIGHT BIOCHEMISTRY

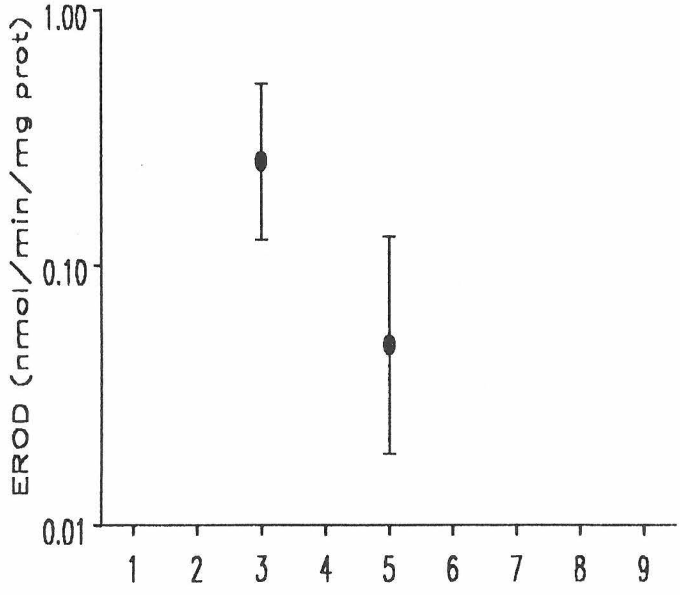
Addison, Hansen and Renton
Female Dab Liver



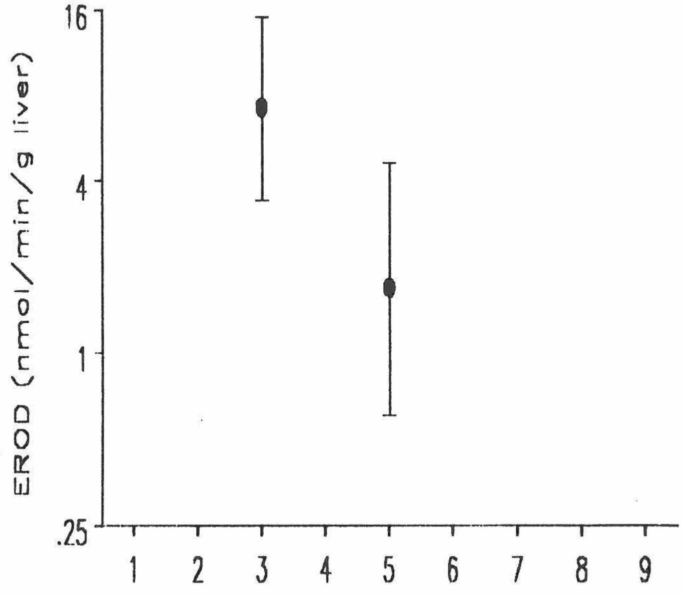
Addison, Hansen and Renton
Female Dab Liver



Addison, Hansen and Renton
Female Dab Liver

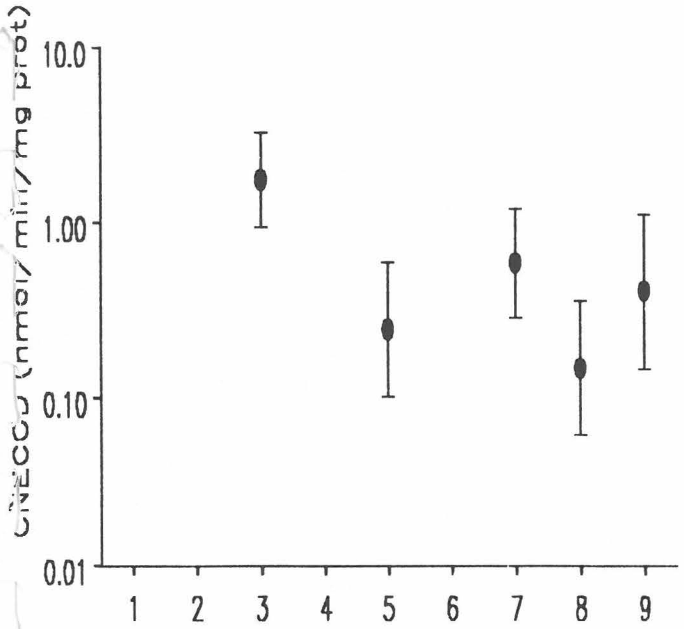


Addison, Hansen and Renton
Female Dab Liver

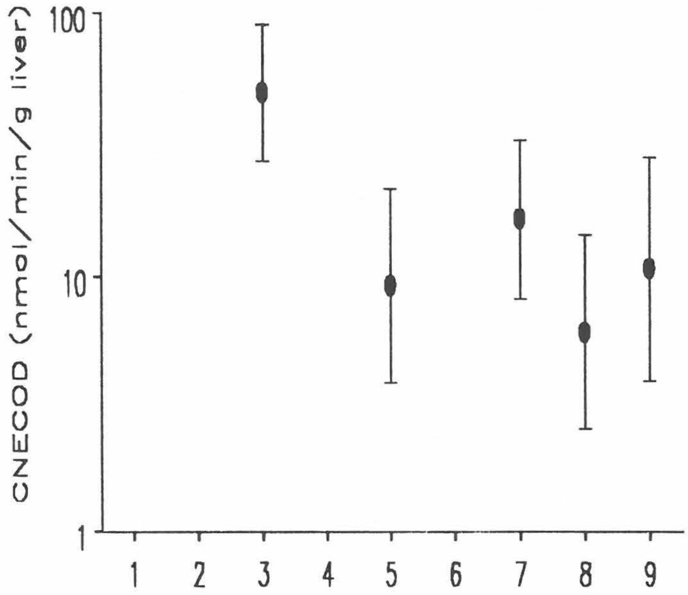


GERMAN BIGHT BIOCHEMISTRY

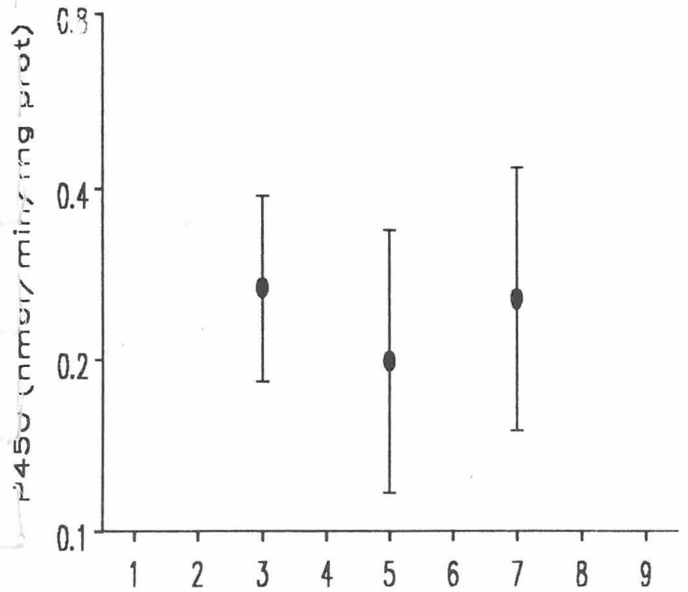
Addison,Hansen and Renton
Female Dab Liver



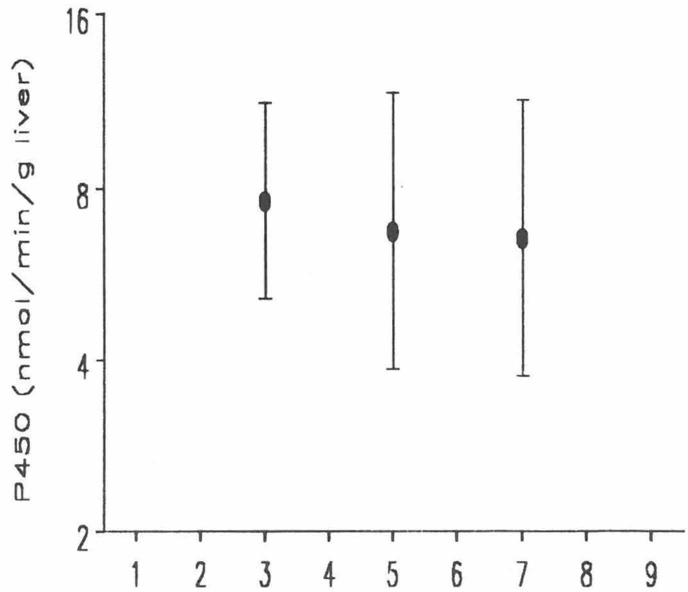
Addison,Hansen and Renton
Female Dab Liver



Addison,Hansen and Renton
Female Dab Liver

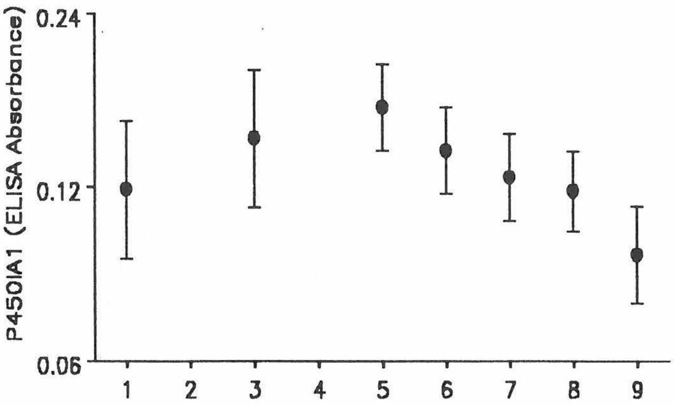


Addison,Hansen and Renton
Female Dab Liver

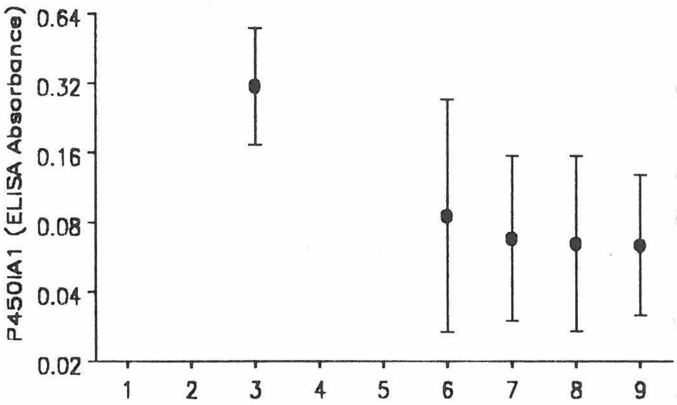


GERMAN BIGHT BIOCHEMISTRY

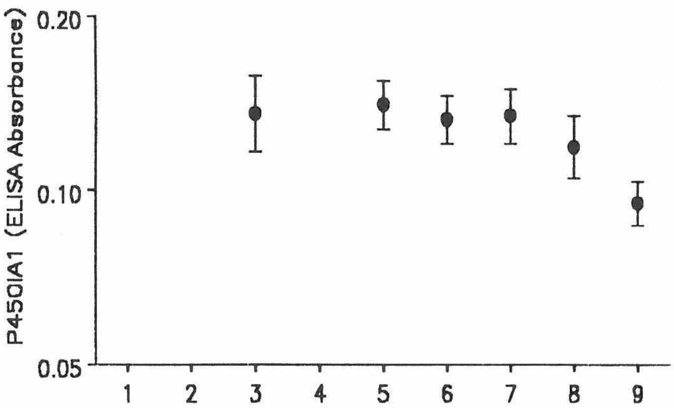
Goksoyr and Larsen
Dab Head Kidney (microsomes)



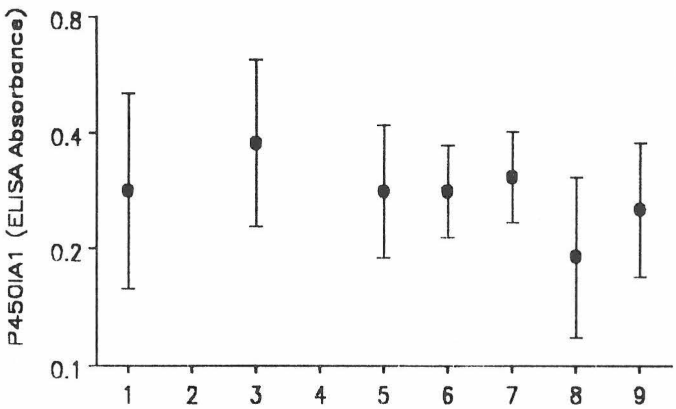
Goksoyr and Larsen
Dab Liver (microsomes)



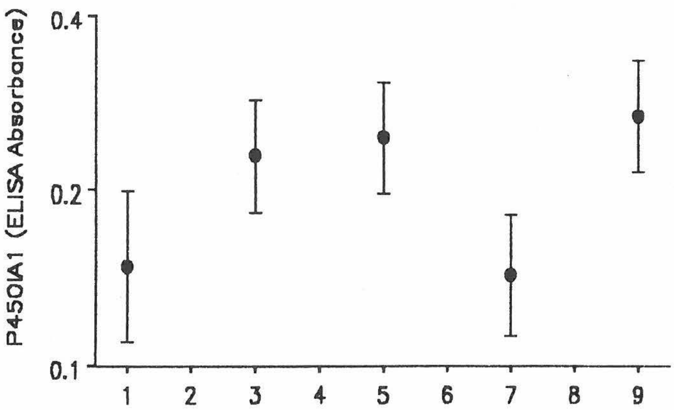
Goksoyr and Larsen
Dab Trunk Kidney (S9 Fractions)



Goksoyr and Larsen
Dab Liver (S9 Fractions)

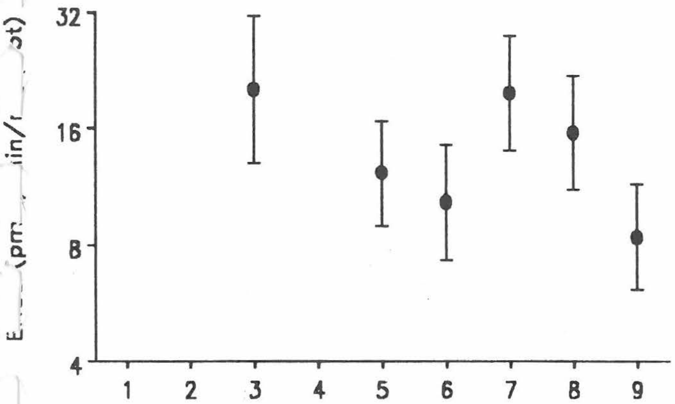


Goksoyr and Larsen
Dab Gills (S9 Fractions)

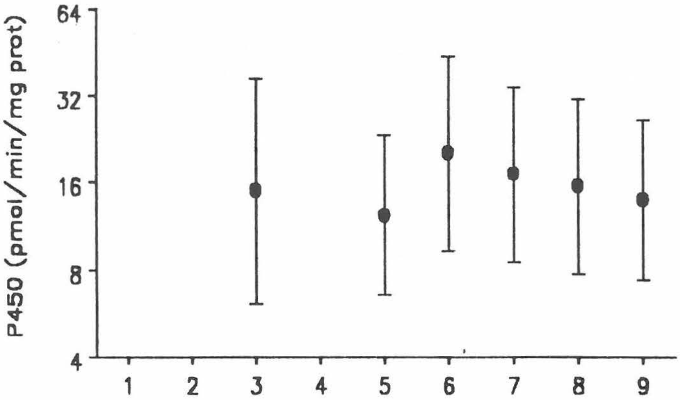


GERMAN BIGHT BIOCHEMISTRY

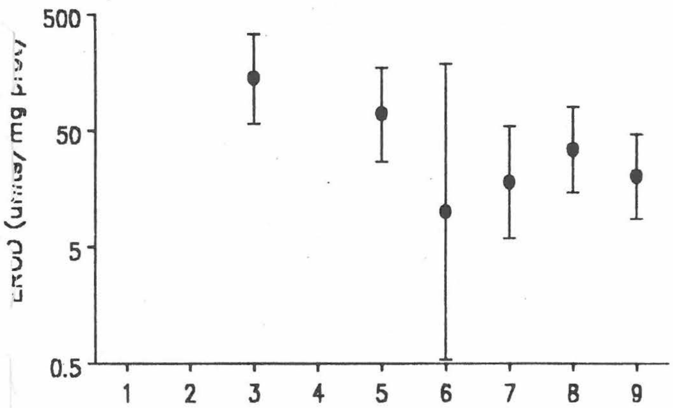
Blom and Forlin
Male Dab Trunk Kidney



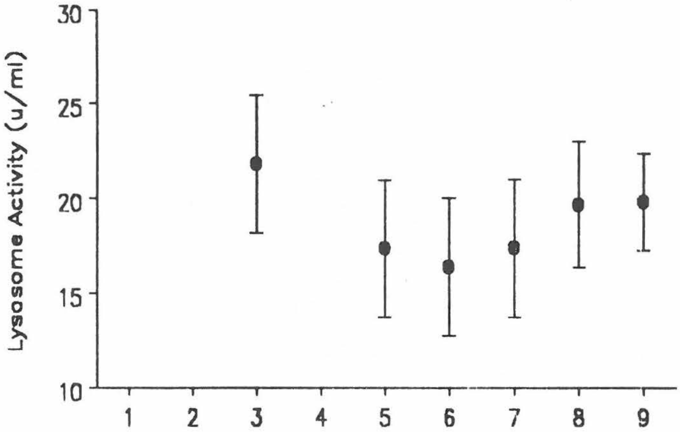
Blom and Forlin
Male Dab Head Kidney



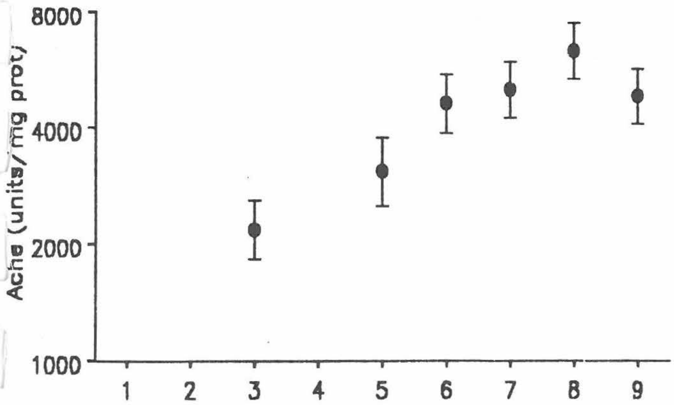
Martin Eggens
Female Dab Liver



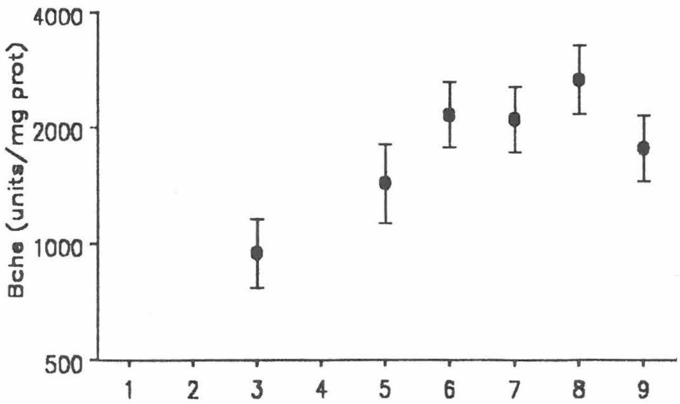
Andreas Mock
Female Dab Blood Plasma



Galgani and Bocquene
Dab Muscle

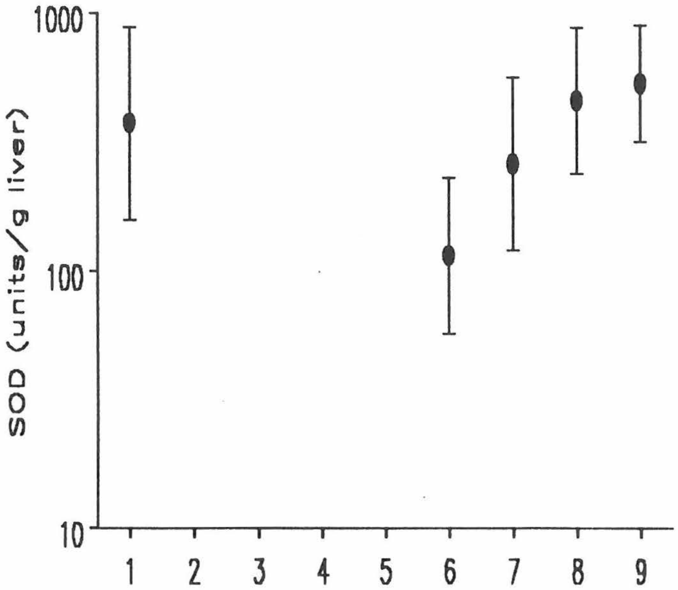


Galgani and Bocquene
Dab Muscle

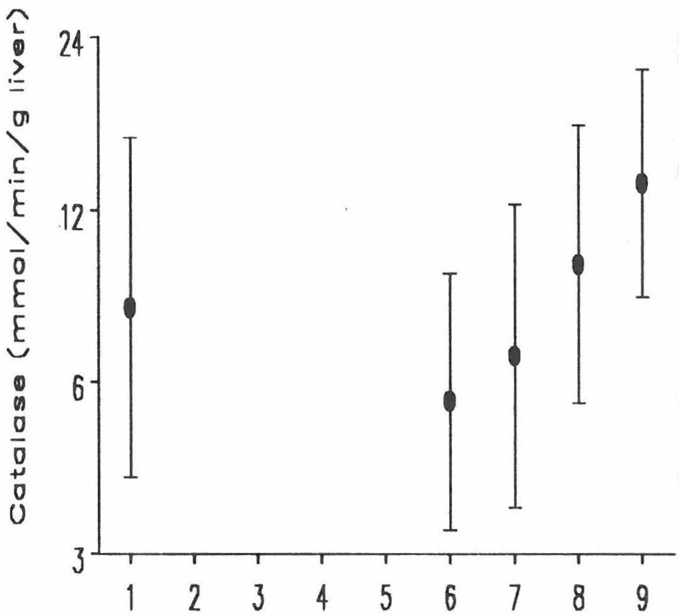


GERMAN BIGHT BIOCHEMISTRY

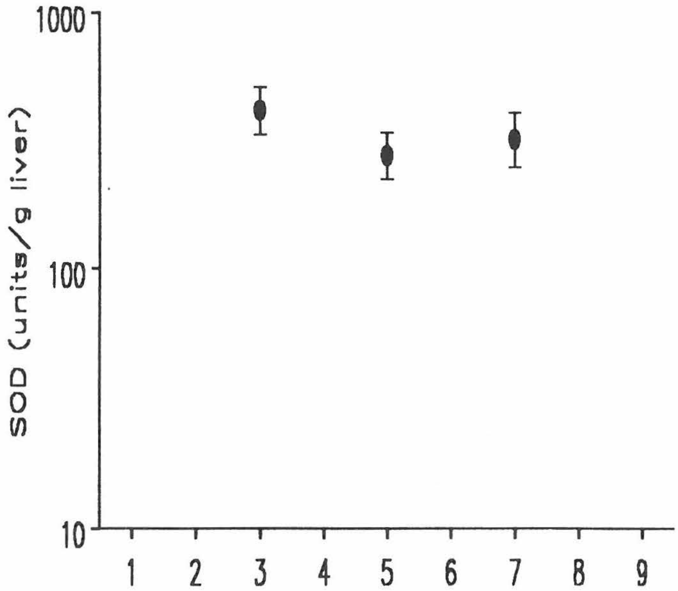
Livingstone and Archibald
Frozen Dab Liver



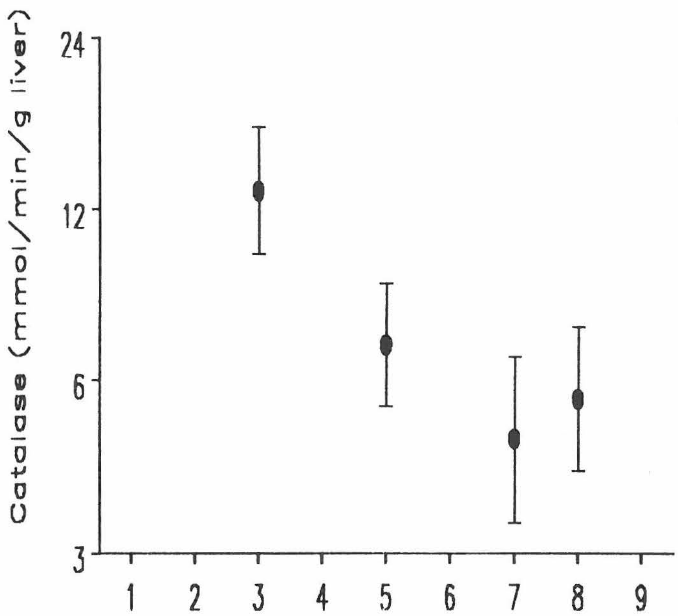
Livingstone and Archibald
Frozen Dab Liver



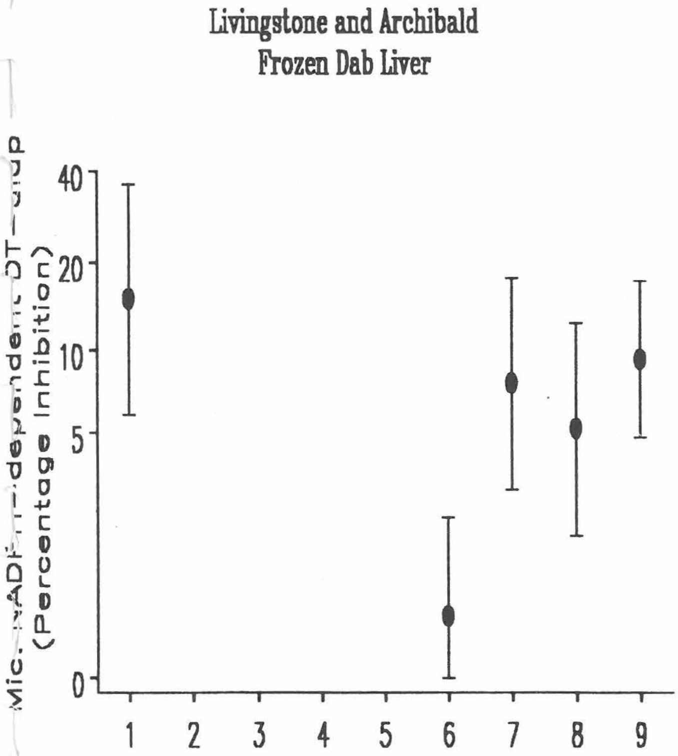
Livingstone and Archibald
Fresh Dab Liver

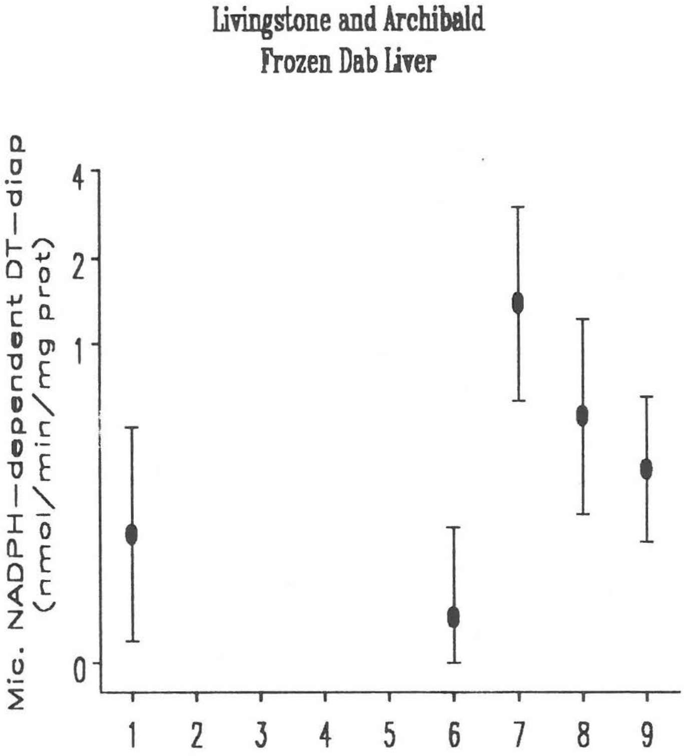


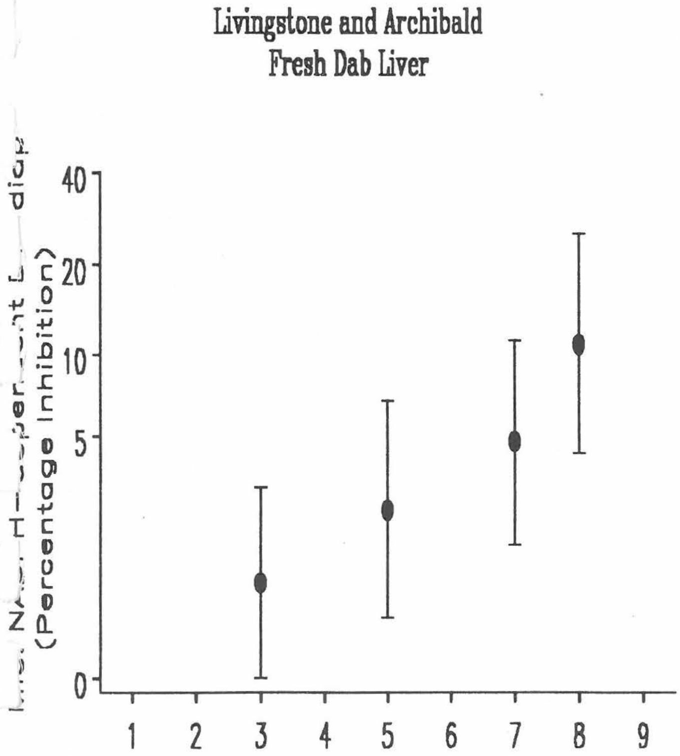
Livingstone and Archibald
Fresh Dab Liver

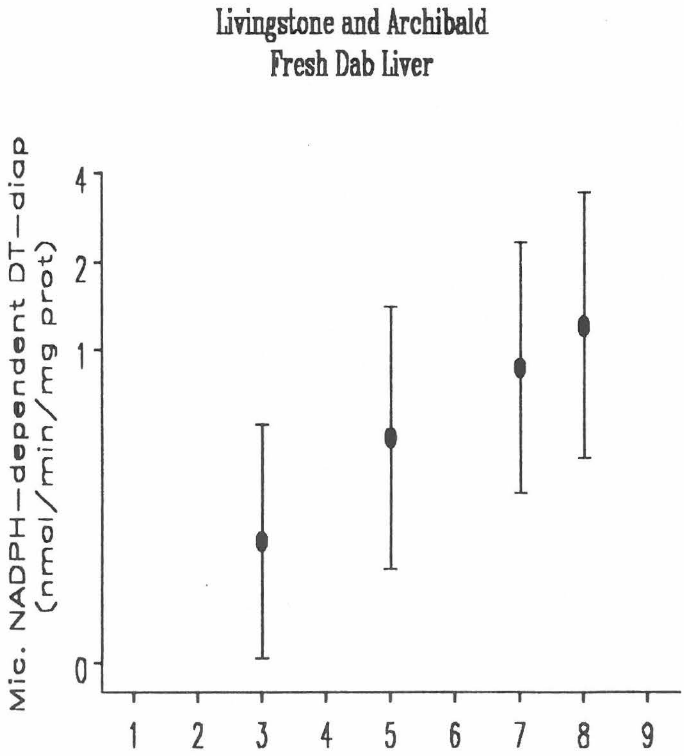


GERMAN BIGHT BIOCHEMISTRY

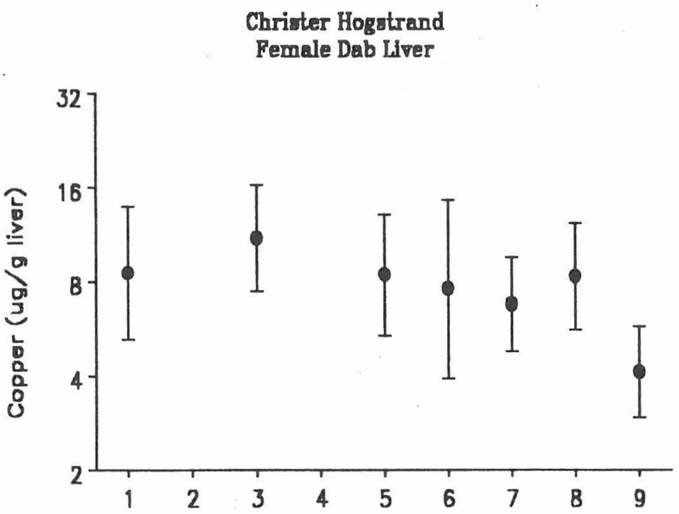


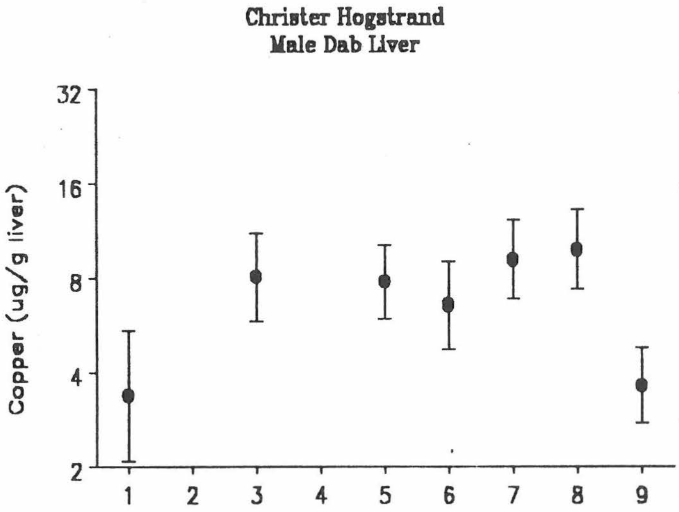


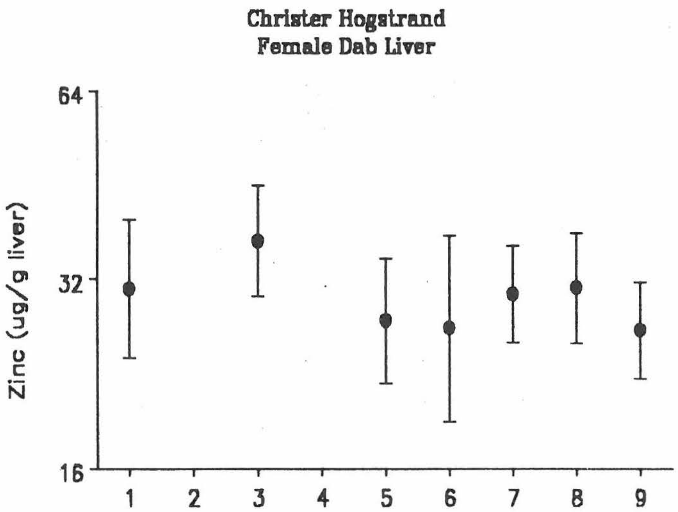


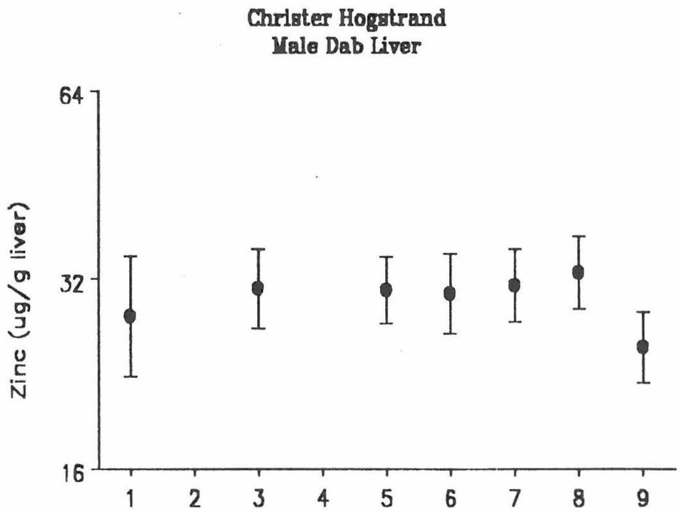


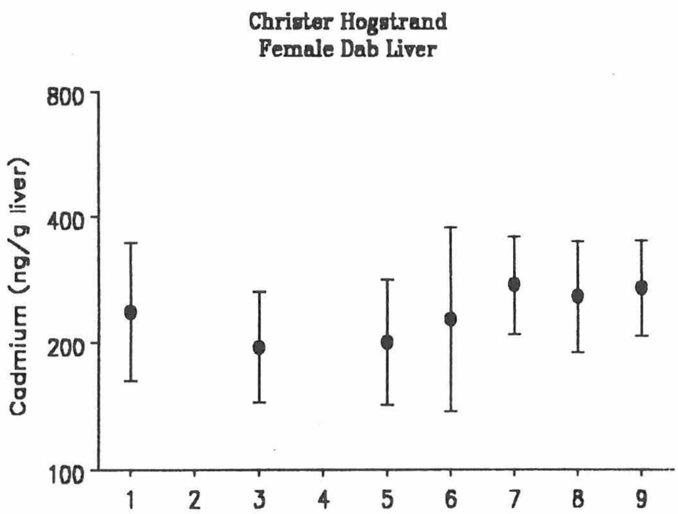
GERMAN BIGHT BIOCHEMISTRY

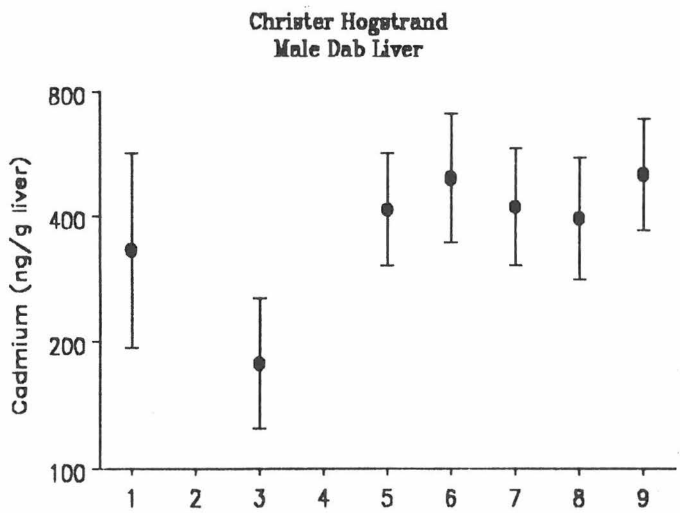






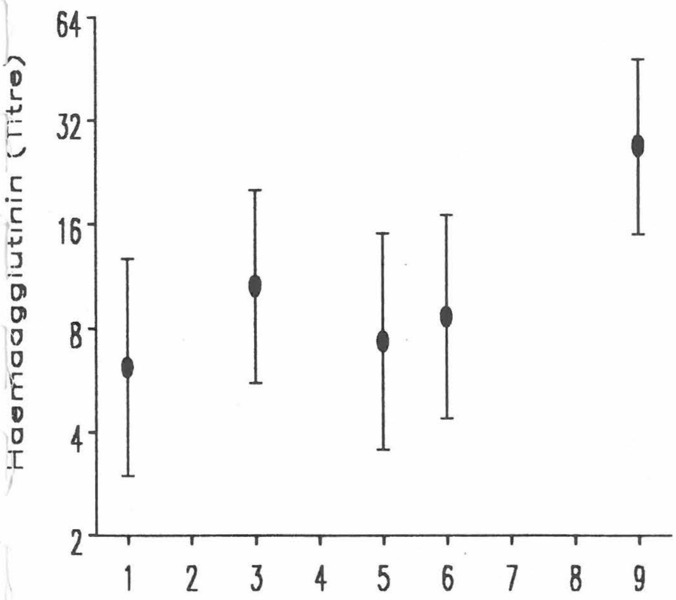




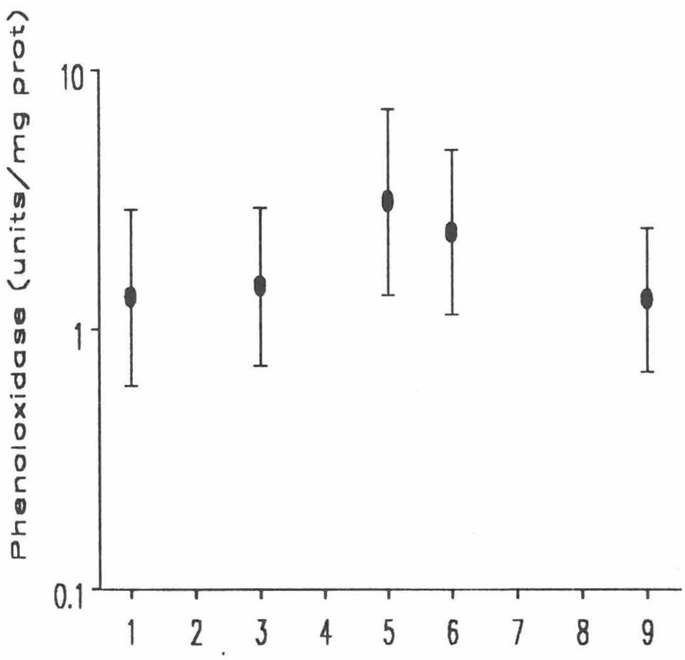


GERMAN BIGHT BIOCHEMISTRY

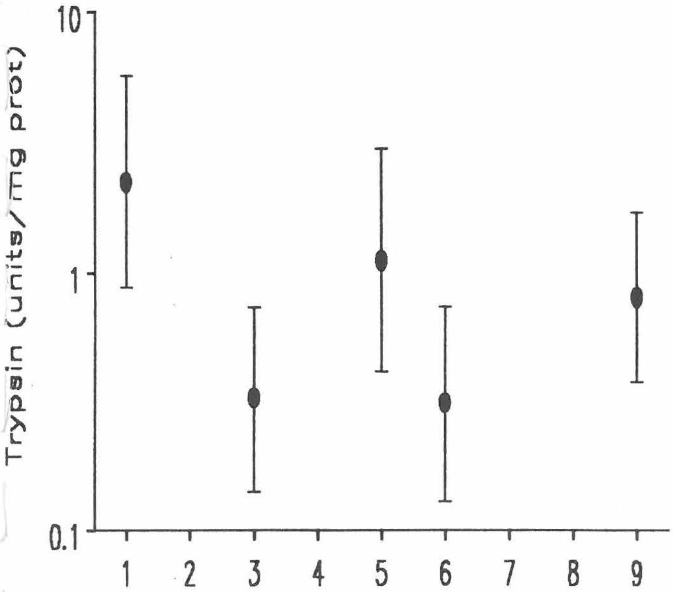
John McHenery
Crab Blood Plasma



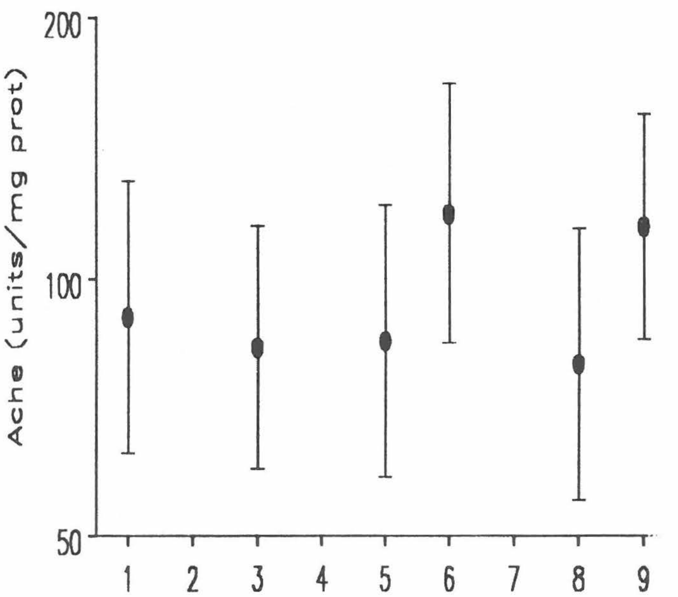
John McHenery
Crab Blood Cells



John McHenery
Crab Blood Cells



John McHenery
Crab Pincer Tissue



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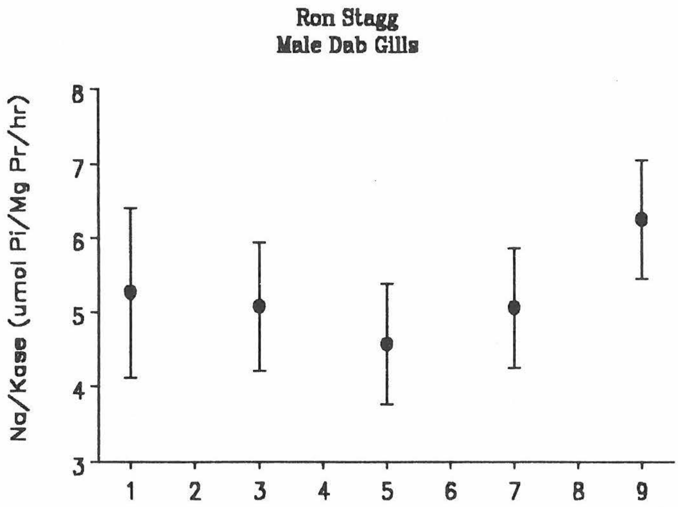
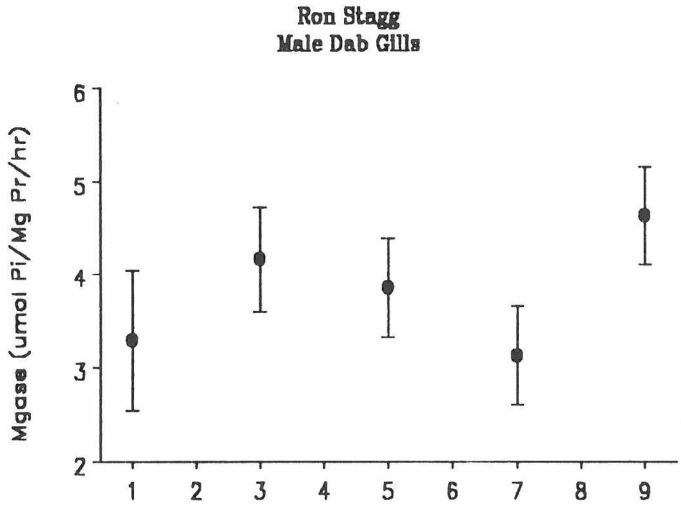
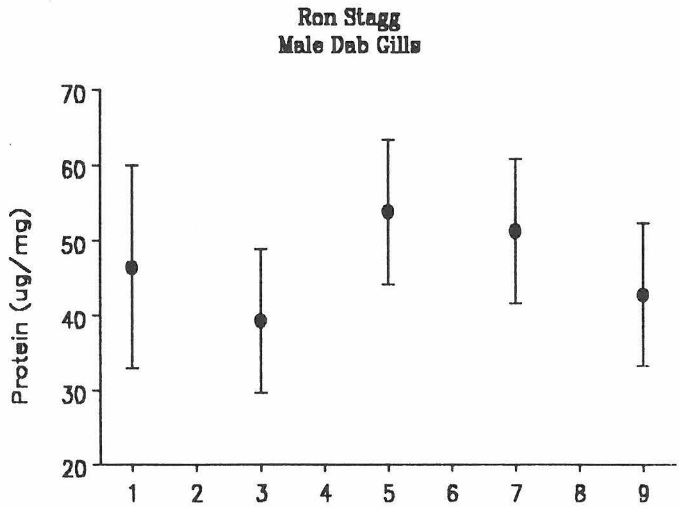
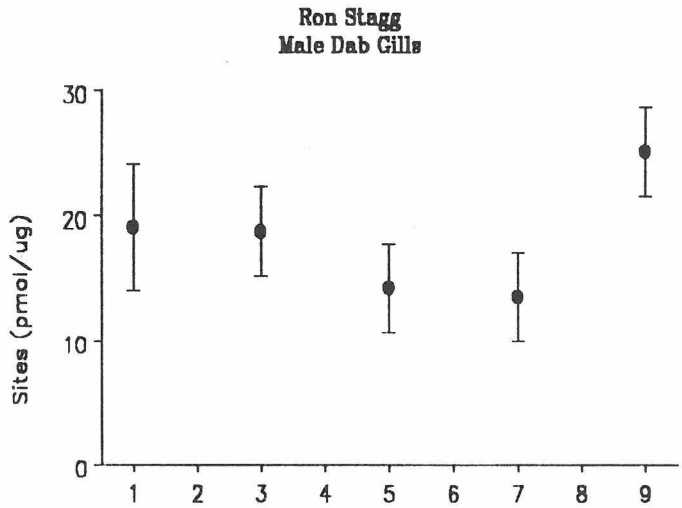
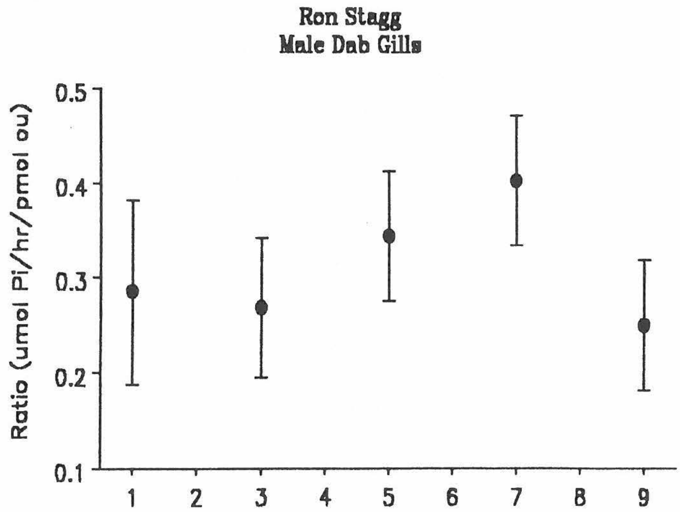




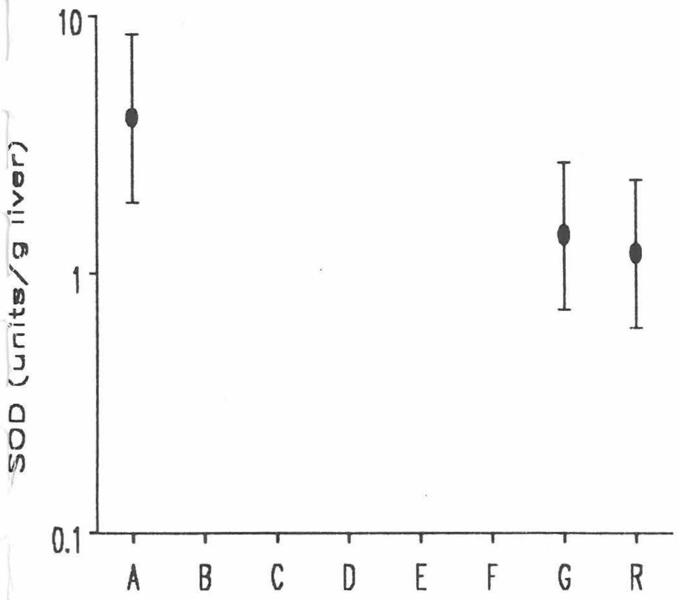




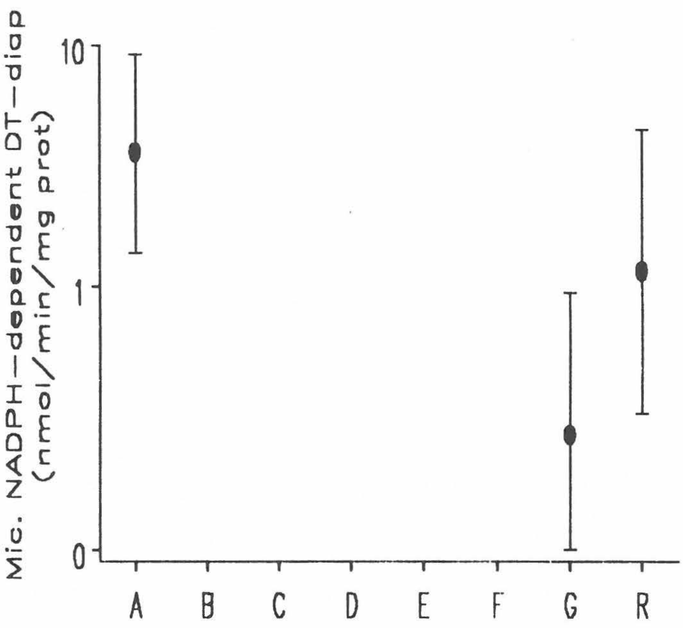


OIL PLATFORM BIOCHEMISTRY

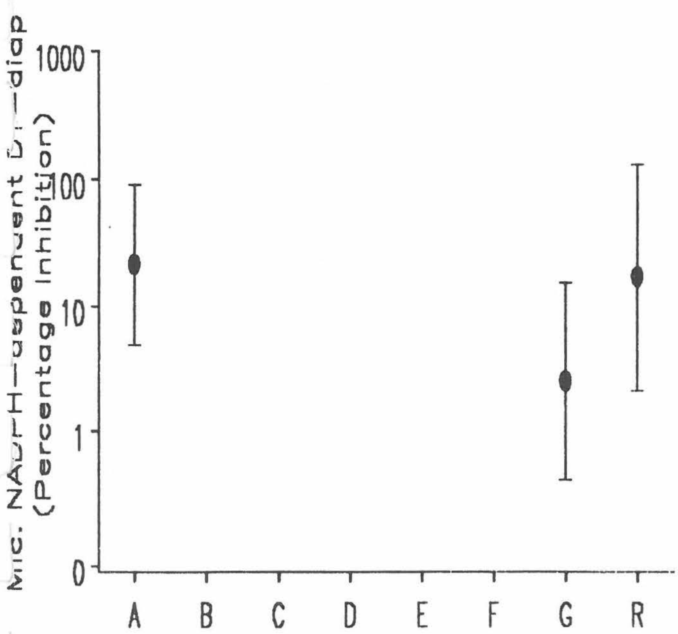
Livingstone and Archibald
Dab Liver



Livingstone and Archibald
Dab Liver

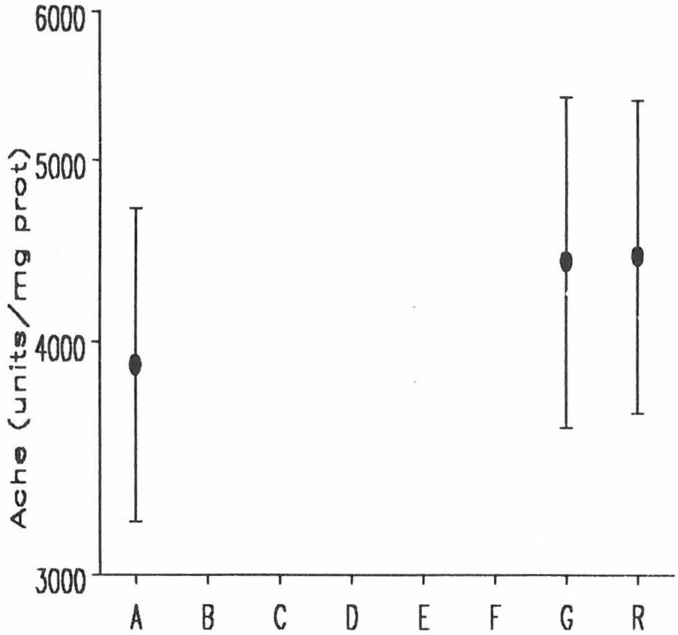


Livingstone and Archibald
Dab Liver

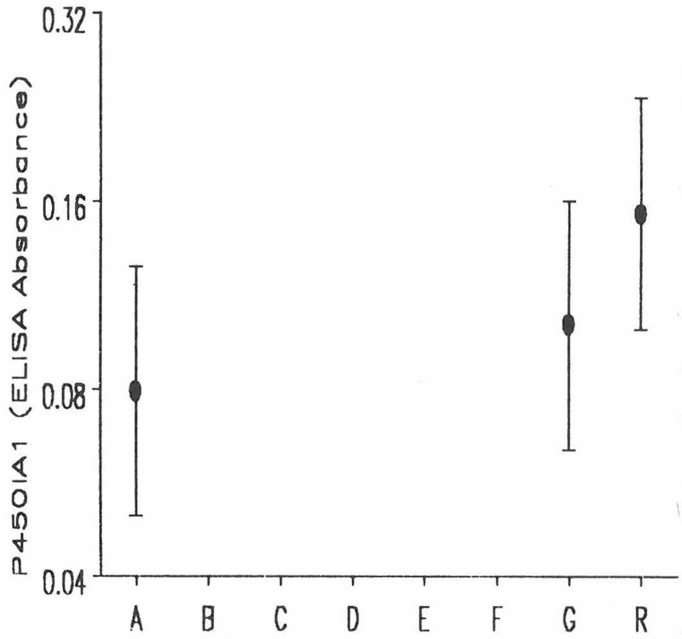


OIL PLATFORM BIOCHEMISTRY

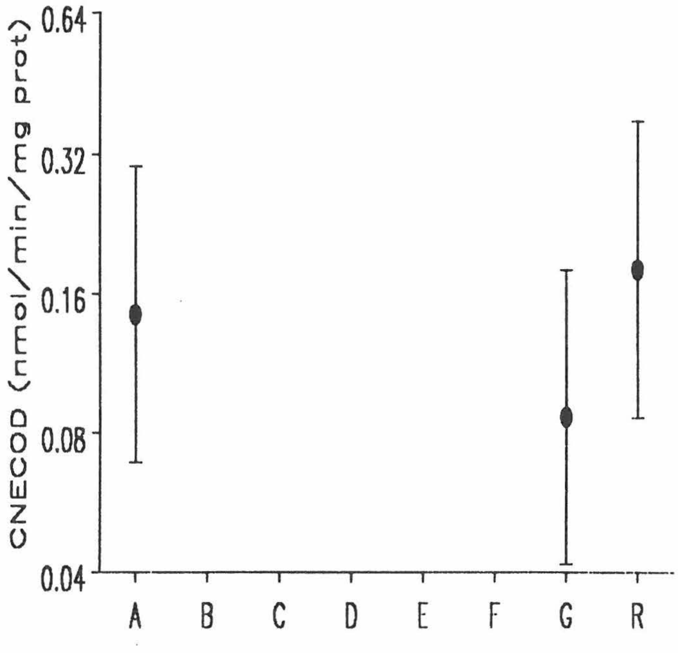
Galgani and Bocquene
Dab Muscle



Goksoyr and Larsen
Dab Liver (microsomes)

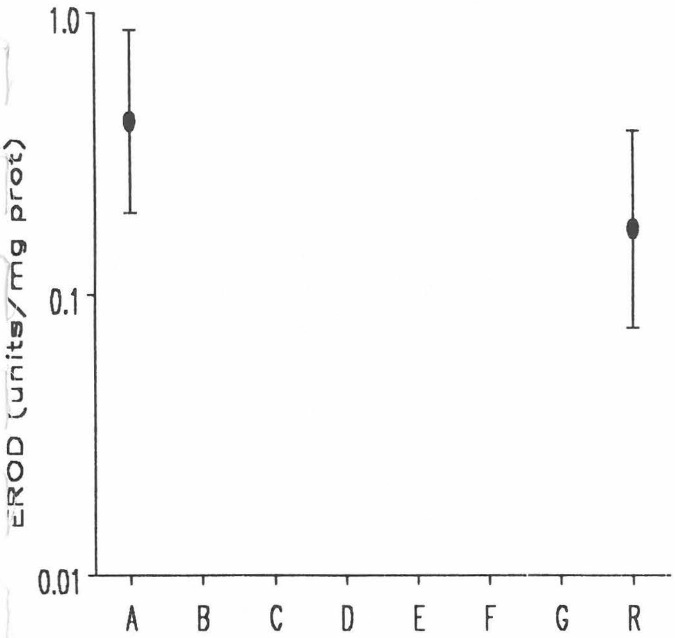


Addison,Hansen and Renton
Dab Liver

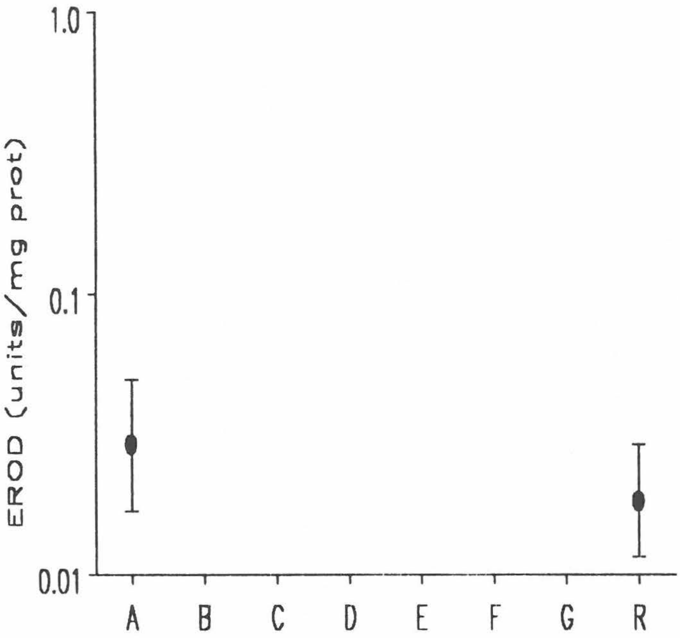


OIL PLATFORM BIOCHEMISTRY

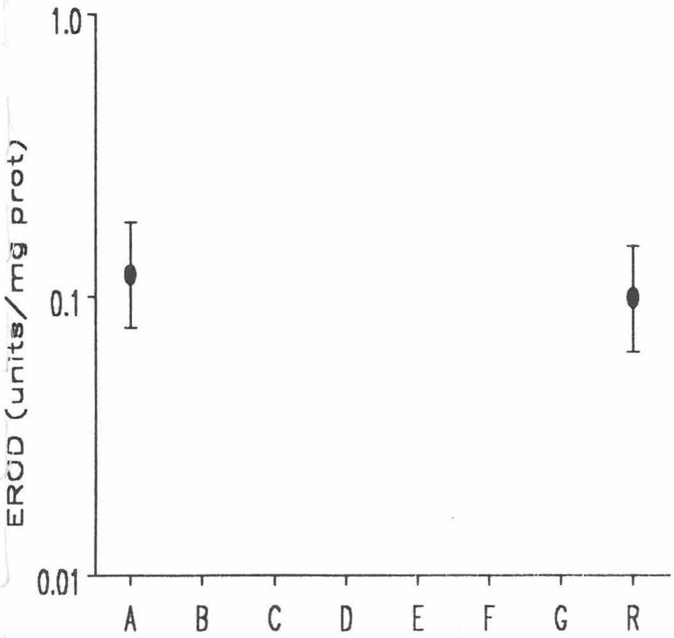
Martin Eggens
Male Dab Liver (Mic. Susp.)



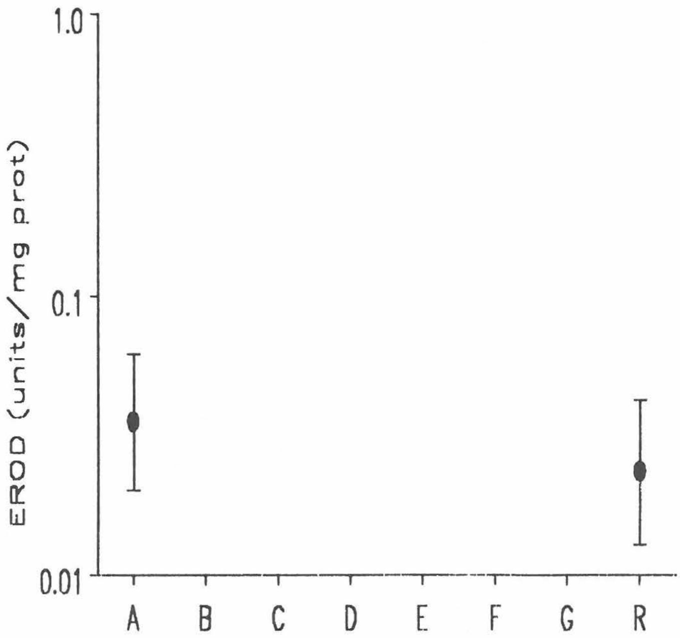
Martin Eggens
Female Dab Liver (Mic. Susp.)



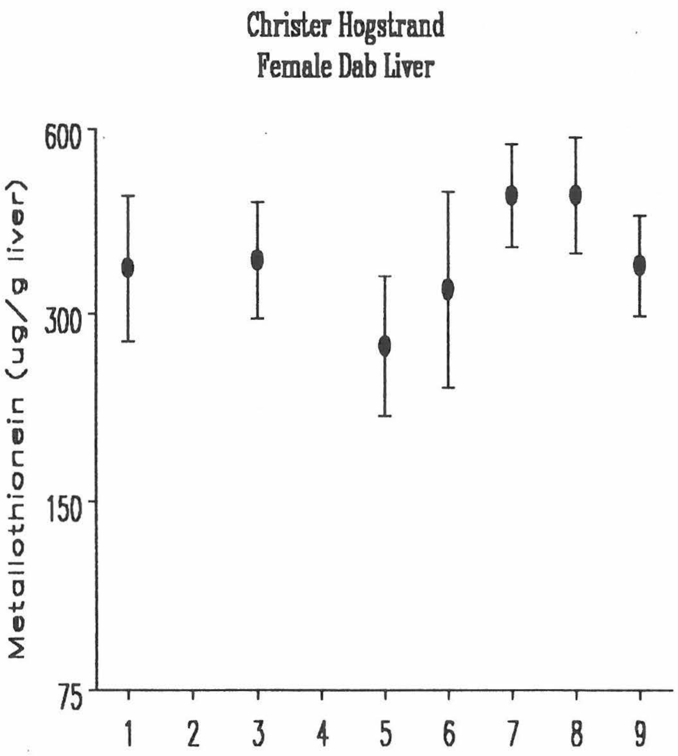
Martin Eggens
Male Dab Liver (12.5 g sup.)

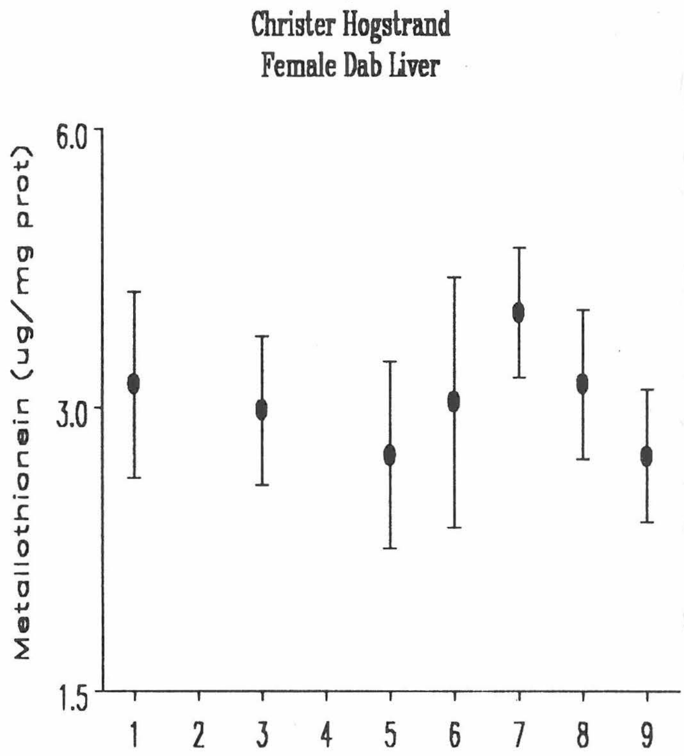


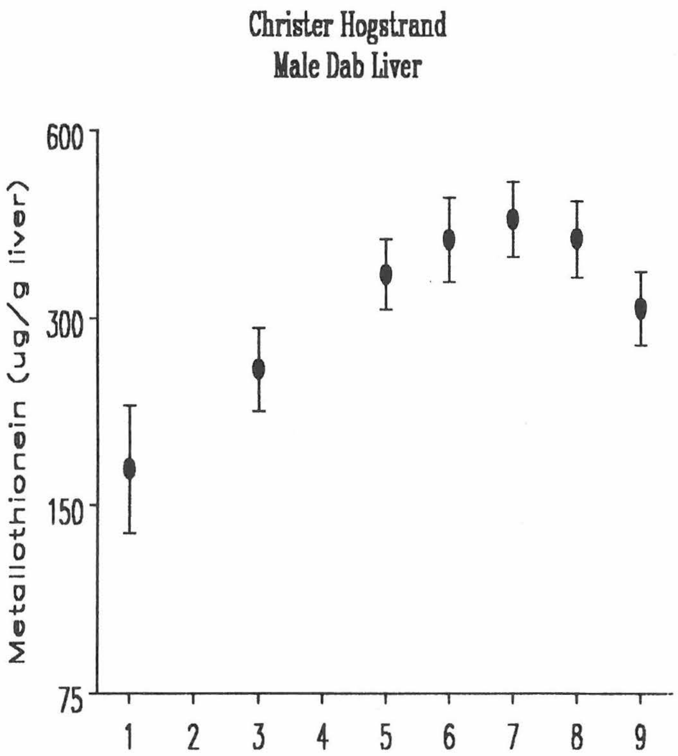
Martin Eggens
Female Dab Liver (12.5 g sup.)

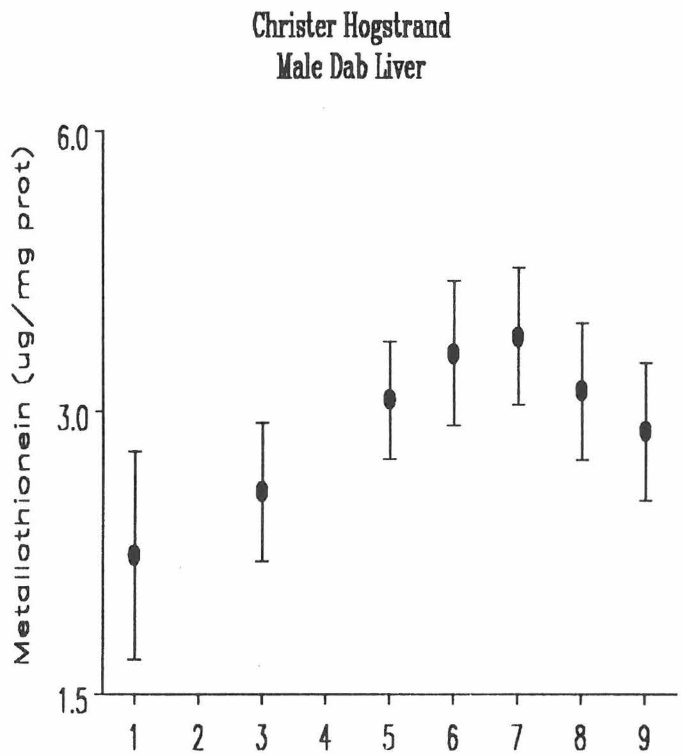


GERMAN BIGHT BIOCHEMISTRY









7. MOLECULAR AND CELLULAR PATHOLOGY (Coordinator: M.N.Moore)

Objectives The aim of the molecular and cellular pathology group in this Workshop was to test for evidence of biological changes which were indicative of exposure to toxic chemicals, as well as to their adverse effects. Much of the study focussed on the liver of the flatfish dab (*Limanda limanda*). The liver was chosen as it is an integrator of many functions including detoxication/activation of toxic chemicals, digestion and storage, excretion and synthesis of the egg yolk protein vitellogenin. There is also a considerable body of literature on pollutant chemical impact on the cellular pathology of fish liver to aid interpretation.

The approach was based on the detection of molecular and cellular changes resulting from chemical contamination. The identification of early onset changes, which may ultimately lead to overt disease, is a key requirement in assessment of the effects of environmental pollution. Diseased fish are likely to be rapidly eliminated from the population, hence assessment based on such fish may prove difficult, and even if samples are available higher level complications resulting from the primary lesion will be a confounding factor. In fact, it is only through the mechanistic linking of changes in molecular and cellular processes with the later pathological endpoints that it will be possible to establish causal relationships.

Results The methods used included techniques for the detection of genotoxic damage, through subcellular injury to detailed histopathology and embryo abnormalities. Non-overtly diseased female dab were used through this study.

Genotoxic assays. A battery of assays was used for the detection of genotoxic damage and production of metabolites of chemical pro-mutagens. Liver samples were analysed for bulky hydrophobic DNA adducts and oxidised bases (8- hydroxydeoxyguanosine); no significant differences were seen between stations along the transect. Mutagenicity assays did not detect mutagens in liver extracts or deconjugated bile samples. There was no evidence of pollution- related unscheduled DNA synthesis in dab liver cells. Liver enzymes, however, activated 2-acetylaminofluorene to mutagenic derivatives to a significantly greater extent in fish from stations 3 and 5 than from stations 6 and 7.

Molecular probes inserted into live isolated liver cells showed biochemical alterations as well as changes in some of the membranous compartments within the liver cells. These included an increase in production of oxyradicals (reactive forms of oxygen which can damage cellular macromolecules), as well as in the amount of endoplasmic reticulum (ER) at stations 3 and 5 (contaminated) as compared with station 7. The ER is responsible for manufacture of proteins and detoxication of xenobiotics (ie organic chemical contaminants). Activity of an ER associated detoxication enzyme, EROD, was significantly elevated in live cells from the contaminated station 3. Elevated activity of this enzyme is widely accepted as a biomarker of exposure to toxic xenobiotics. Metabolic derivatives of xenobiotics can redox cycle in the ER and generate oxyradicals.

Another membranous compartment, the lysosomes, was also adversely affected. Lysosomes are responsible for degrading and recycling both endogenous and exogenous macromolecules and are highly sensitive to the toxic effects of xenobiotics and metals. The functional integrity of this compartment was significantly impaired at all stations relative to station 7. The most pronounced damage was seen at stations 3 and 5.

Lysosomes. The lysosomal compartment is closely linked with the endocytic system (responsible for bulk transport into the cell by invagination of the cell surface). The endocytic system transports macromolecules from the cell surface to the lysosome for degradation and involves a complex molecular sorting system for the recycling of cell surface receptors. Non-specific endocytosis of labelled protein was significantly reduced at stations 3, 5 and 6. However, there was a marked increase in the cellular content of a special form of fat (lipid) known as low-density lipoprotein (LDL); this LDL enters the cell by endocytosis following binding to a specific cell surface receptor. The inference is that endocytosis of LDL is enhanced and this in turn contributes to the degenerative fatty change observed in the liver cells at stations 1, 3 and 5. Much of this lipid accumulation was associated with enlarged lysosomes and constitutes a toxic lipidosis. This type of degenerative change appears to be an important step on the path to liver disease such as tumour formation.

An increase in cell surface receptors for epidermal growth factor (EGF- stimulates cell growth) at station 3 may indicate attempted regeneration of the damaged liver. Once again, the EGF-receptor complex, like LDL, enters the cell by receptor-mediated endocytosis. This type of endocytosis requires a specific protein (clathrin) which is also elevated at station 3.

Histopathological analysis confirmed that the liver was the major target organ for toxic damage. Dividing liver cells were observed in samples from stations 1 and 3 and may be associated with regenerative foci. Livers from the inshore sites also showed an increase in foci of cellular alteration. These foci are an established indicator of preneoplastic change and as such are a biomarker for potential tumour development. A cancer-inducing protein (*ras*-oncoprotein) was also detected in small groups of liver cells and foci of cellular alteration (preneoplastic lesions). The incidence of positive livers was 100% at station 3, 0% at station 6 and 30 % at station 7 (reference).

Immunological parameters included plasma lysozyme activity and various measurements on melanomacrophage centres (MMC) in spleen and liver. No significant differences were detected between the inshore sites and station 7.

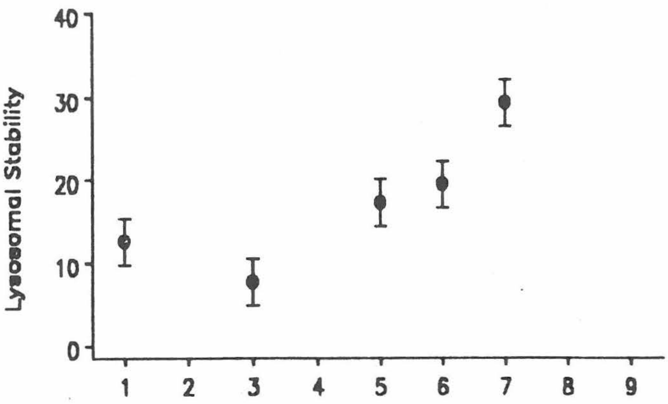
Dab embryo abnormalities and chromosomal aberrations were highest at the inshore contaminated stations 3 and 5, as well as station 9 on the eastern side of the Dogger Bank, and lowest at station 7 and 8. Chromosomal aberrations were highest at stations 6 and 7, with an upward trend in frequency at station 9.

Conclusions The findings clearly demonstrate degenerative changes in liver cells and embryos from the most contaminated inshore sites. The most obvious "distress signal" was given by the various **measurements of lysosomal damage** (membrane stability, neutral red retention, binding of acridine orange, enlargement and toxic lipidosis). These alterations together with the higher-order lesions such as fatty change and preneoplastic foci are all **biomarkers of liver degeneration** in the "non- overtly" diseased female dab used in this study. The increased endoplasmic reticulum, its associated EROD activity and activation of pro-mutagens, together with the increased production of oxyradicals can be considered as probable **biomarkers of exposure** to contaminant xenobiotics, as indicated by the liver PCB data.

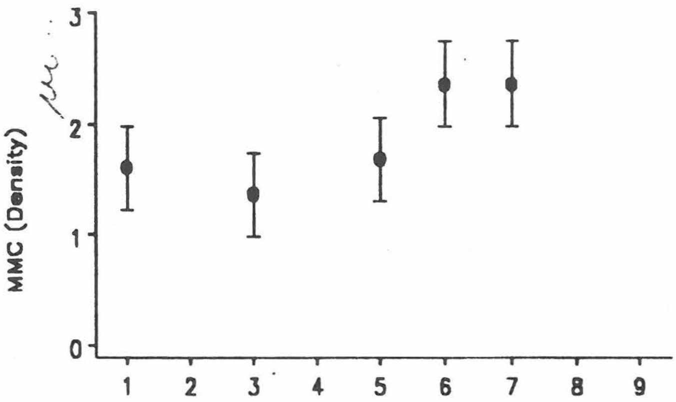
The results indicate that future monitoring studies should include a suite of molecular and cell pathological techniques, which would provide a link between biochemical and histopathological results. This would provide a better understanding of the sequence of events between contaminant exposure and its probable effects, opening a pathway for environmental decision making. A further benefit would be to aid in the assessment of recovery during environmental bioremediation. No single technique provides an adequate index of pollutant effect, rather we advocate the use of a comprehensive suite of clinical tests from those now available to cellular pathologists.

GERMAN BIGHT CELLULAR PATHOLOGY

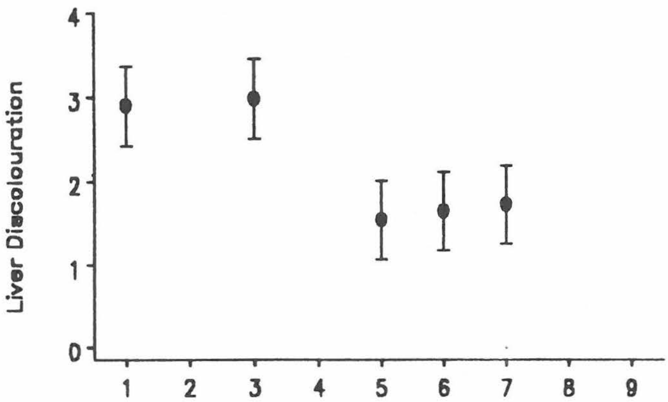
Angela Kohler-Gunther
Dab Liver



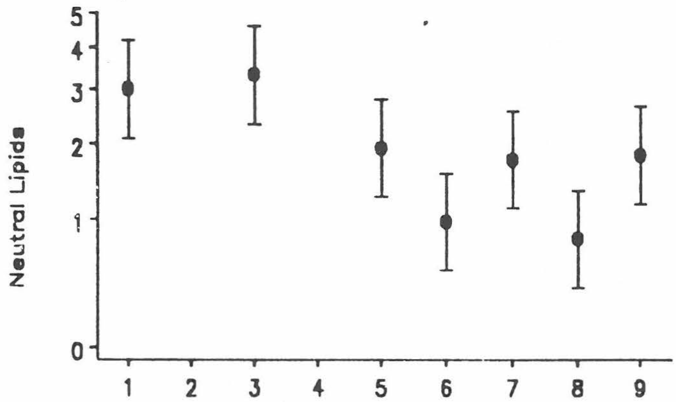
Angela Kohler-Gunther
Dab Liver



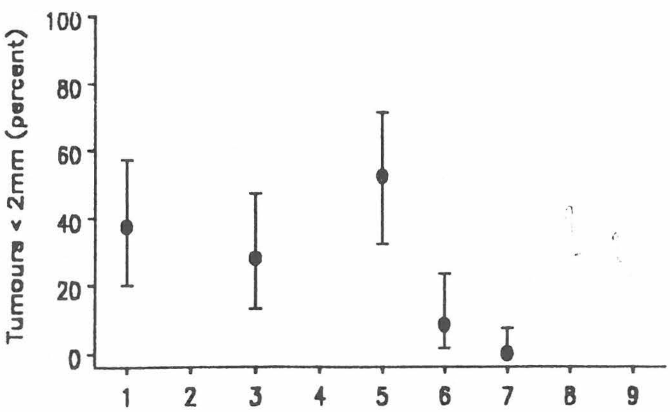
Angela Kohler-Gunther
Dab Liver



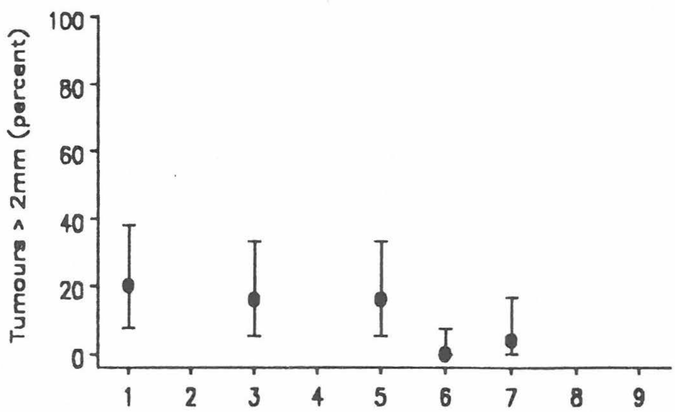
Angela Kohler-Gunther
Dab Liver



Angela Kohler-Gunther
Dab Liver

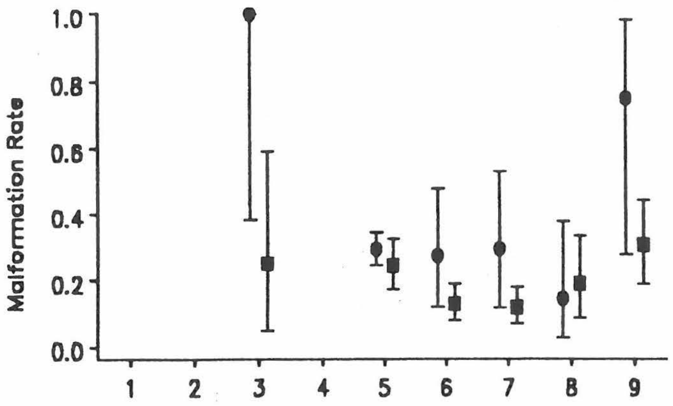


Angela Kohler-Gunther
Dab Liver

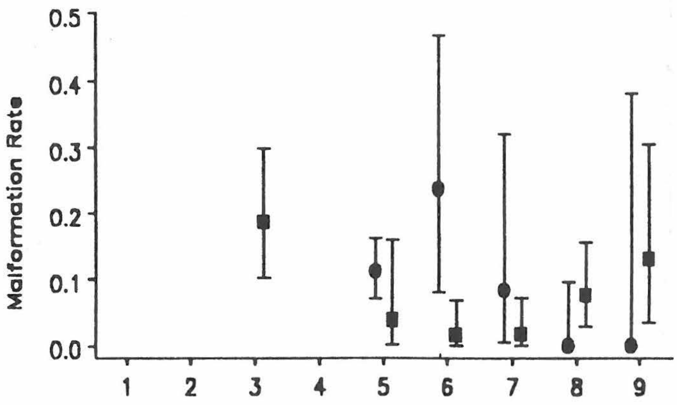


GERMAN BIGHT CELLULAR PATHOLOGY

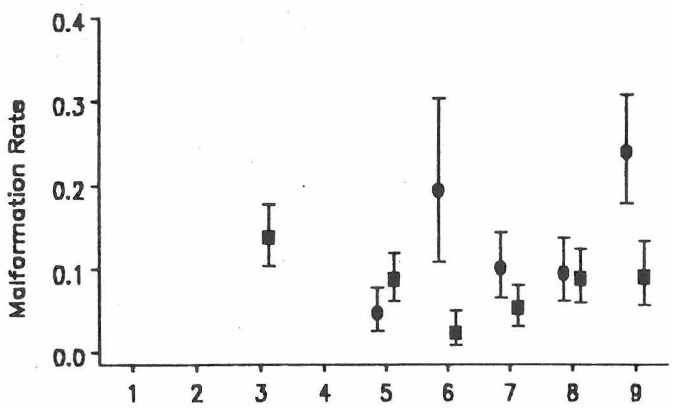
Cameron and Berg
Dab Embryos – Stage Ia



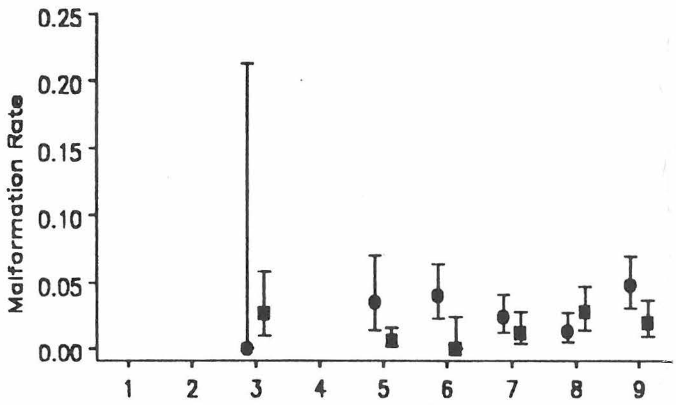
Cameron and Berg
Dab Embryos – Stage Ib



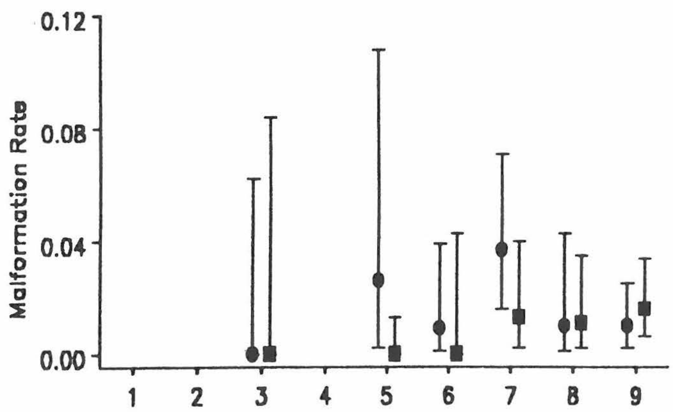
Cameron and Berg
Dab Embryos – Stage II



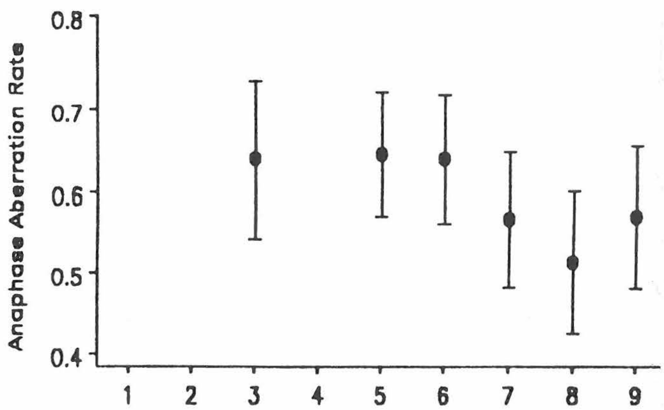
Cameron and Berg
Dab Embryos – Stage III



Cameron and Berg
Dab Embryos – Stage IV



Cameron and Berg
Pelagic Fish Embryos



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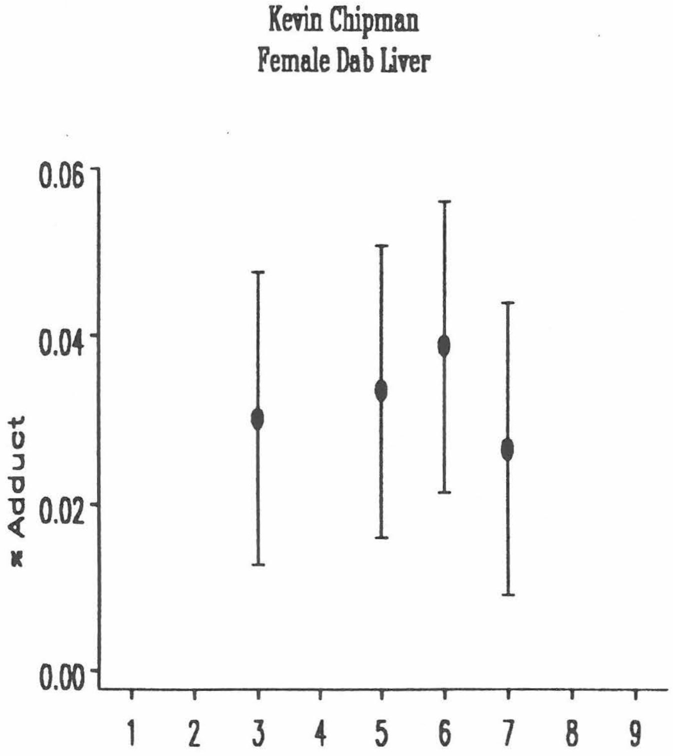
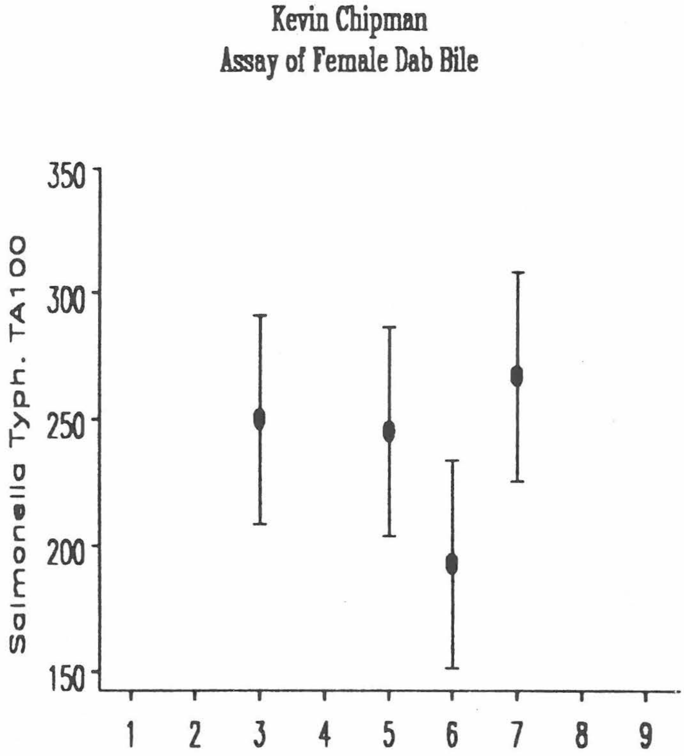
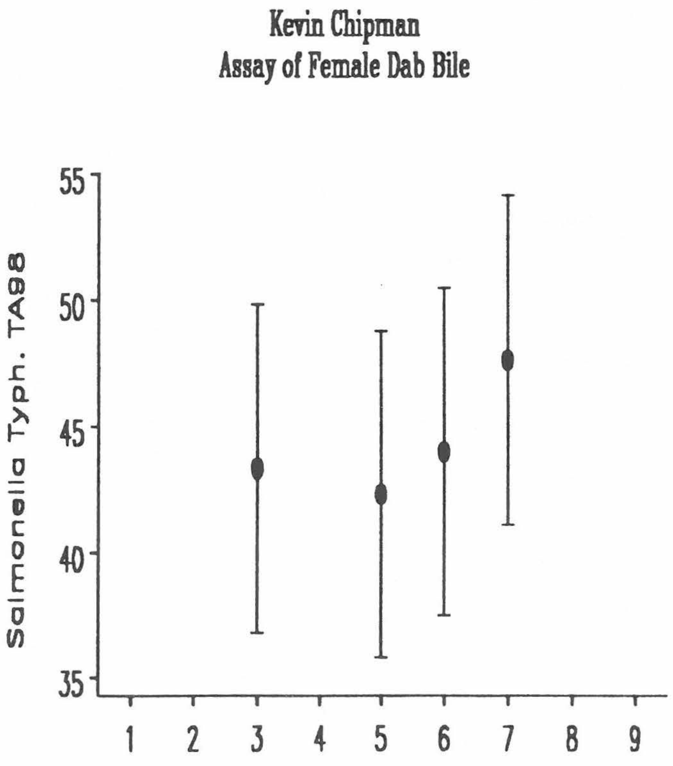
GERMAN BIGHT CELLULAR PATHOLOGY

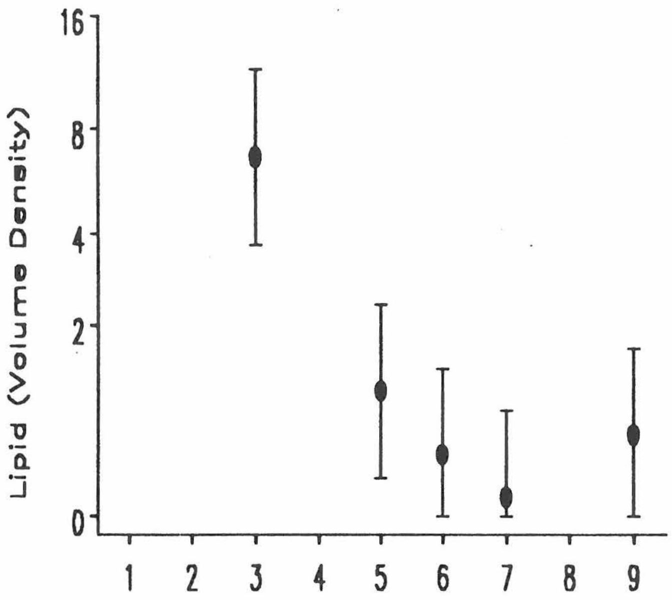




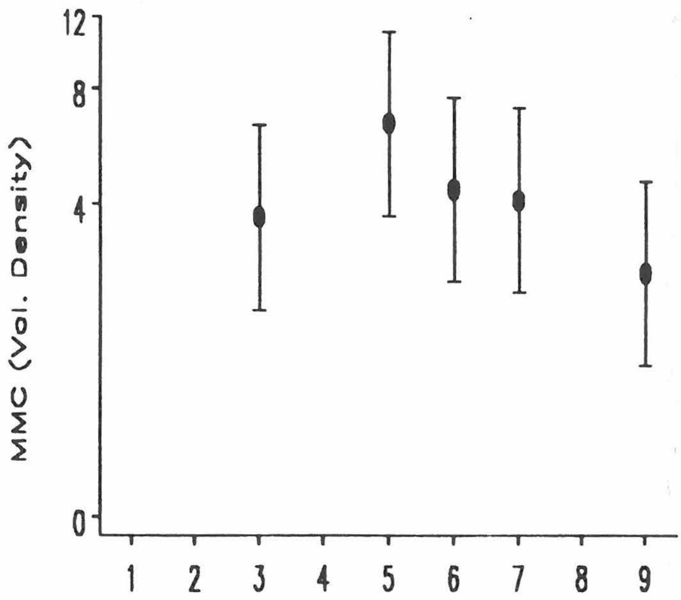


GERMAN BIGHT CELLULAR PATHOLOGY

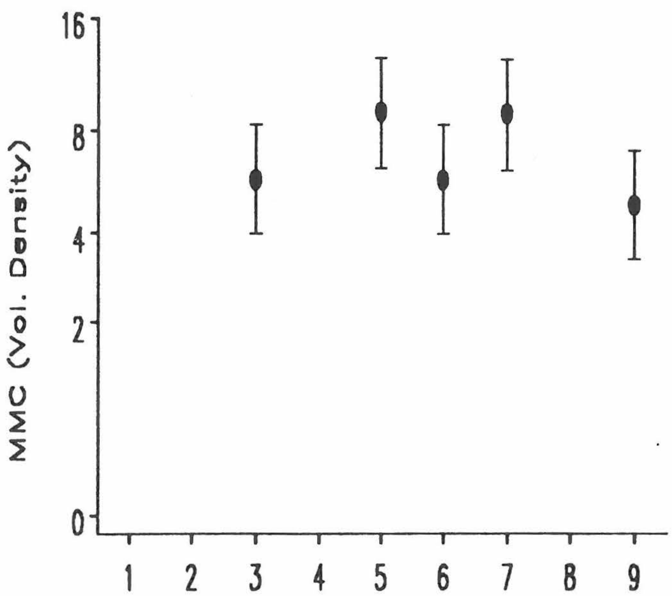
David Lowe
Female Dab



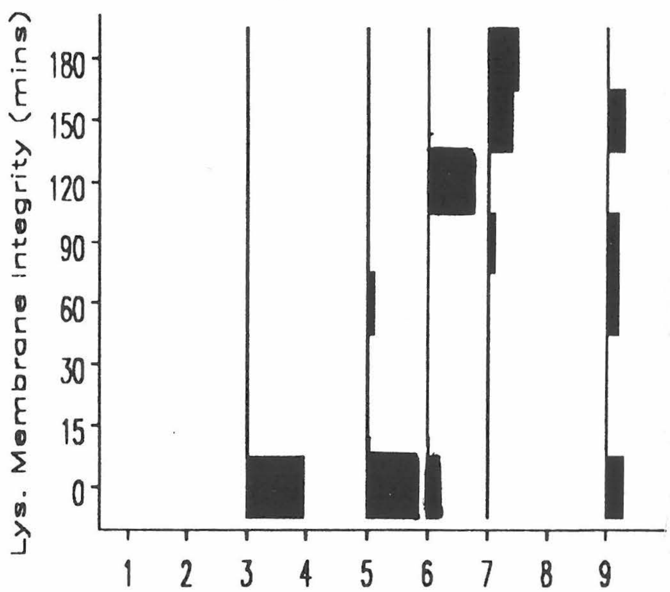
David Lowe
Female Dab Spleen (LMB/BF)



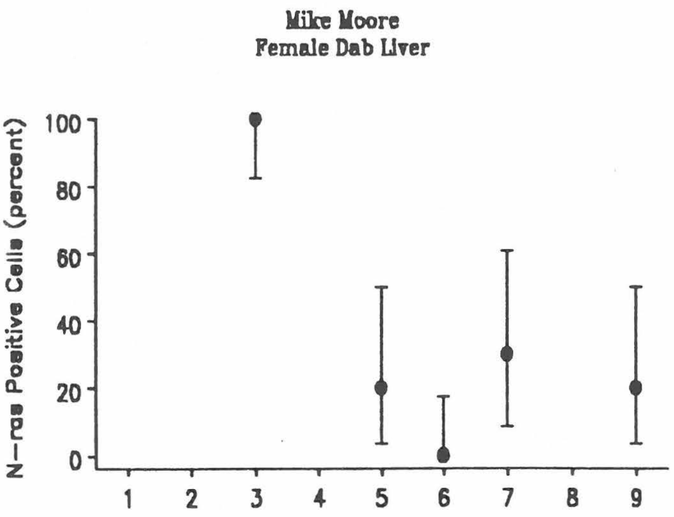
David Lowe
Female Dab Spleen (Perls)

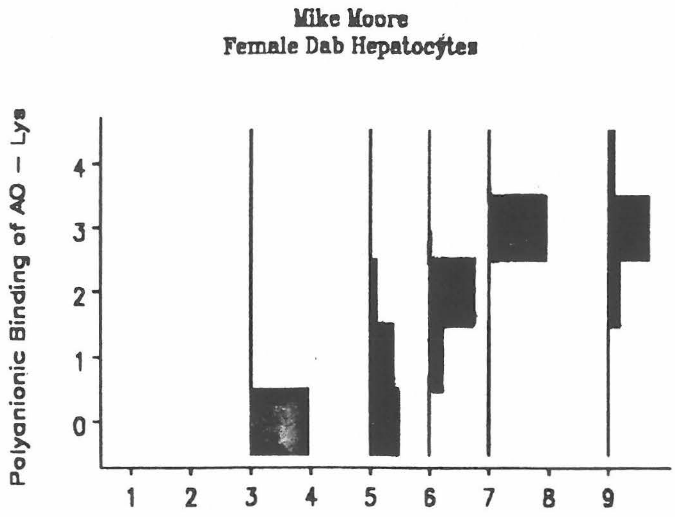


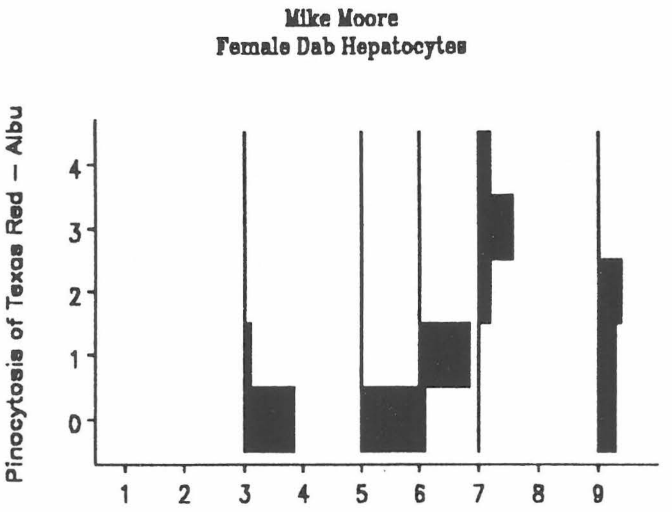
David Lowe
Female Dab

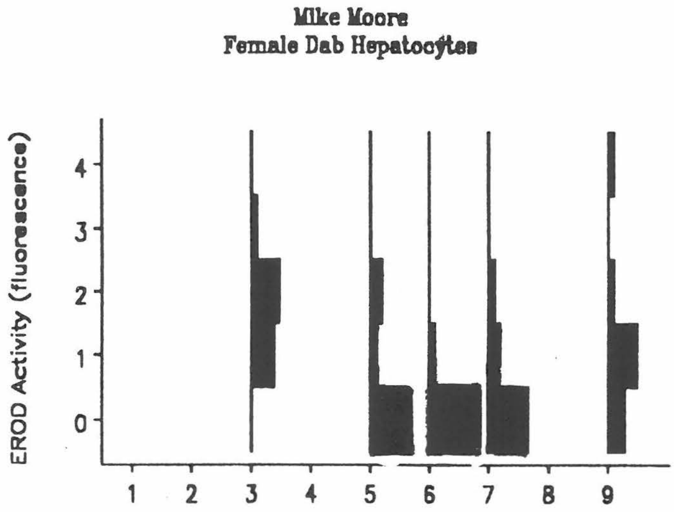


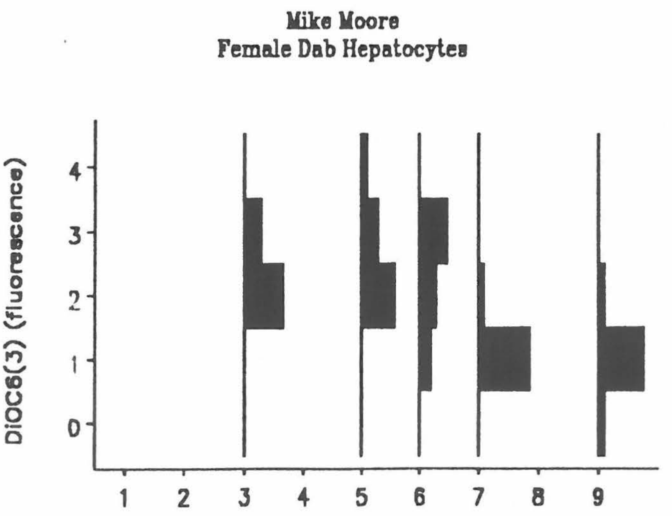
GERMAN BIGHT CELLULAR PATHOLOGY

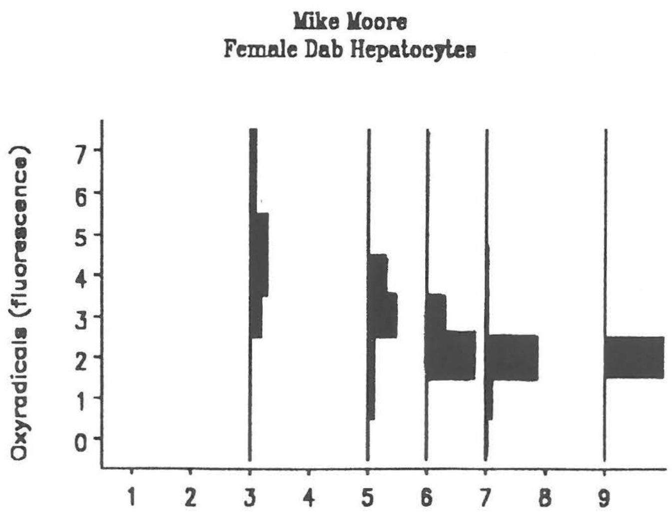




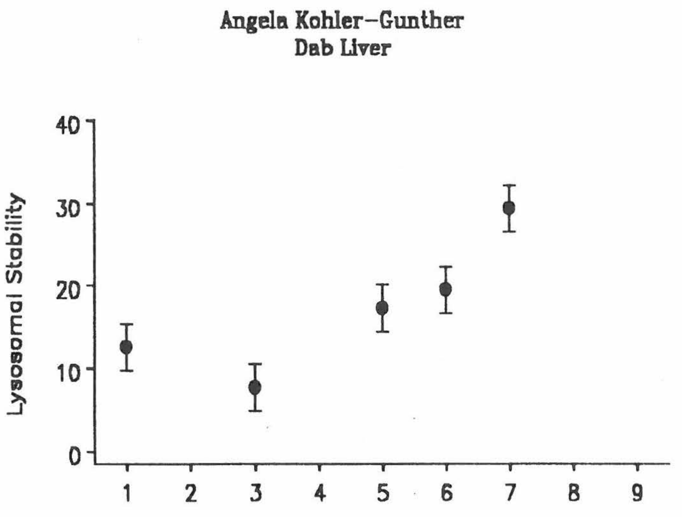


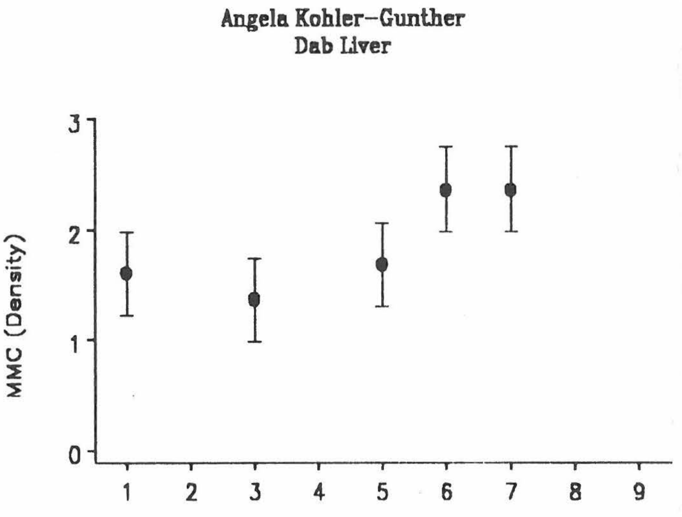


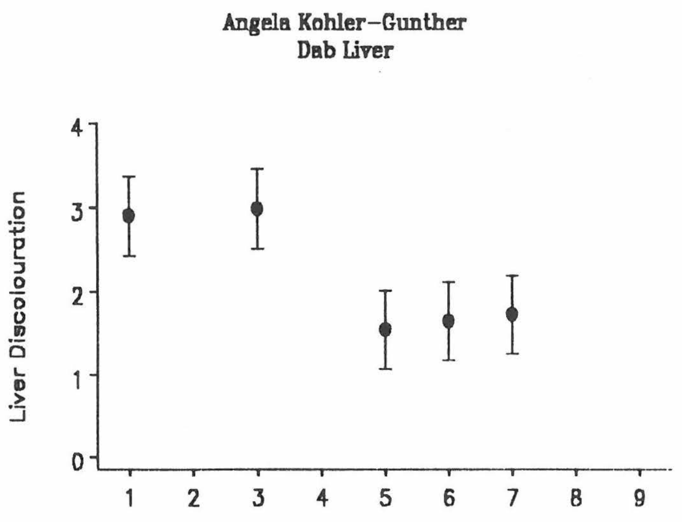


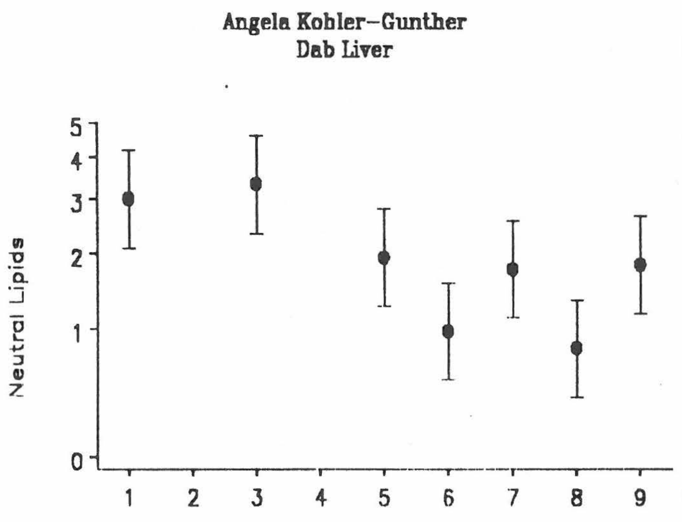


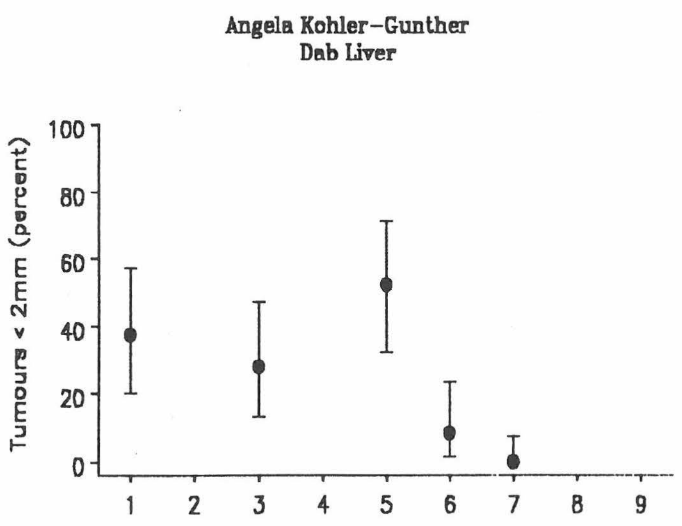
GERMAN BIGHT CELLULAR PATHOLOGY

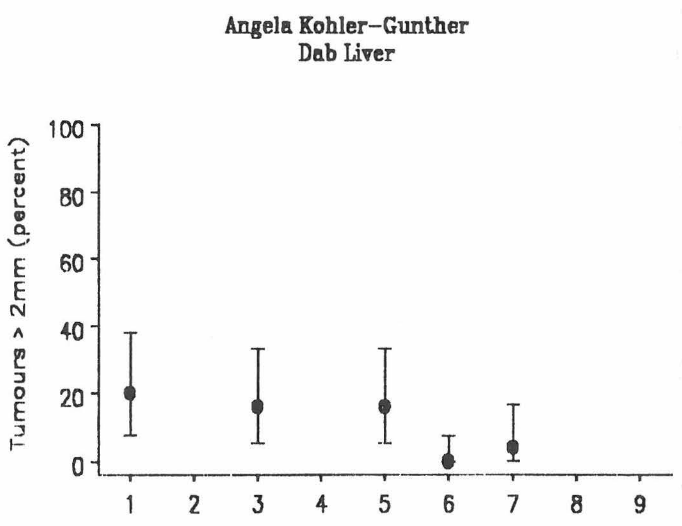






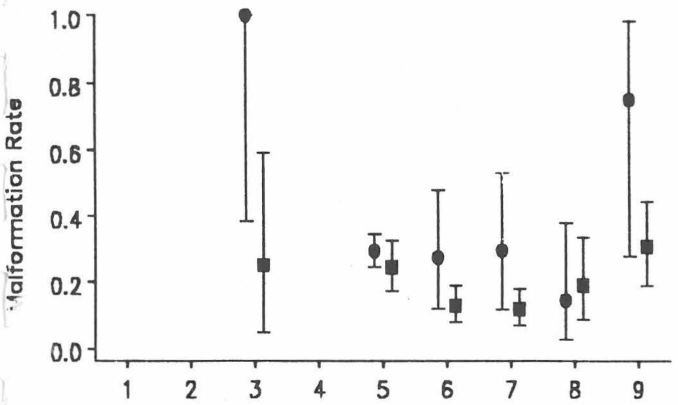




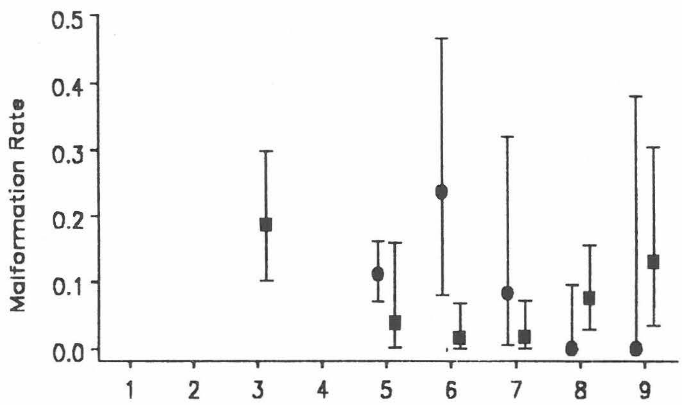


GERMAN BIGHT CELLULAR PATHOLOGY

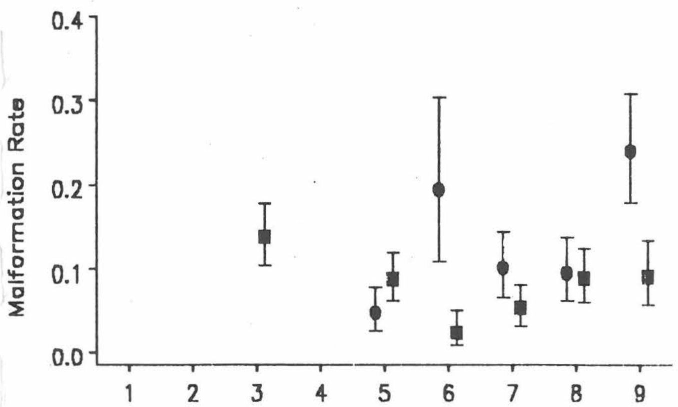
Cameron and Berg
Dab Embryos – Stage Ia



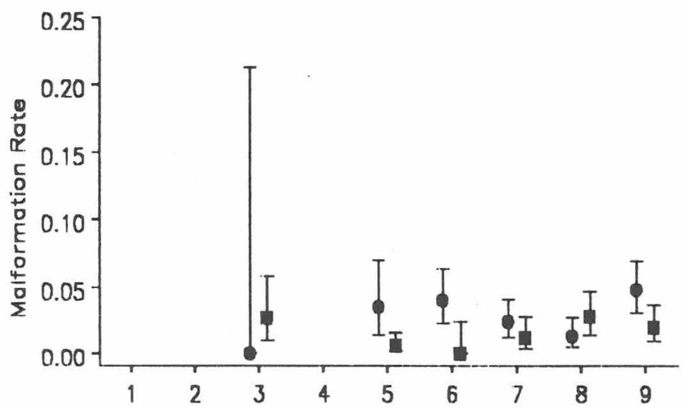
Cameron and Berg
Dab Embryos – Stage Ib



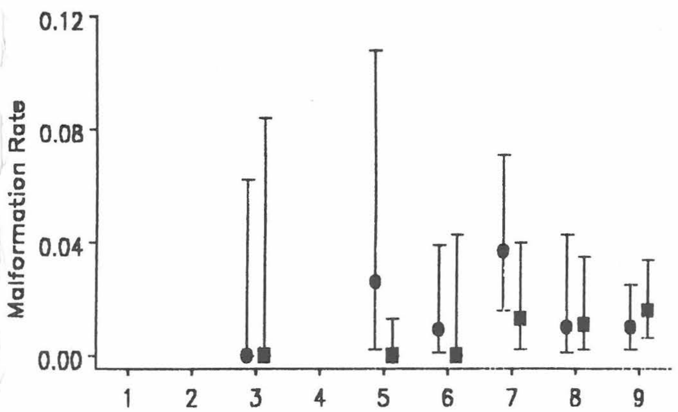
Cameron and Berg
Dab Embryos – Stage II



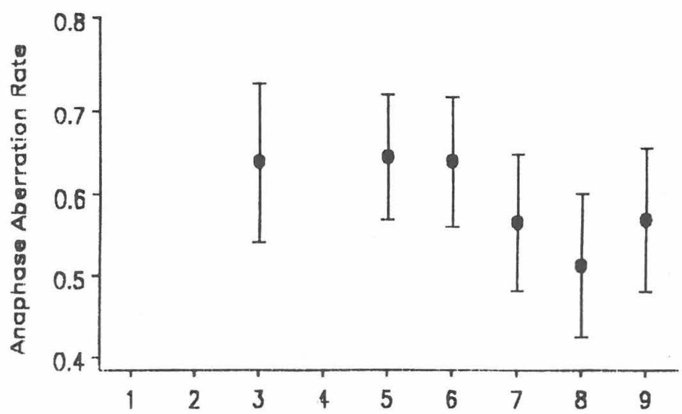
Cameron and Berg
Dab Embryos – Stage III



Cameron and Berg
Dab Embryos – Stage IV



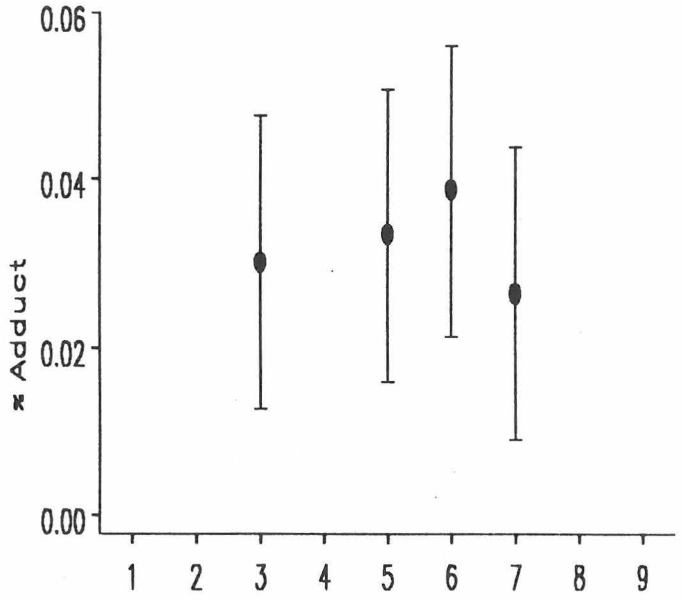
Cameron and Berg
Pelagic Fish Embryos



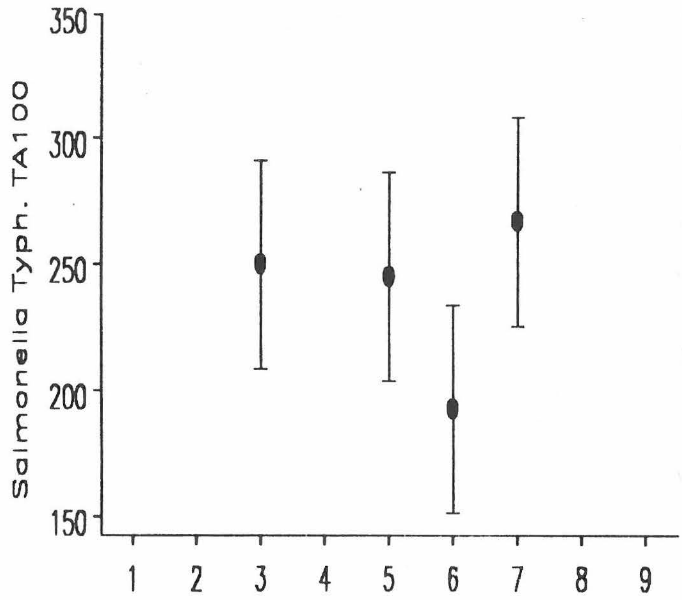
● = outward ■ = return

GERMAN BIGHT CELLULAR PATHOLOGY

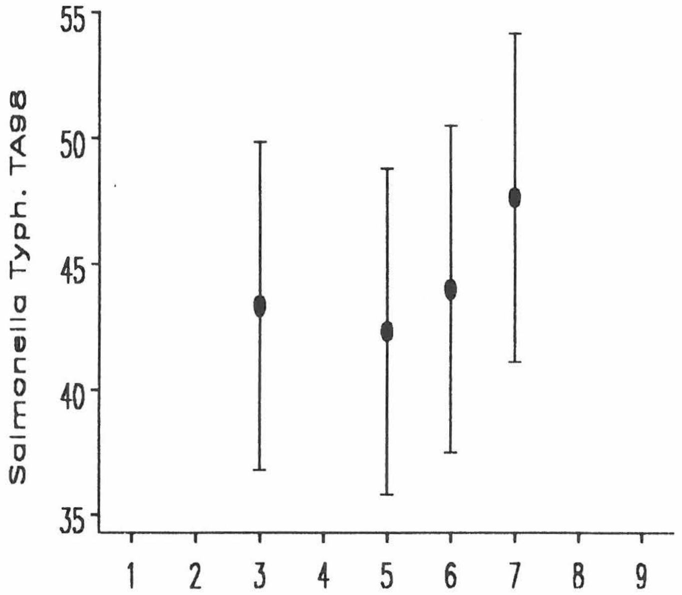
Kevin Chipman
Female Dab Liver



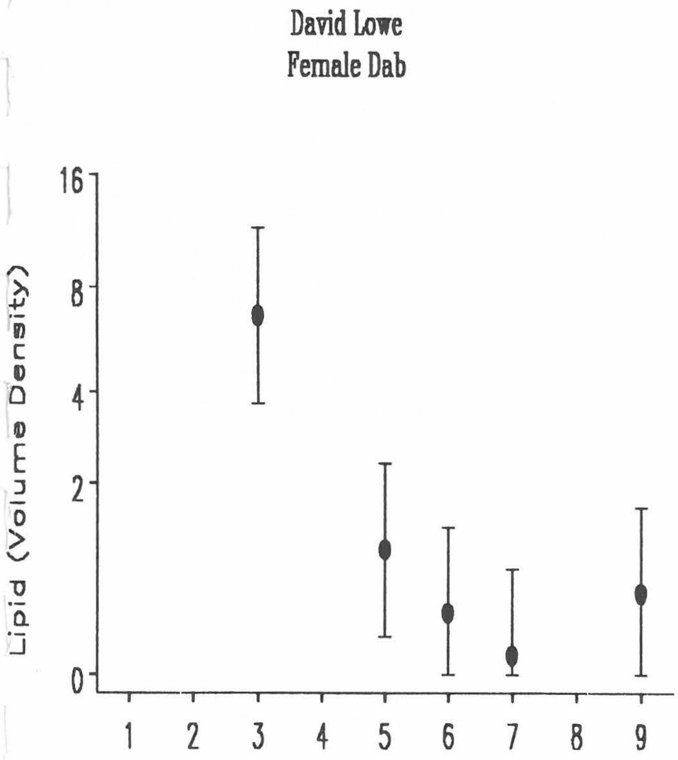
Kevin Chipman
Assay of Female Dab Bile

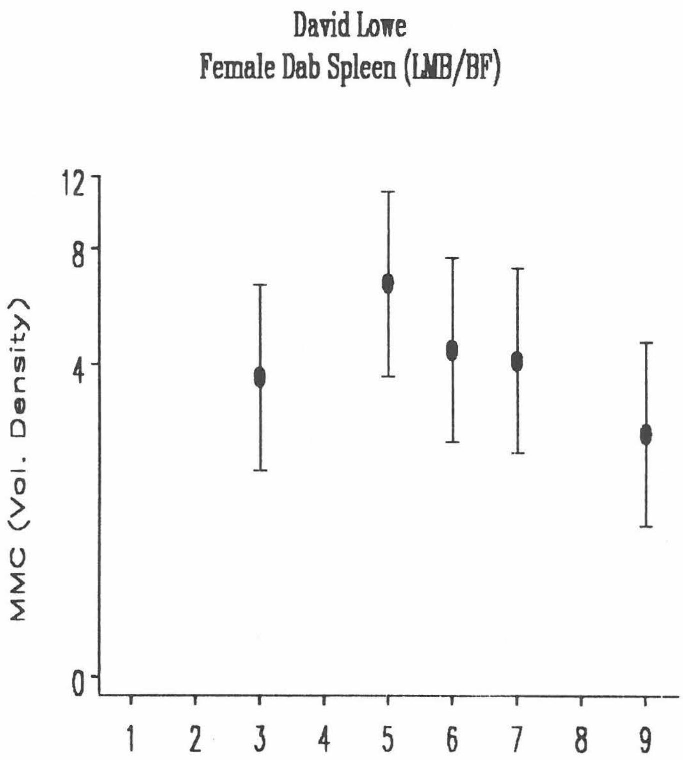


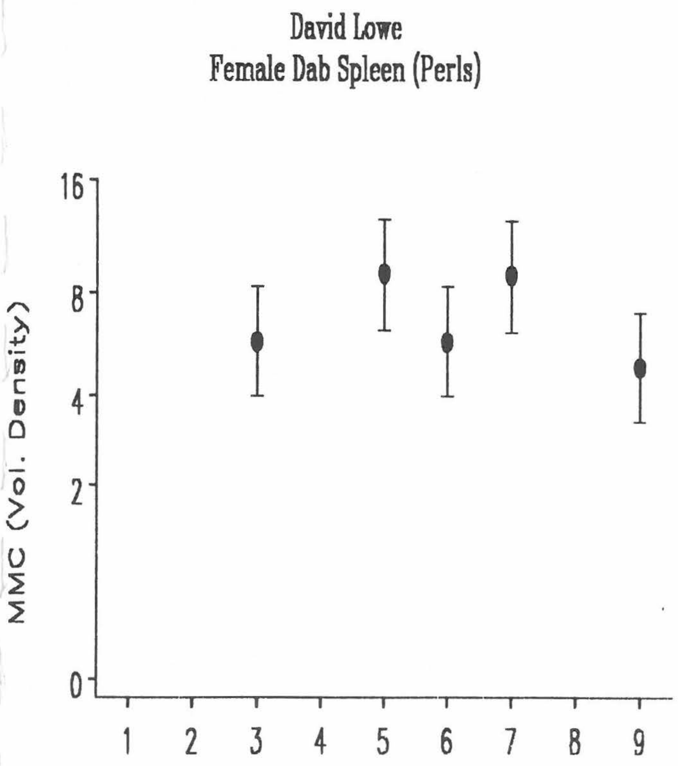
Kevin Chipman
Assay of Female Dab Bile

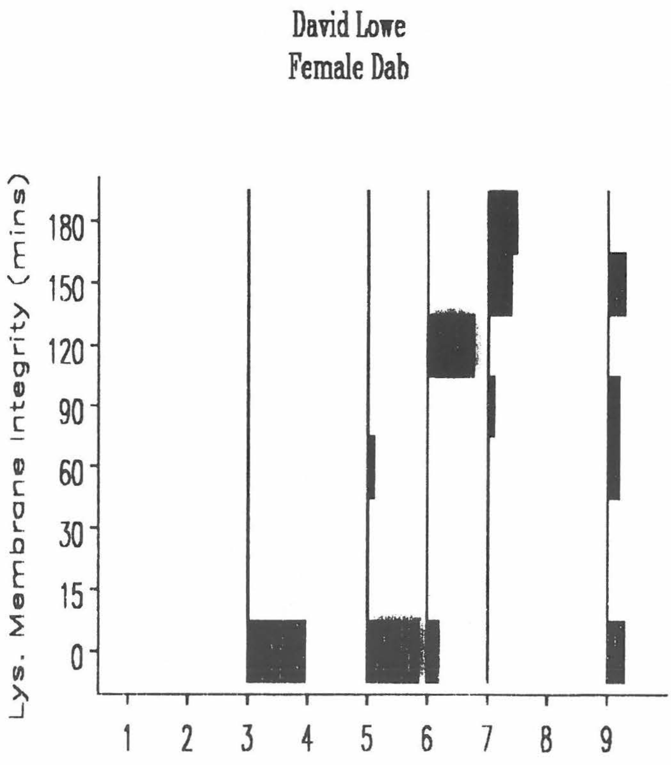


GERMAN BIGHT CELLULAR PATHOLOGY

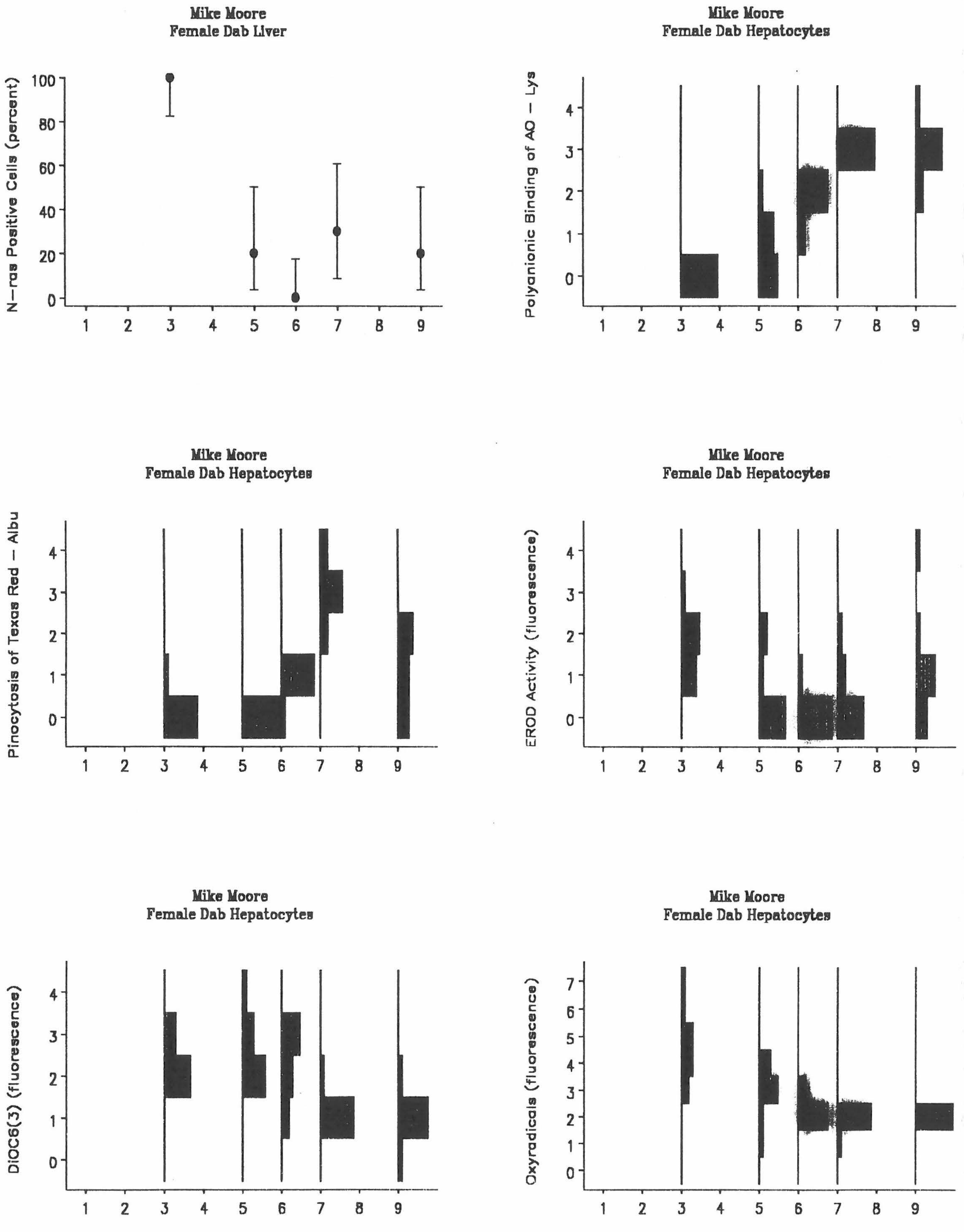








GERMAN BIGHT CELLULAR PATHOLOGY



8.GROSS PATHOLOGY AND HISTOPATHOLOGY (Coordinator: A. D. Vethaak)

Introduction. The principal objective of this study was to test the use of fish diseases in biological effects monitoring largely by following the recommendations provided by ICES (1988) on the methodology of fish disease surveys. A subsidiary objective was to provide background data on pathological conditions of dab (*Limanda limanda*) for those groups in the workshop using techniques at lower levels of biological organisation.

Techniques The work of this group was divided into the following projects:

1. An epidemiological study of selected pathological conditions in dab sampled at 6 stations along the German Bight transect; spatial trends of prevalence were determined and correlations sought with important disease risk factors including contamination. The lesions, examined post-mortem, included lymphocystis, epidermal hyperplasia/papilloma, and skin ulcerations. The liver was the only internal organ examined for gross pathology.

2. A histopathology study comprising:
a) Histological confirmation of all gross lesions detected in the epidemiological study;
b) Histological investigation of further liver samples from dab from the above stations and two additional stations on the Dutch drilling site gradient; these livers were selected because they did not show obvious lesions at the gross level.

3. Quantitative assessment of the density of melanomacrophage centres (MMCs) in the spleen of dab from the German Bight sampling stations. This project aimed to detect any influence of environmental contaminants on one of the many non-specific defence mechanisms in these fish.

Results.
1. The prevalence of epidermal hyperplasis/papilloma decreased with distance offshore (Figure 1).

2. Lymphocystis and skin ulcerations showed the opposite trend, with highest prevalences at the outermost station (Figures 2, 3).

3. The prevalence of grossly detected liver nodules (> 2 mm diameter) in large female dab was found to be highest at stations 3 and 8 (Figure 4), but no statistically significant trend was apparent. These nodules, present in 2.1% of livers sampled, were histologically confirmed to be hepatocellular adenoma. In addition, two cases were diagnosed as hepatocellular cholangioma.

In those livers of large female fish which did not show gross lesions, significant histological abnormalities were mostly related to fat storage in hepatocytes, and to foci of cellular alteration, which did not show the characteristics of hepatocellular adenoma. These occurred in 12.7% of 110 livers examined. They were also found to account for about half of the smaller spots and lesions (< 2 mm diameter) observed during the gross examination of the livers.

Quantitative analysis of the occurrence of MMCs in individual sections of dab spleen showed a spatial pattern, with significant differences between stations 8 and both stations 5 and 9 along the German Bight transect (Figure 5).

Conclusions The findings of the group indicate that the most promising external disease, in terms of biological effect monitoring, seems to be epidermal hyperplasia/papilloma and the most promising internal disease preneoplastic and neoplastic liver lesions. In studies of liver neoplasia, it is recommended that histological techniques be included, in order to confirm the identity of grossly visible lesions and to conduct routine investigations of apparently healthy livers.

Disease data from the workshop will contribute to the data base of relationships between disease and contamination, and can not demonstrate clear causality. Before these diseases can be used as reliable techniques, there are several areas that need further research:

1. Migration patterns and age distribution of the fish;

2. Natural background levels of disease;

3. Cause-and-effect relationships, to be elucidated by laboratory experiments.

Recent data on migration and reproduction (see Section 4) have shown that the use of dab as an indicator species has limitations, especially during the main spawning period of the species (January-April). Long-term monitoring programmes using dab should continue, but it is essential that sampling is carried out consistently during the same period of the year in order to produce comparable data in which trends might be detected.

FIGURE 1 EPID. HYPERPLASIA/PAPILLOMA

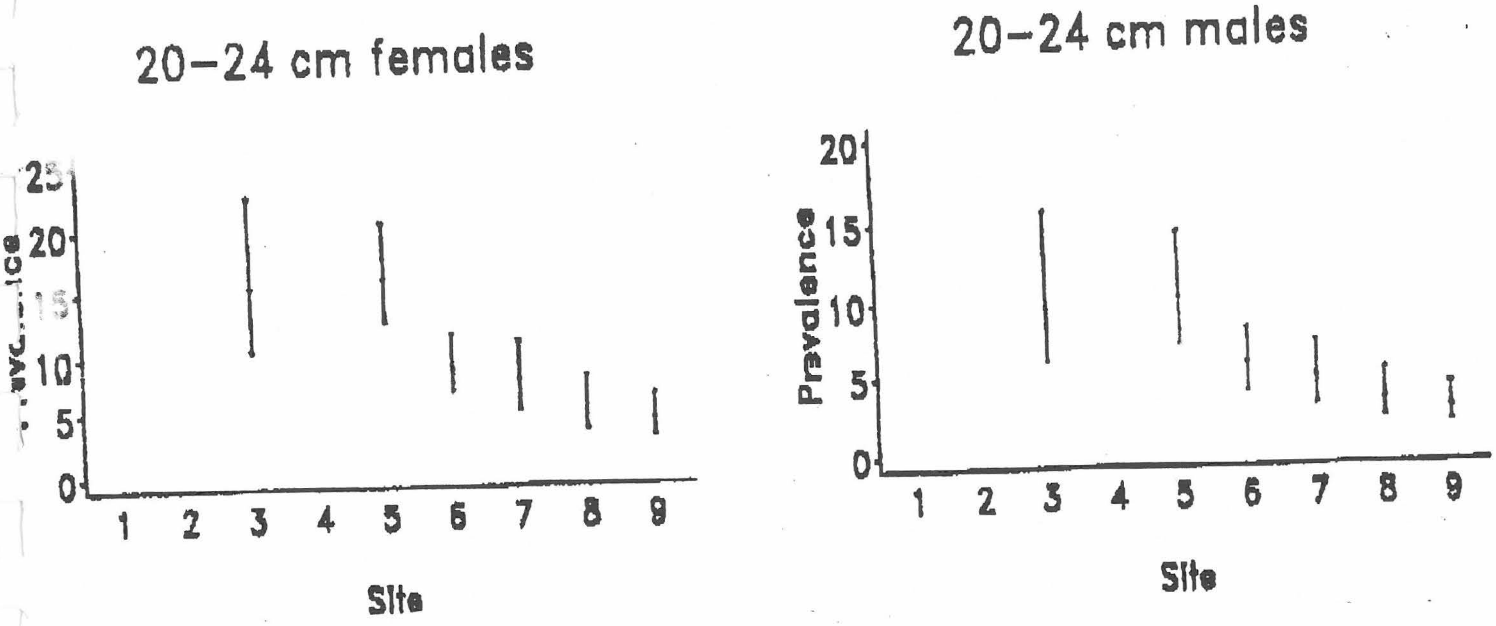


FIGURE 2 LYMPHOCYSTIS

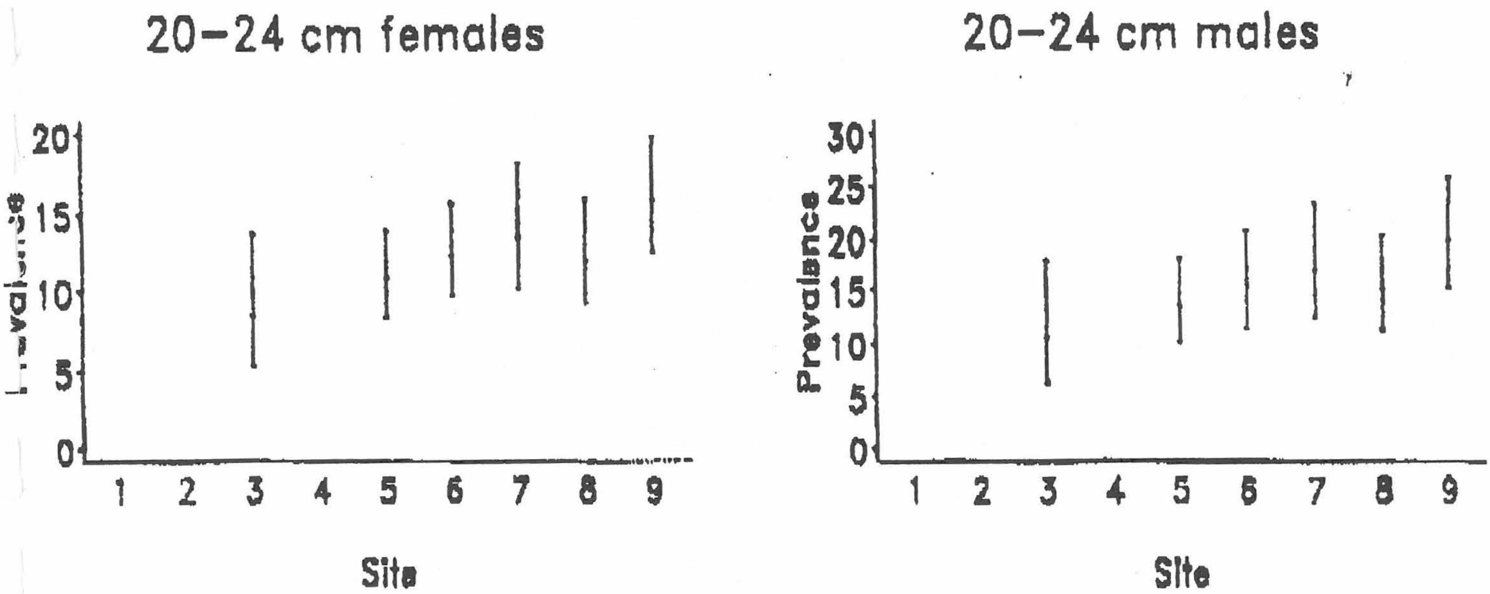


FIGURE 3 SKIN ULCERS

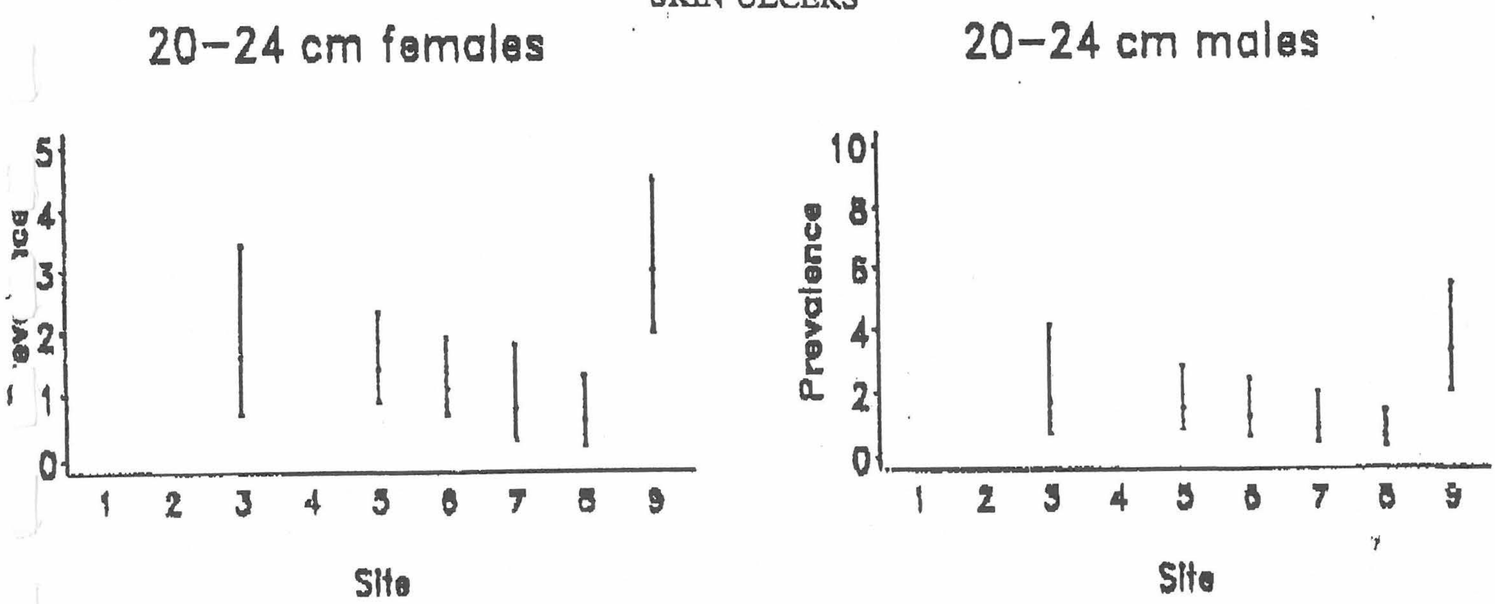


FIGURE 4

LIVER NEOPLASIA > 2MM

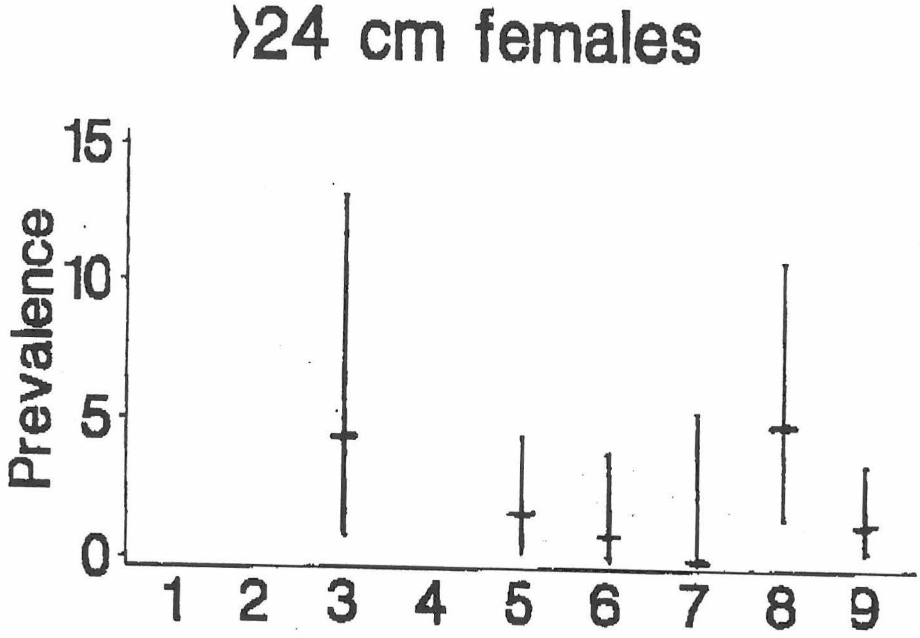
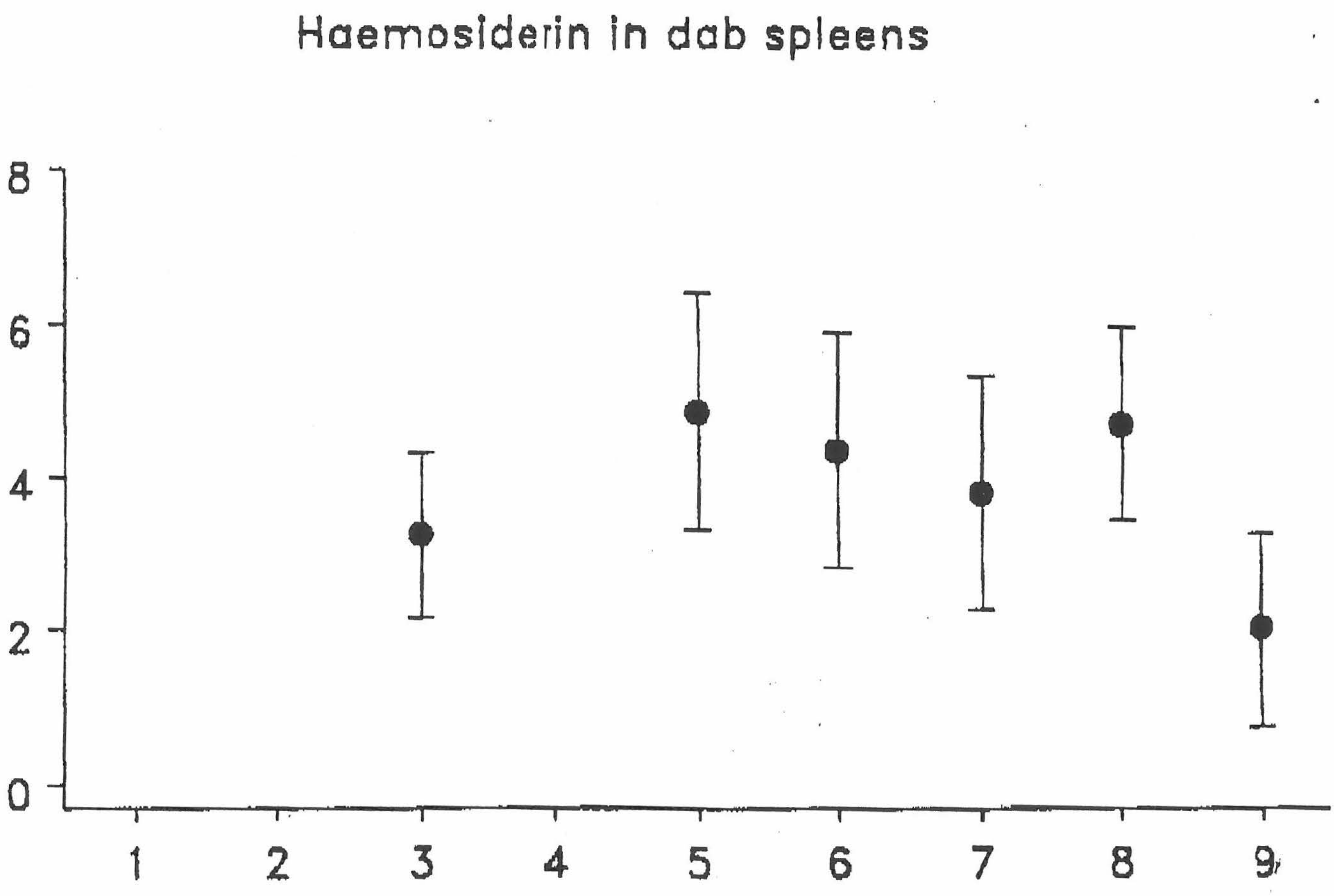


FIGURE 5



9. WATER QUALITY BIOASSAYS (Coordinator: L.Karbe)

Objectives. The studies conducted by the participants of the group were designed to:

a. compare and to intercalibrate water quality bioassays which can be used at sea;
b. to use bioassays measure effects in different matrices (surface microlayer, subsurface water, water from intermediate depth and sediment elutriates) using the same technique, at the same location, and at the same time,
c. to compare results obtained in this way along a pollution gradient in the German Bight.

(The group did not use any samples from the Drilling Site gradient.)

Samples used for bioassays. All the samples used for water quality bioassays were taken during a cruise of the RV Valdivia with two traverses of the German Bight transect: (1) from Hamburg to the Dogger Bank (Tails End) and to Bremerhaven, and (2) from Bremerhaven to the Dogger Bank and back to Hamburg. Different types of samples were taken from all the stations along the German Bight transect, and subsamples of the same batches were used for different types of bioassay techniques.

Sampling techniques
a. Surface microlayer: Two types of equipment were used to sample material from the surface microlayer (SMIC) at sea: Garret type screens and a Teflon Drum Sampler. To deploy the sampling equipment away from the parent vessel a rubber boat was used driven by an electric motor. During the cruise, priority was given to SMIC sampling, but this was only possible at Beaufort Force 4 or less so, SMIC sampling had to be restricted to a training station off Helgoland (0) and the German Bight transect stations 2, 4 and 6. At sea, sampling was possible only during times of day light (security reason) and relatively low wind speeds. An intercomparison of the various microlayer samplers was conducted in the outer harbour of Wilhelmshaven.

b. Subsurface water: At all stations a water sample was taken with a glass bottle 0.5 m below the surface, to compare bioassay responses to the SMIC samples with those to the bulk water. At all stations water samples were taken at 3 m by a rosette water sampling system connected to a CTD-Multiprobe.

c. Sediment Elutriates: At all stations sediment samples were taken by a box grab, to prepare sediment elutriates. Water from station 7 was used for elutriation of all sediment samples. The sediment elutriates from each station were tested simultaneously with the water samples.

d. XAD-resin extracts: During the first leg, large volume water samples were passed through through an XAD ion exchange column to extract and concentrate organic contaminants from sea water. The volumes required and the pumping rate of the system made it necessary to pump for long periods, so it was run continuously while under way between stations. Samples obtained are composites from stations 1 to 4, 4 to 5, 6 to 8, 8 to 9 to 8, and 5 to 2. On the way from Bremerhaven back to Hamburg the German Bight transect was resampled at stations 1, 5, 7, 9 and at a reference station within the outer Elbe estuary.

Bioassay techniques The following bioassay techniques measuring lethal effects and sublethal endpoints were deployed:

a. Phytoplankton, *Tetraselmis suecica* and *Isochrysis galbana*, population growth.
b. Hydroid, *Eirene viridula*, colony growth rate.
c. Oyster, *Crassostrea gigas*, embryo survival and abnormality frequency.

d. Cryopreserved oyster, *C. gigas*, larval survival and growth
e. Cryopreserved clam, *Tapes philippinorum*, larval survival and growth.
f. Copepod, *Tisbe battagliai*, nauplii survival and development to copepodite, length of nauplii/copepodite.
e. Echinoderm embryo development to pluteus larvae.

Results

Surface microlayer: Enhanced concentrations of metals and organotins occurred in the SMIC samples relative to the subsurface bulk water samples taken at 0.5 m. Enrichment was greatest for organotins at station 0 and 4, and for copper and cadmium at station 6. It is relevent that microlayer enhancement is often greatest when conditions are calm and a surface film is visible, as at station 4.

At station 4 the survival of oyster embryos was significantly reduced for both types of SMIC samples (Screen and Drum Sampler), while the bulk water did not elicit a response. One interesting response was an increase of copepod larval growth as a response to an exposure to SMIC drum sample from Station 4, which is not an uncommon response to low levels of toxicants. At all stations, SMIC samples resulted in significant toxic effects with toxicity decreasing from inshore to offshore.

Water: The most significant regional differences in responses of water quality bioassays to water samples are those of cryopreserved oyster and clam larvae. Survival and growth of both was significantly reduced at stations 1 to 5 and only slightly affected at the more offshore stations 6 to 9. The results at the inner stations may be in response to the Continental Coastal Water (see Section 2) influenced by the effluents of the contaminated waters of the Elbe/Weser plumes. Unaffected larvae were exposed to the less contaminated Central North Sea Water. There are indications that larval growth was influenced by differences in food supply from the natural plankton.
Regional differences of the trophic conditions are shown by the results of a phytoplankton bioassay (two species) exhibiting a high rate of reproduction at the most inshore stations 1 to 3 followed by a gradient of decreasing growth to a lower level at the offshore stations 6 to 9. The results probably indicate the elevated concentrations of nutrients carried offshore in the Elbe/Weser plumes.

XAD extracts: The material obtained by liquid-solid adsorption to XAD resin shows significant differences corresponding to the those obtained with oyster larvae exposed to water samples described above. XAD extracts from stations 1 to 4 caused the greatest inhibition of hydroid colony growth rates and much less inhibition to offshore samples from stations 6 to 9. The results agree with those from cryopreserved larvae and reflect the two major water masses traversed by the German Bight transect.

Sediment elutriates: The results obtained using the water quality bioassays for testing the sediment quality are not yet fully evaluated. However, there are significant differences in response that relate to the amount of sediments eluted per unit of water and the origin of the sediment sample. The survival of larvae of the bivalves and copepods was generally very low in elutriates of sediments sampled at the most inshore station 1 (100% mortality in the case of higher concentrations of sediment elutriated) and intermediate survival at stations 2 and 3. Along the transect, an increase of larval survival was observed with distance offshore, which related to the increasing grain size and decreasing concentrations of various organic contaminants measured in the bulk sediment. The sediments exhibited a growth promoting effect on the microalgae that decreased from inshore to offshore, reflecting the nutrient content of the elutriates.

Conclusions

a. Techniques deployed are sufficiently robust to be used at sea, yet sensitive enough to respond to contamination in the offshore marine environment.

b. Regional differences in effects of contaminants can best be investigated using samples from substrates where contaminants accumulate to higher concentrations than the ambient water. Of most interest are the interfaces: the surface microlayer enriched by contaminants due to hydrophobic lipophilic interactions within the water, and the wet and dry deposition of contaminants from atmospheric inputs, and the sediment surface layer enriched by freshly deposited fines and decomposing organic matter with their associated organic and inorganic contaminants.

c. For the purpose of toxicity studies, it is recommended to separate fractions that differ in sorption capacity and the bioavailability of contaminants (eg liquid and solid fractions of surface microlayer samples, top surface layer and deeper layers of sediment cores).

e. Evaluation the bioassay data, it has to be accepted that the organisms are responding to numerous interrelated cariables (a complex mixture of contaminants and factors that determine their bioavailability) that are not understood in a mechanistic sense.

The future

a. Monitoring programmes should make use of a variety of different bioassay techniques and not rely on any one technique.

b. There is scope for integrating the use of biomarkers with the more usual sublethal and lethal bioassays reported here.

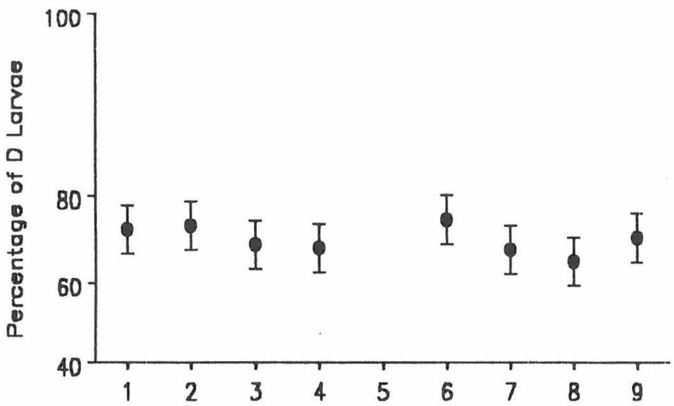
c. Practical monitoring of biological effects should combine
field studies of indigenous fish and invertebrates, with standard bioassays, such as the oyster embryo bioassay.

d. Cryopreserved bioassay oyster and clam larvae have been successfully used at sea for the first time, although further development to guarentee consistency between batches of crypreserved larvae is necessary before they could be used routinely in monitoring programmes.

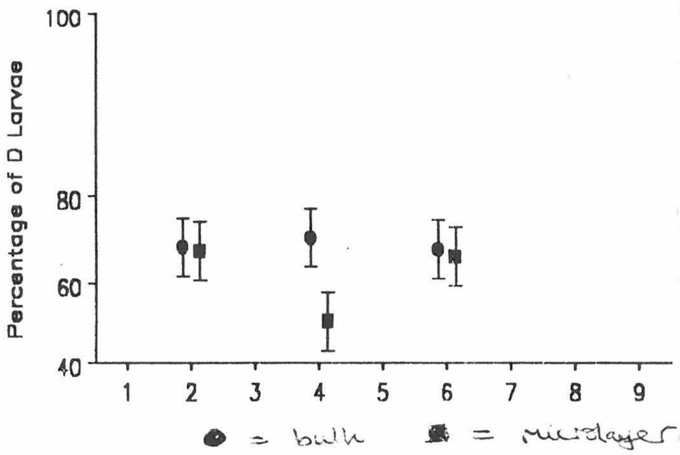
e. Positive as well as negative controls are to be considered an essential component of any bioassay study.

GERMAN BIGHT WATER BIOASSAY

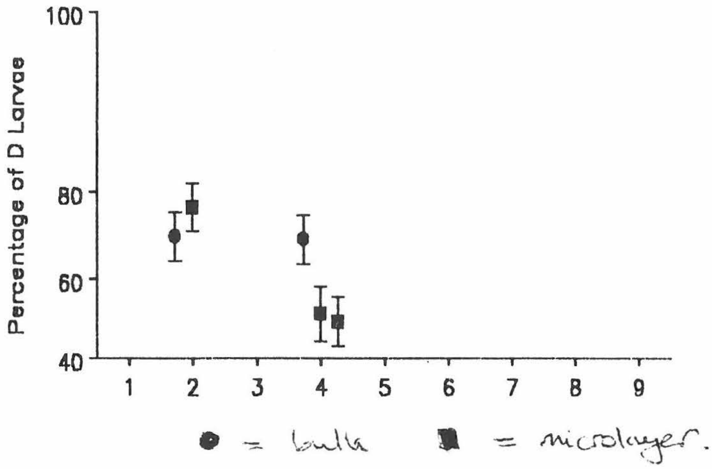
John Thain
Oyster – 3 m water sample



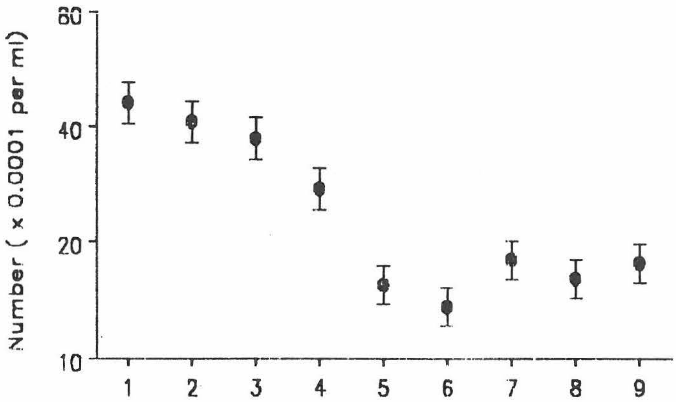
John Thain
Oyster – PML Microlayer



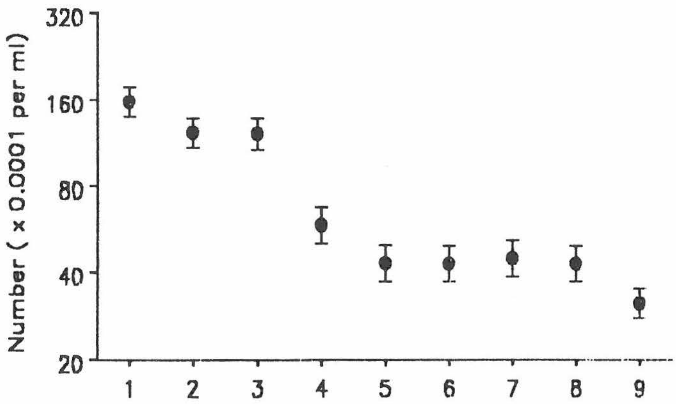
John Thain
Oyster – Hardy Microlayer



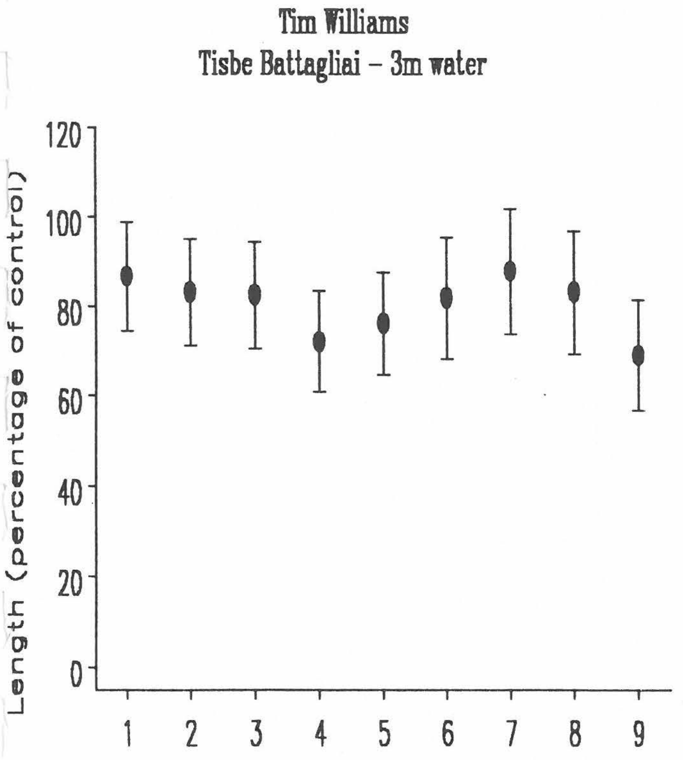
John Thain
Tetraselmis – 3m Water

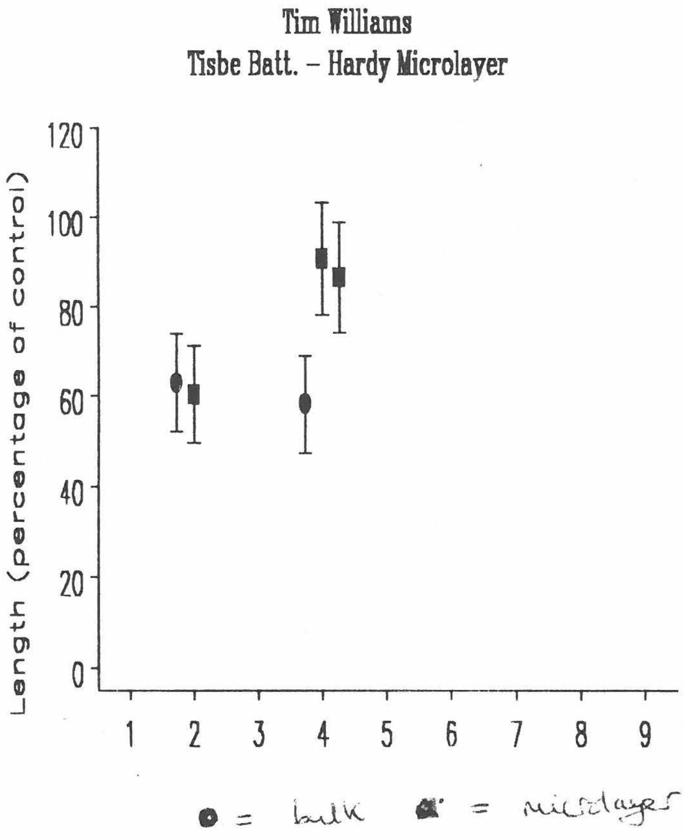


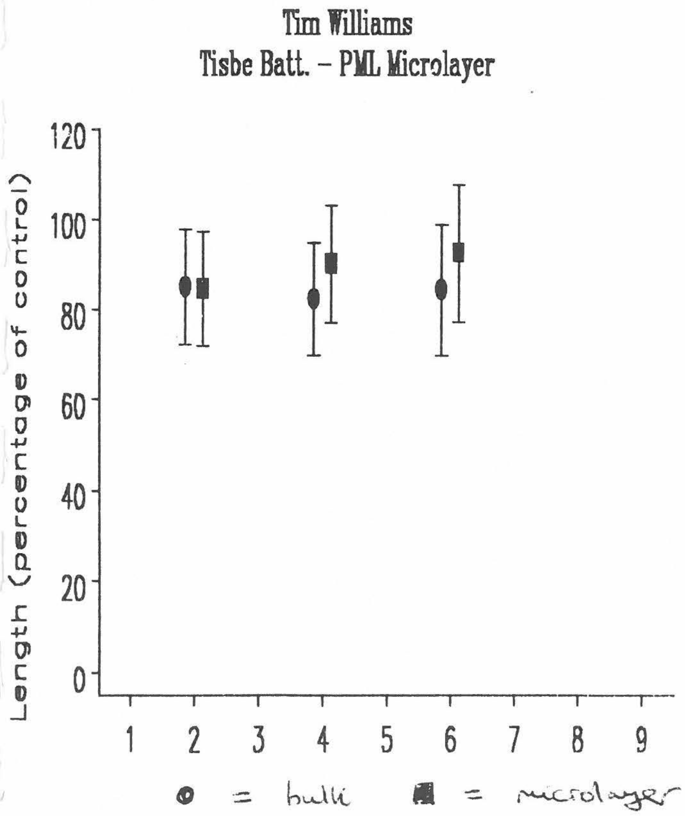
John Thain
Isochrysis – 3m Water



GERMAN BIGHT WATER BIOASSAY







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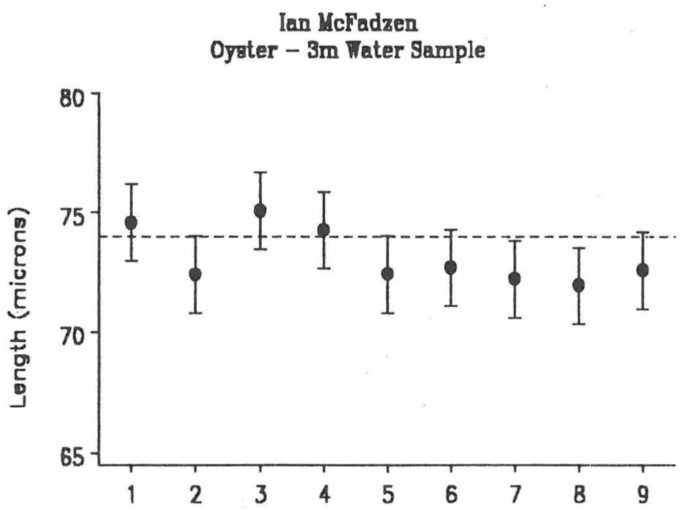

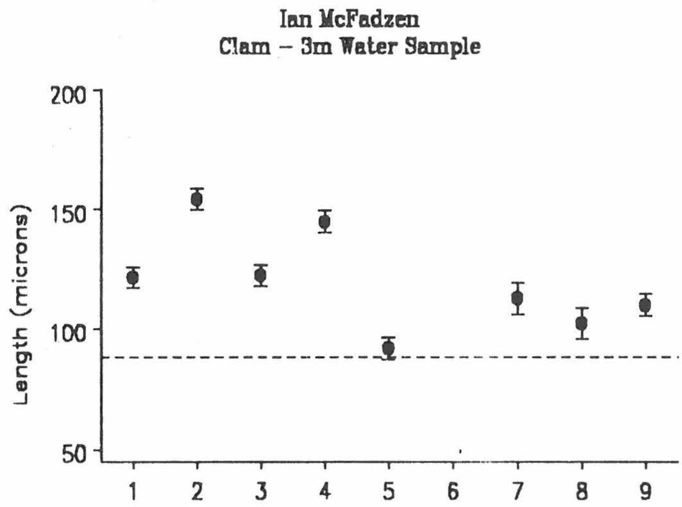

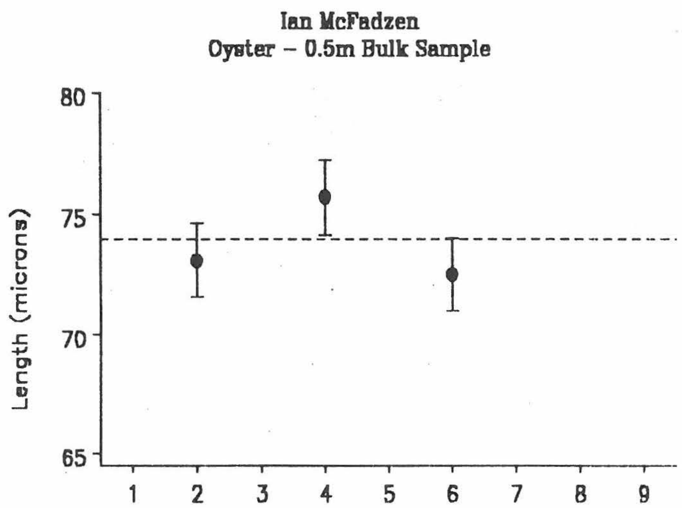

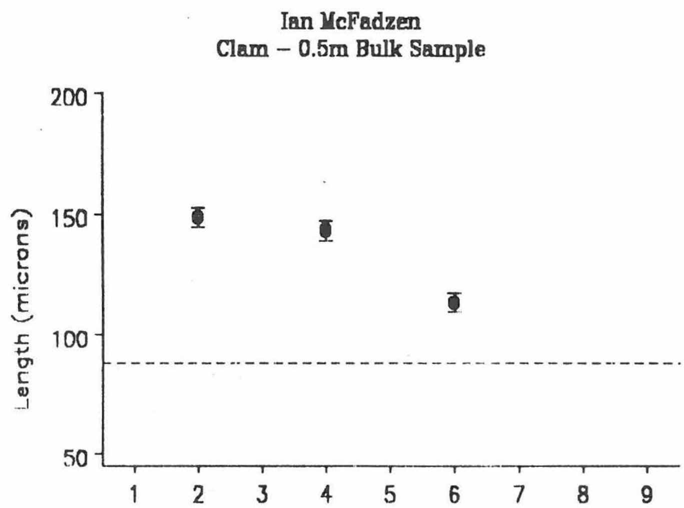

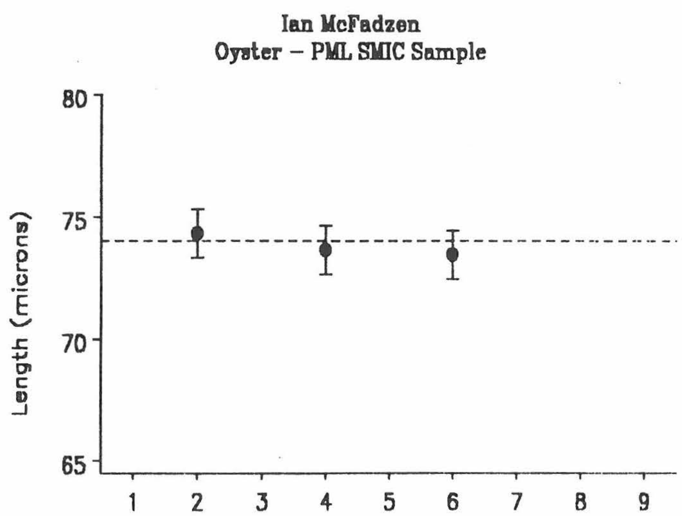

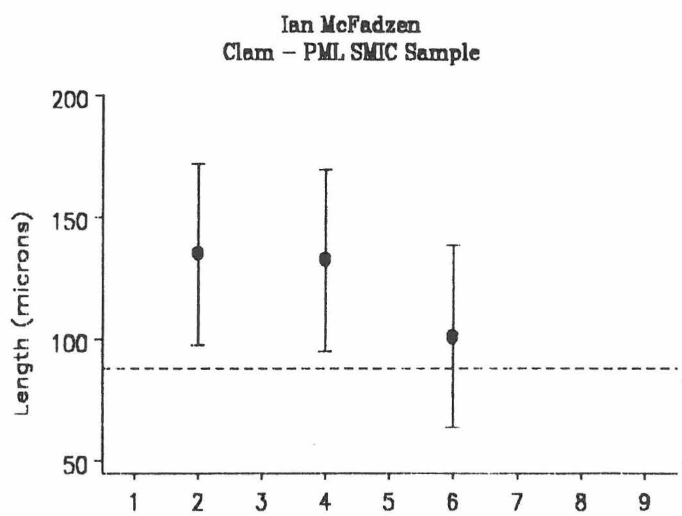

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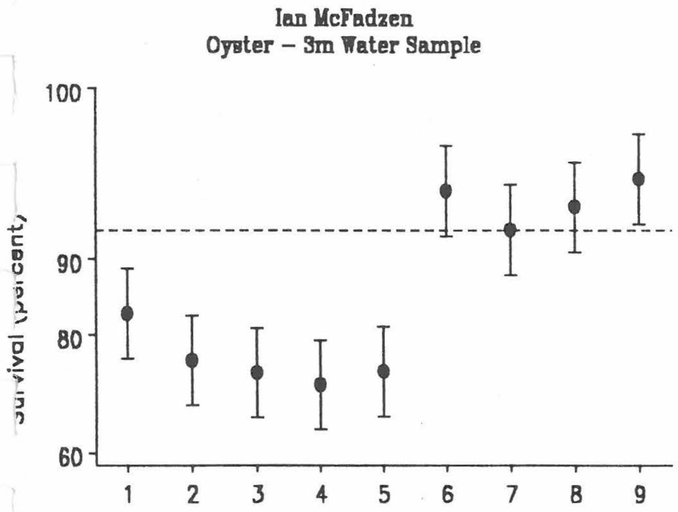
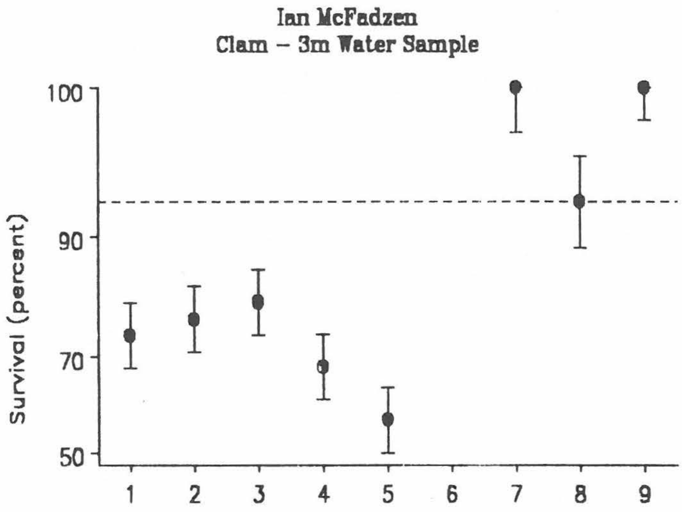
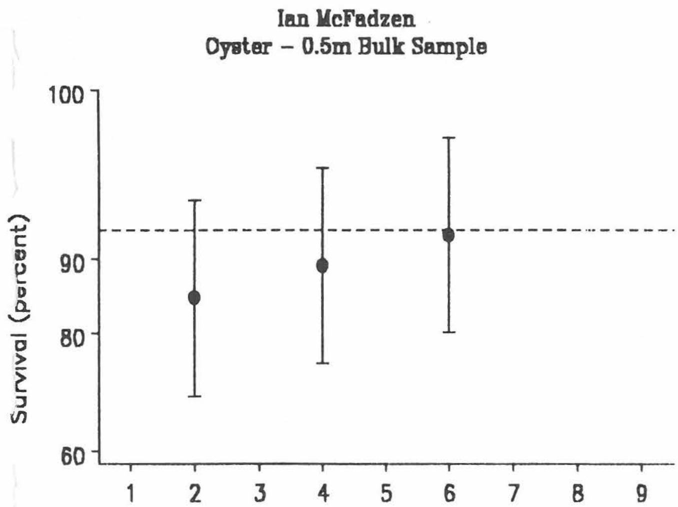
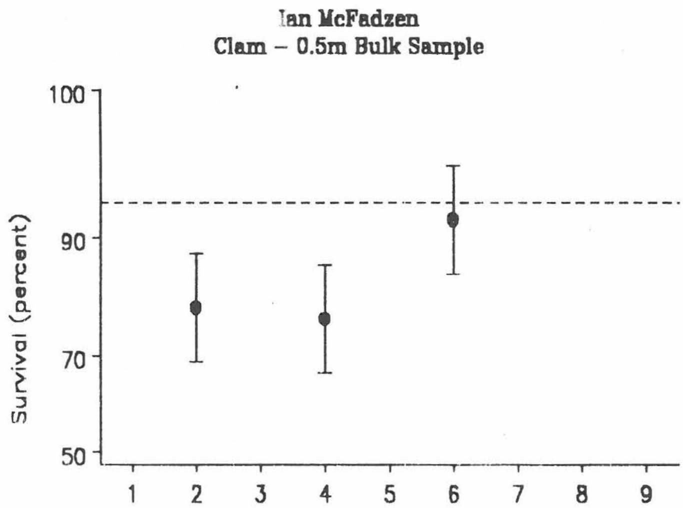
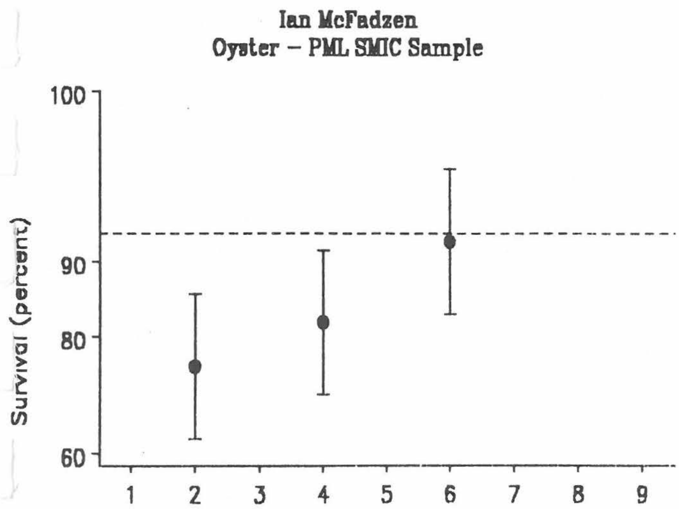
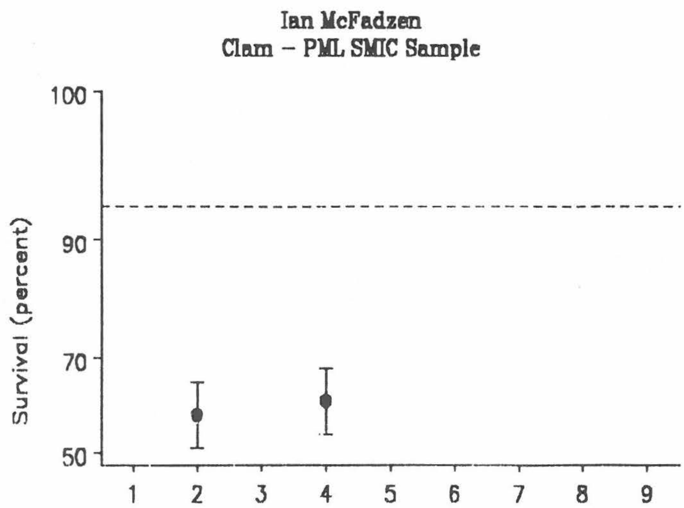






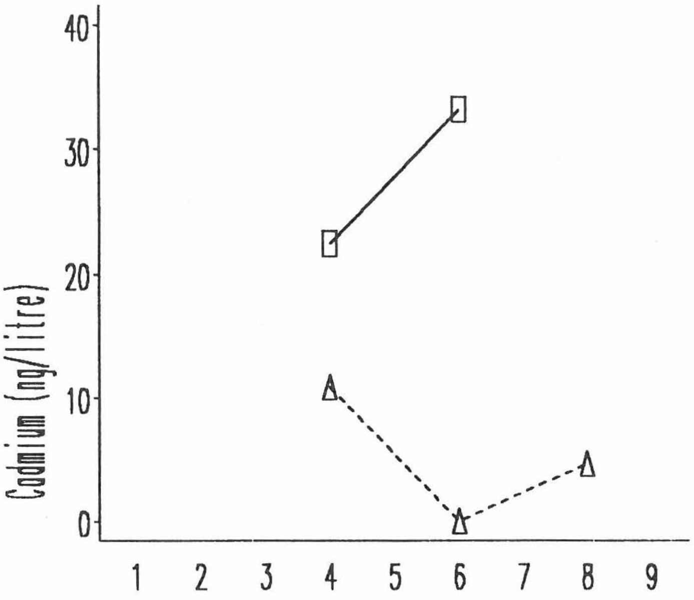




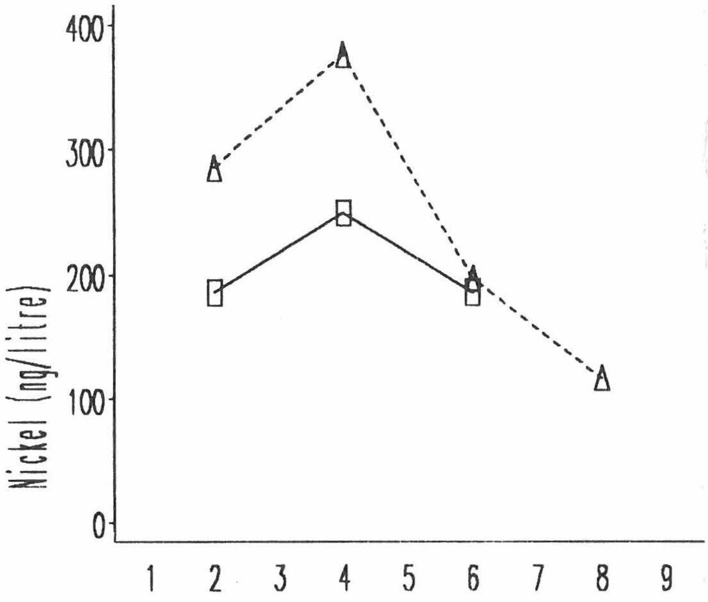


GERMAN BIGHT WATER BIOASSAY

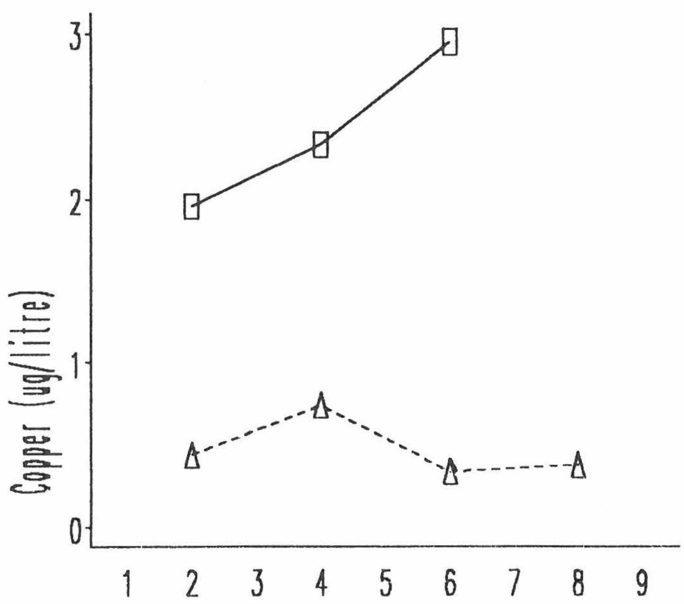
John Cleary
Surface Microlayer Chemistry



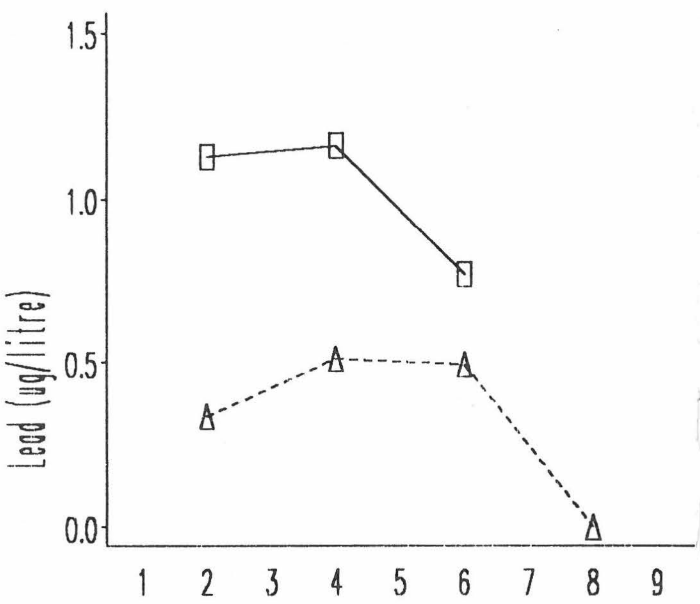
John Cleary
Surface Microlayer Chemistry



John Cleary
Surface Microlayer Chemistry



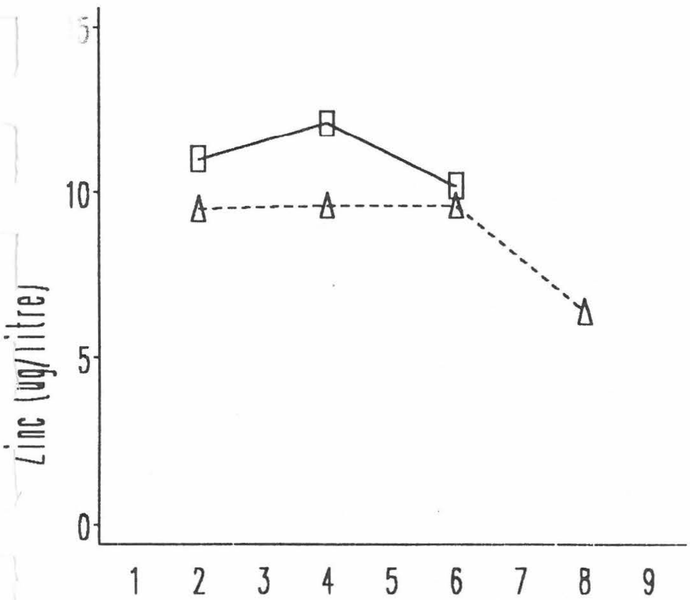
John Cleary
Surface Microlayer Chemistry



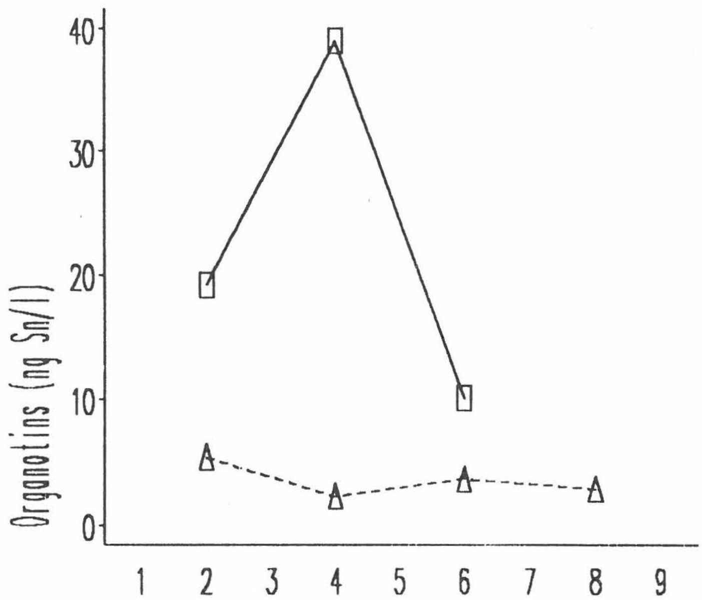
□ = Surface microlayer   Δ = 0·5m bulk

GERMAN BIGHT WATER BIOASSAY

John Cleary
Surface Microlayer Chemistry



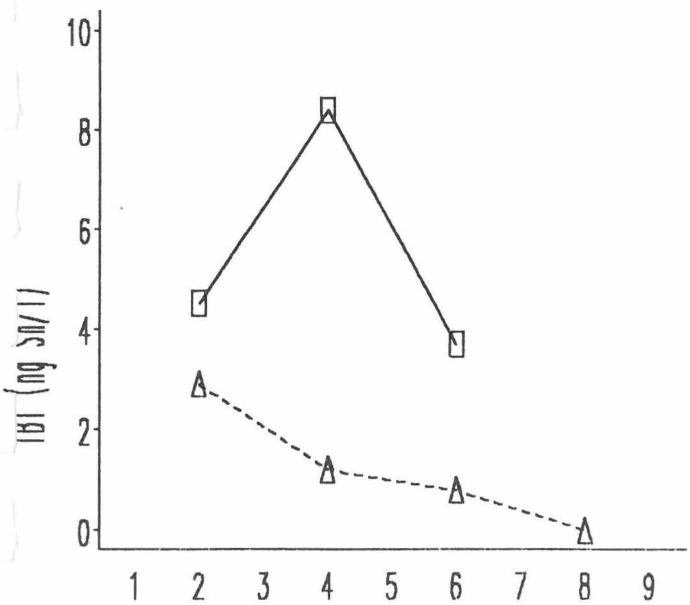
John Cleary
Surface Microlayer Chemistry



□ = surface microlayer

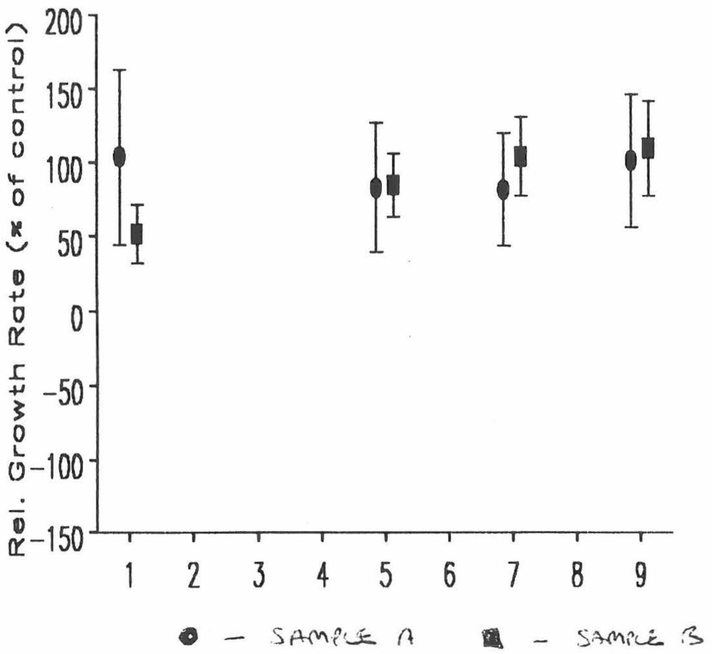
Δ = 0-5 m bulk

John Cleary
Surface Microlayer Chemistry



GERMAN BIGHT WATER BIOASSAY

Jan-C Bening
Cordyl. Caspia (XAD -150)



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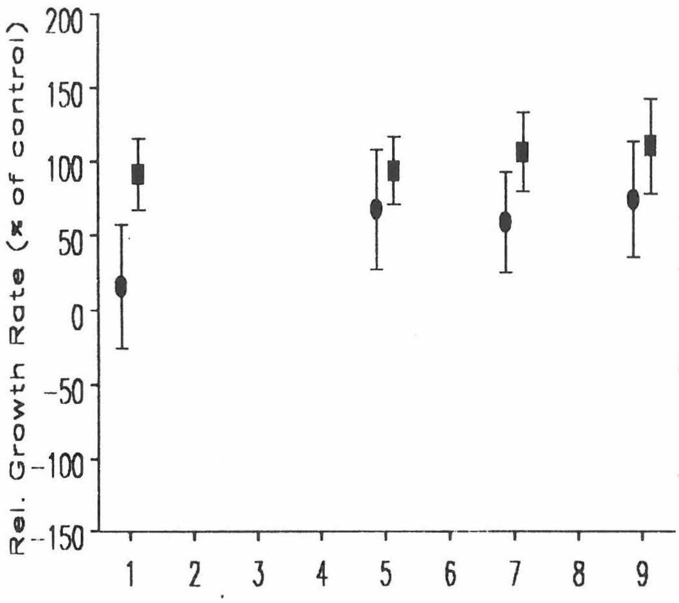

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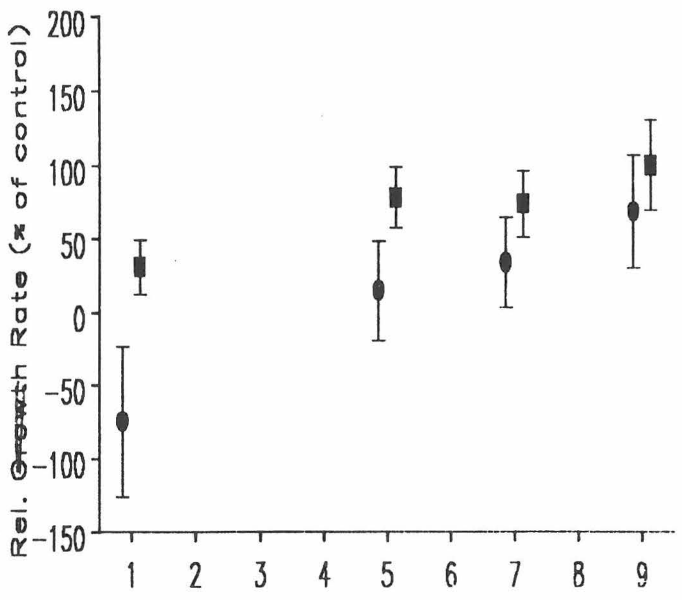

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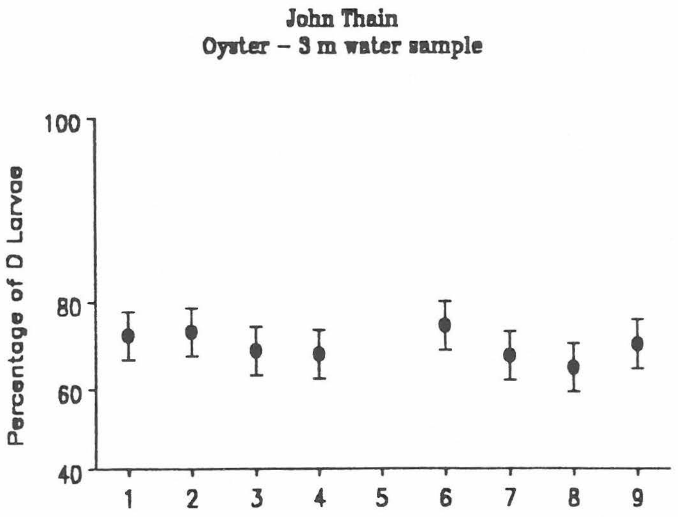
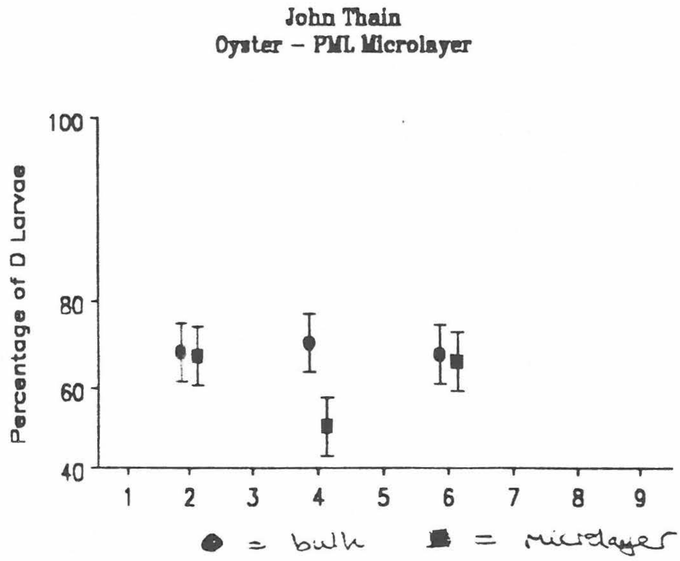
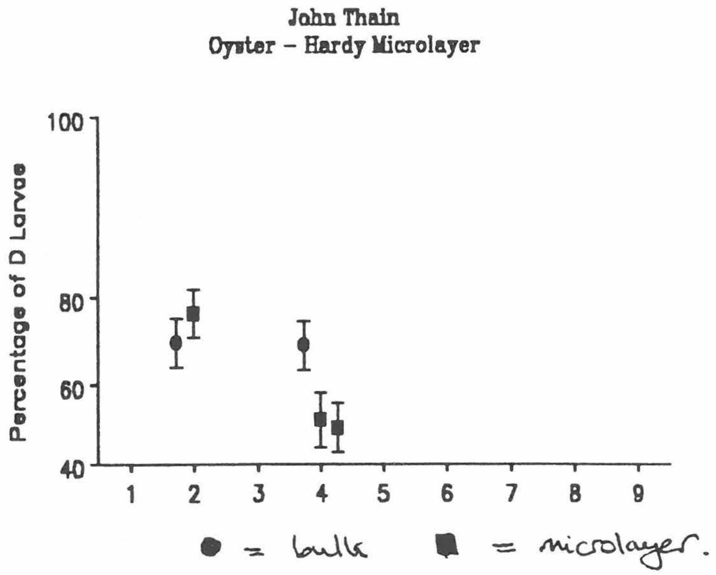
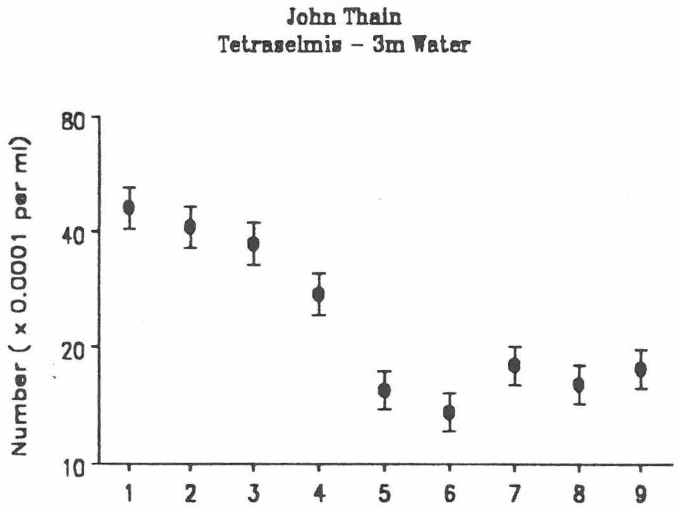
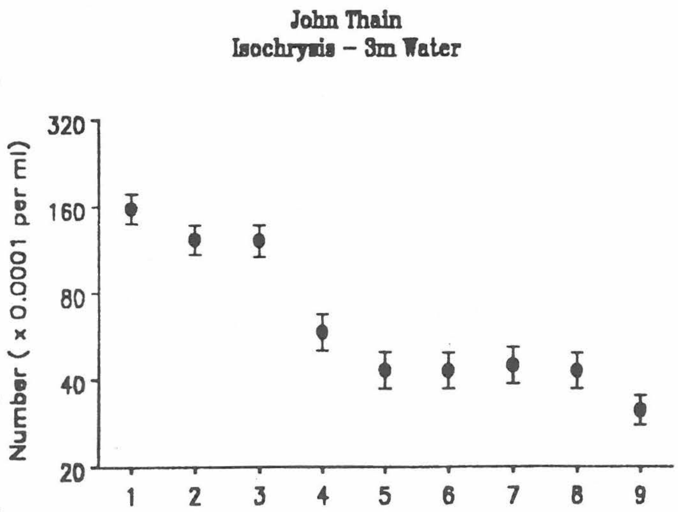




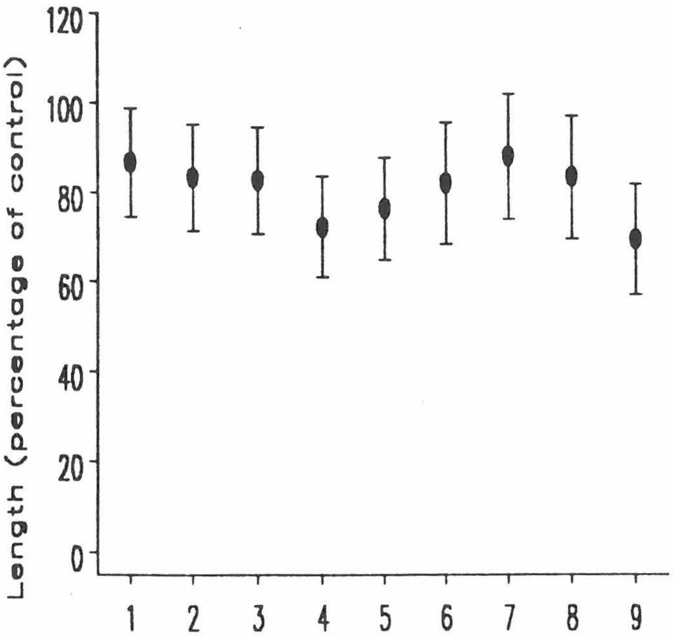




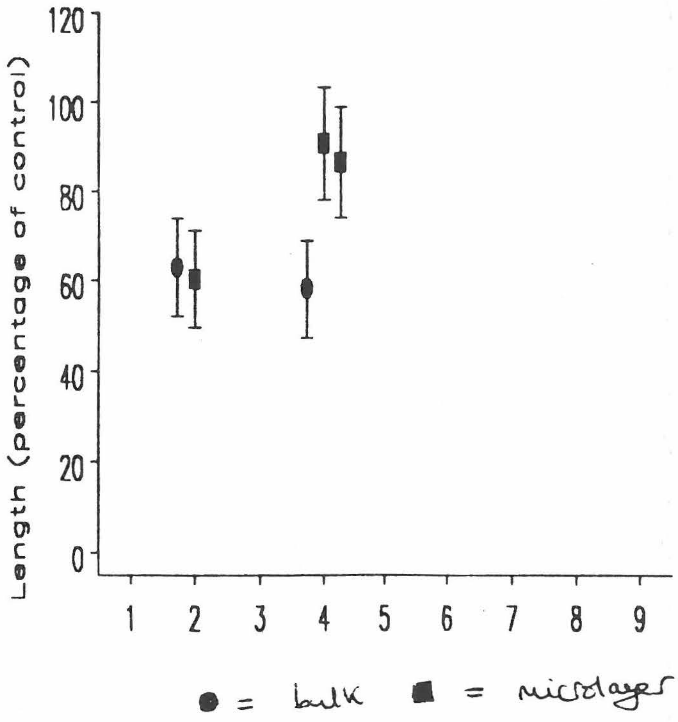


GERMAN BIGHT WATER BIOASSAY

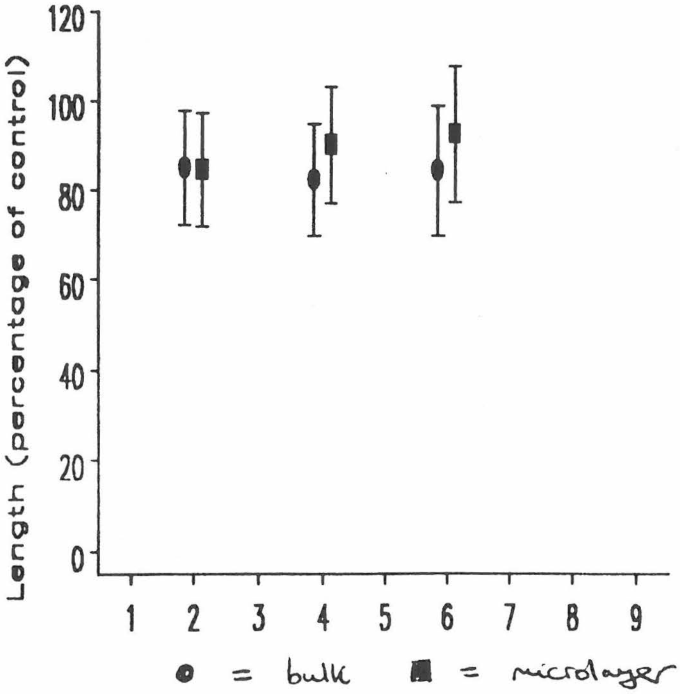
Tim Williams
Tisbe Battagliai - 3m water



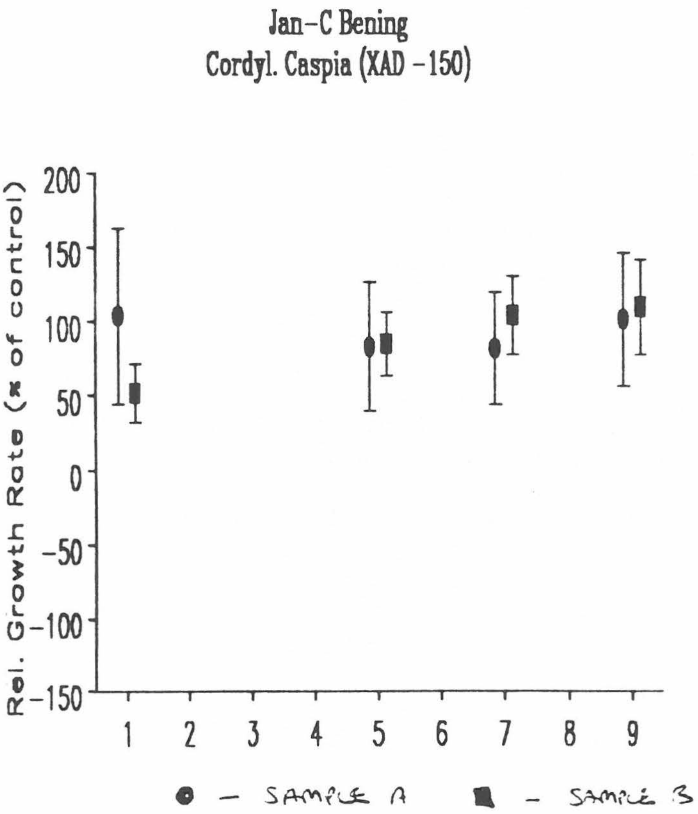
Tim Williams
Tisbe Batt. - Hardy Microlayer

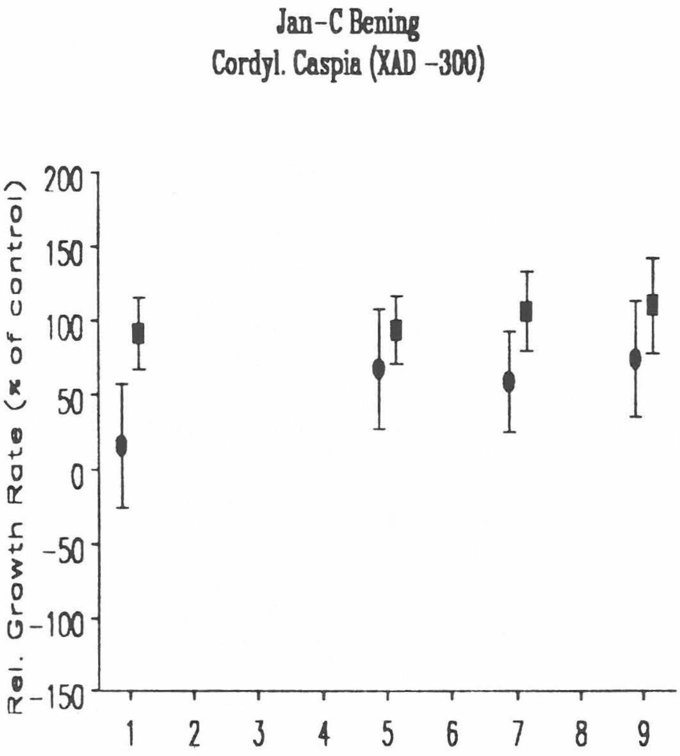


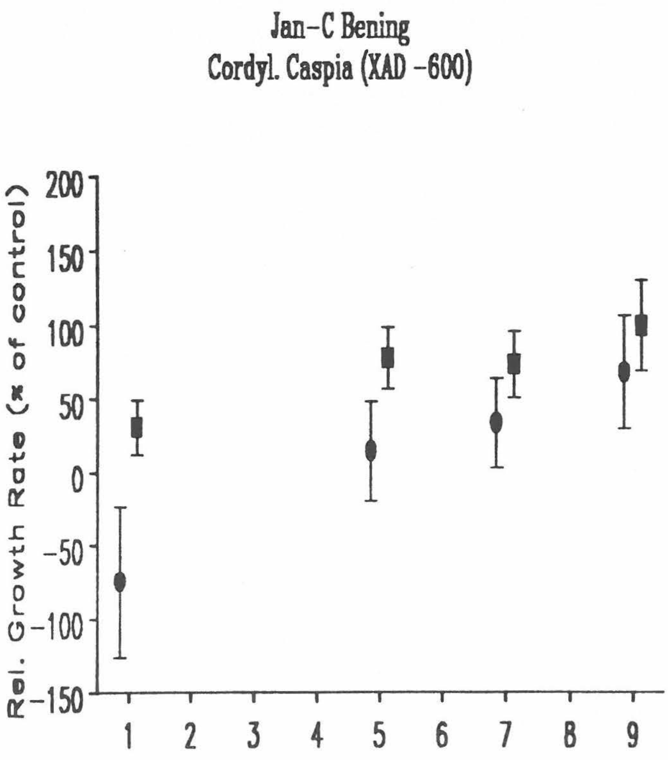
Tim Williams
Tisbe Batt. - PML Microlayer



GERMAN BIGHT WATER BIOASSAY







10. SEDIMENT BIOASSAYS (Coordinator: P. Chapman)

Objectives To compare sediment toxicity tests on samples from contamination gradients, and identify those most suitable sediment toxicity test techniques.

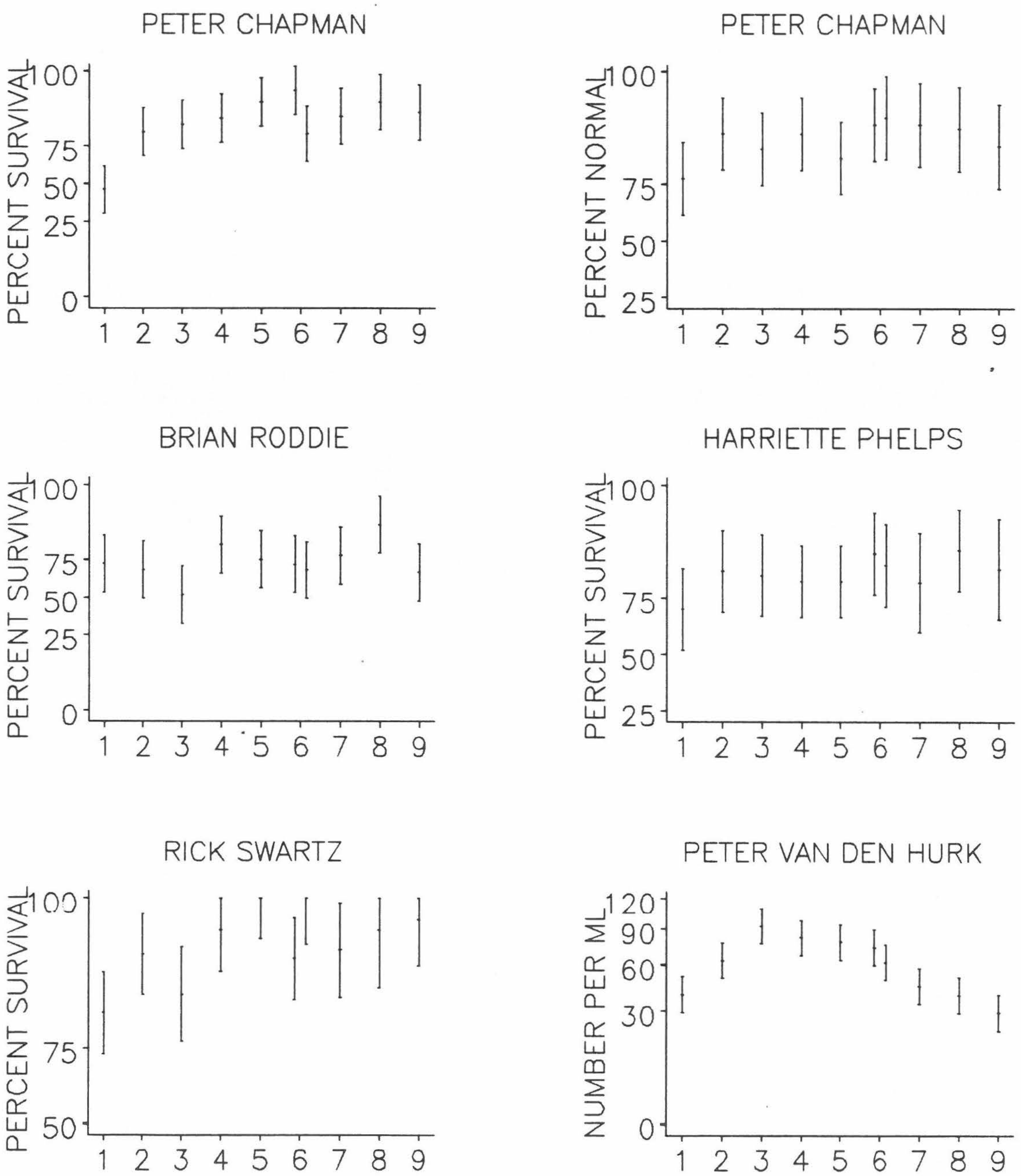
Results The results of the overall toxicity testing, indicate that sediments tested were not highly toxic and were, in fact, moderately to non toxic. Thus, techniques were not tested across as wide a range of toxicity as would have been desirable for a full intercomparison. However, the 10-d infaunal amphipod mortality and 48-h oyster larvae abnormality test proved effective during the workshop. The 24-h oyster larval test was less sensitive. The Microtox direct exposure methods shows promise, but remains to be fully developed. Some tests such as the clam reburial and oyster larval metamorphosis, which can be used rapidly and relatively inexpensively on shipboard, could be useful for initial screening purposes if a range of responses can be demonstrated. However, bivalve embryos are more sensitive than older life stages .

The tests found effective have, as end-points, acute, primarily lethal responses, Chapman has recommended that organism-level responses to pollution include measures of survival, reproduction and growth. The one test used in this study which measured growth (Neanthes) did not provide useful information; no tests used in this study measured reproduction. Swartz and co-workers (unpublished information) have found that survival alone can give a greater than 70% prediction of population response, hence the use of acute lethal tests provides a reasonable but incomplete level of environmental protection. The future development of sediment toxicity tests developed specifically for sediments should measure growth and reproduction as well as survival, and do so over a full life-cycle. Alternatively, testing could use that portion of the life-cycle which is most appropriately sensitive following initial research to determine relative sensitivity of different life-cycle stages.

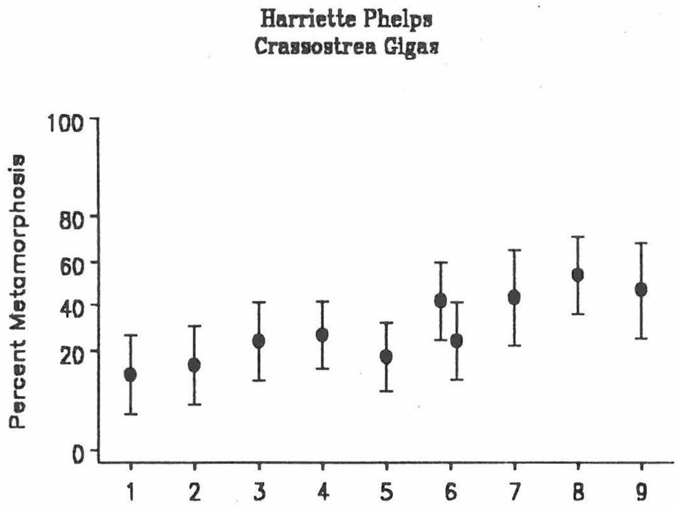
Regardless of which species are used in toxicity tests, investigators should try to account for nonpollutant effects (eg, grain size) by choosing one or more appropriate reference sediments, or by removing such effects by regression. Further, the probability of declaring a sediment significantly different from control or reference depends on the quality of the control or reference data (as well as the number of sediments tested in certain statistical comparisons). Despite statistical significance, there is an area of uncertainty when survival is less than 90% (but greater than 75% for *Rhepoxynius*), where sediments may or may not be toxic. In addition extremely precise data can result in very small and biologically inconsequential changes being found biologically significant. Accordingly, interpretation and use of sediment toxicity test data, derived under "worst case" laboratory exposure conditions, requires use of both best professional judgement and a burden of evidence approach.

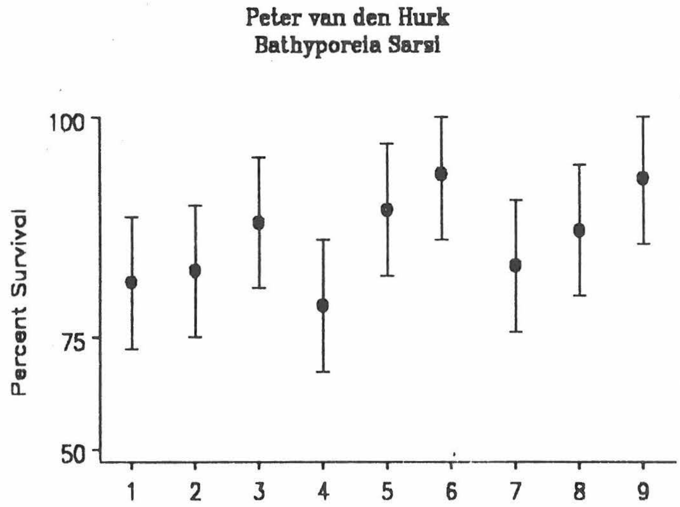
SEDIMENT BIOASSAYS
GERMAN BIGHT

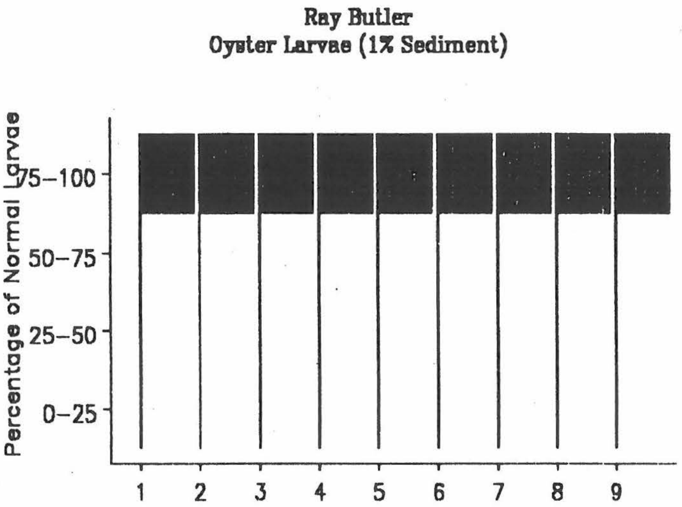
AMPHIPOD   OYSTER

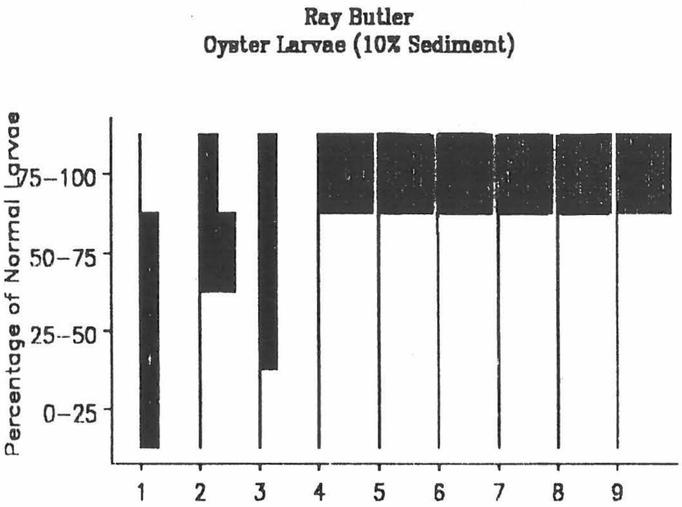


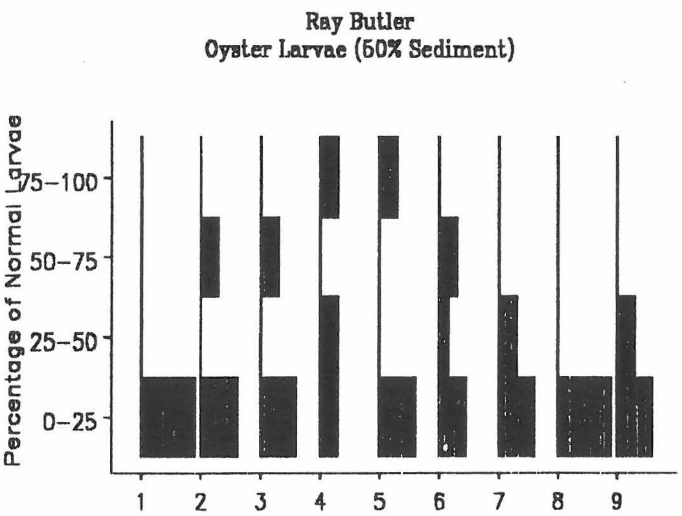
GERMAN BIGHT SEDIMENT BIOASSAY

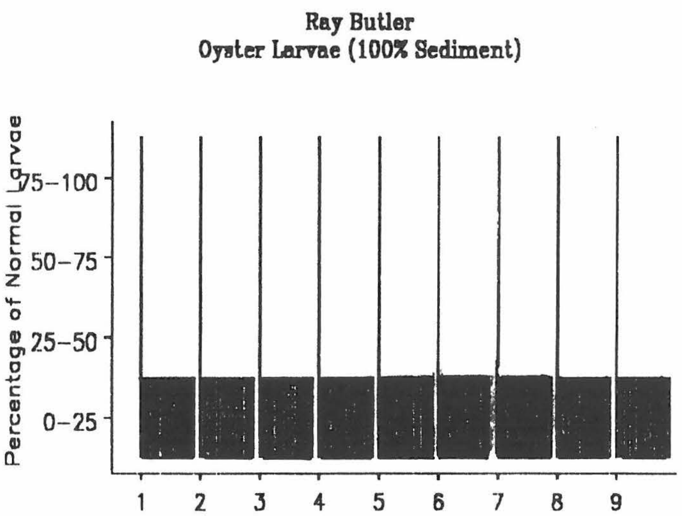








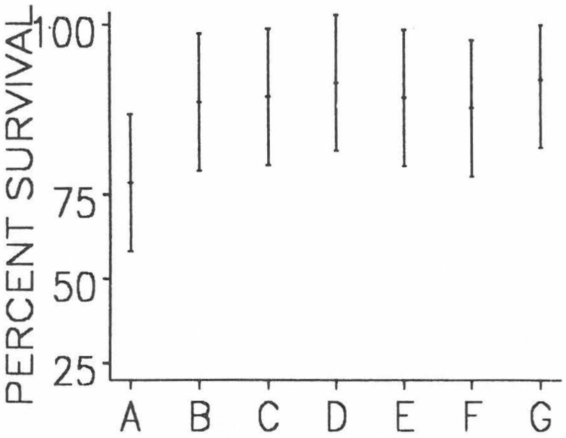




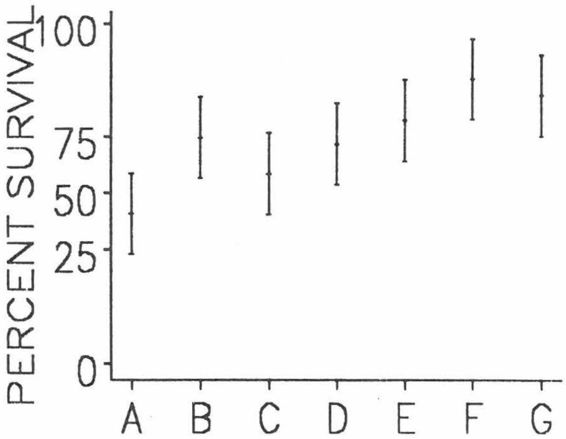
SEDIMENT BIOASSAYS
DRILLING AREA

AMPHIPOD

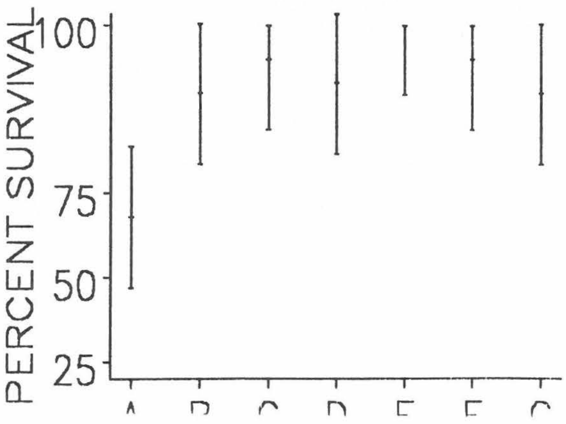
PETER CHAPMAN



BRIAN RODDIE

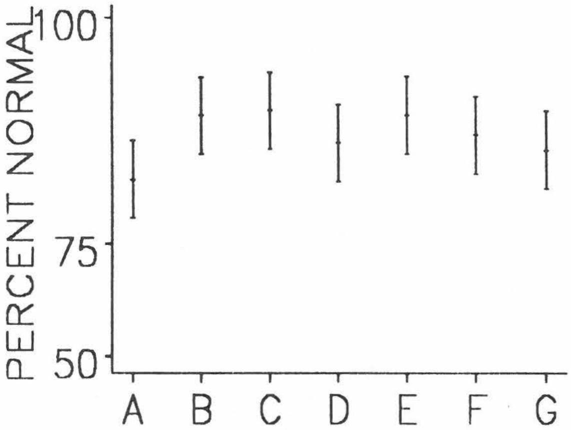


RICK SWARTZ

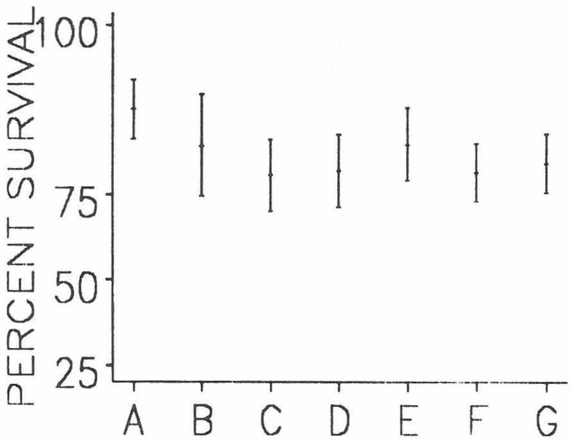


OYSTER

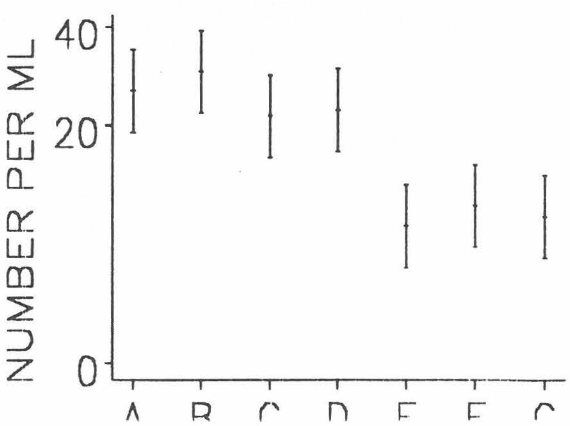
PETER CHAPMAN



HARRIETTE PHELPS



PETER VAN DEN HURK



OIL PLATFORM SEDIMENT BIOASSAY

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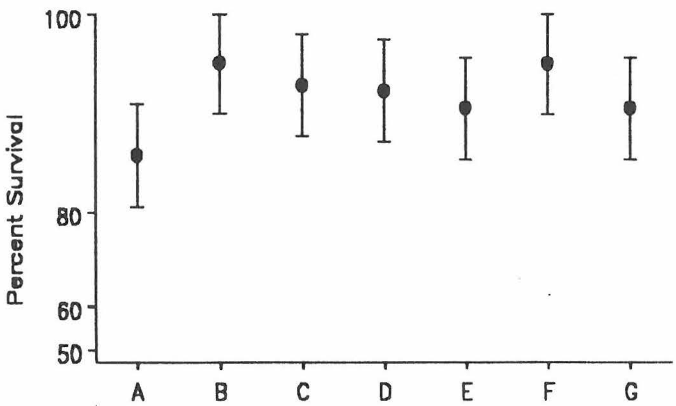
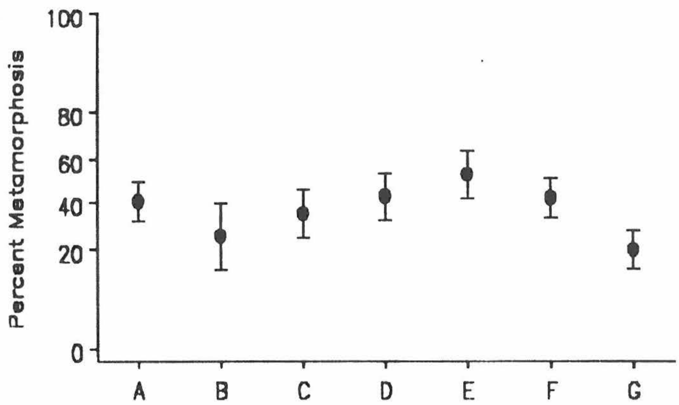
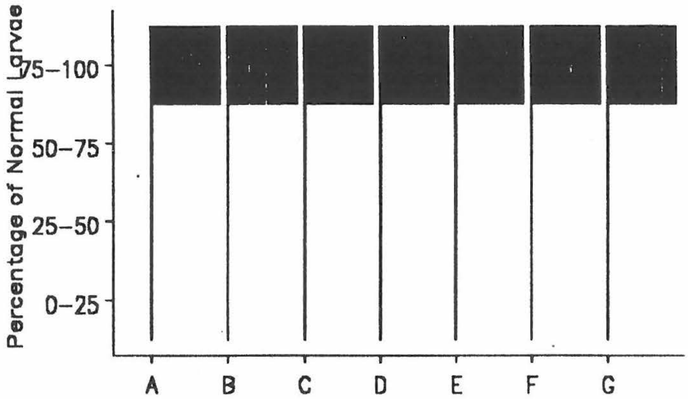
Bathyporeia Sarsi



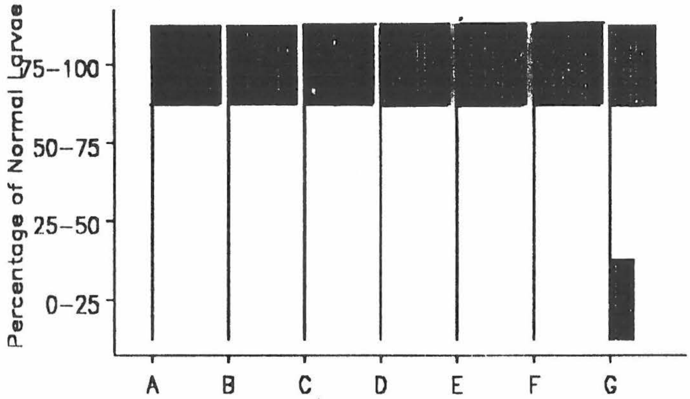
Harriette Phelps
Crassostrea Gigas



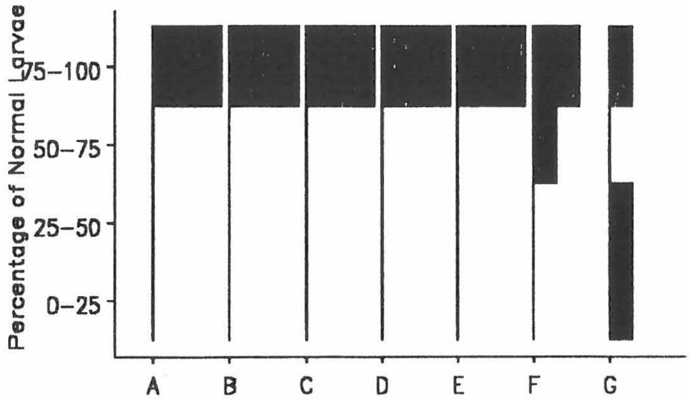
Ray Butler
Oyster Larvae (1% Sediment)



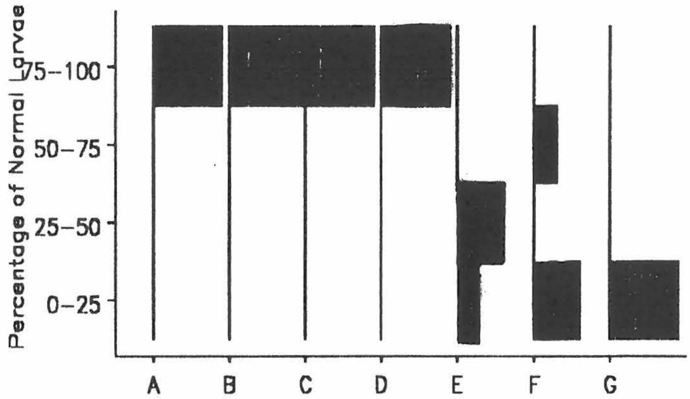
Ray Butler
Oyster Larvae (10% Sediment)



Ray Butler
Oyster Larvae (30% Sediment)



Ray Butler
Oyster Larvae (100% Sediment)



11. BENTHIC COMMUNITY STUDIES (Coordinator: C Heip)

Objectives To use the techniques currently available to detect benthic community changes along the two transects, considering the macrofauna and meiofauna separately.
(The chemical data for sediments from which benthic samples were taken has not been fully evaluated yet by all those working in this group.)

Technique At each station four box cores of sediment were taken, from each of with a sediment core of 10 cm2 surface area was collected to depth of 10 cm. Each core was divided into a top (0-2 cm) and a bottom (2-10 cm) section.

Drilling site- Meiofauna
Separate analyses were performed on the major taxa, copepod and nematode species. Univariate measures that were calculated were species richness, diversity and evenness, and total number of individuals. The significance of differences in univariate measures was tested with ANOVA , and k- dominance curves were plotted. Multivariate methods were also used including classification and multi-dimensional scaling (MDS).

None of the univariate indices showed a trend that could be related to the supposed pollution gradient for any of the meiofaunal components, although in some cases the ANOVA showed that the differences between stations were significant.

The k-dominance curves for the nematodes (and therefore total meiofauna) but not for the copepods are remarkably similar between stations. The differences for the copepods is however not statistically significant. There was no relationship between the form of the curves and the supposed pollution gradient.

The MDS ordinations show no clear discrimation for any of the faunal components. For the major taxa and total nematodes and nematodes in the deeper layers there is a tendency for the three stations nearest to the drilling site to separate from the rest. The dendrograms are very flat with all dichotomies occurring at approximately the same relatively high similarity level. Again there is no obvious discrimination between sites or clear sequencing with respect to the supposed pollution gradient.

In conclusion it can be said that it proved impossible to detect any statistically significant difference between stations in any of the meiobenthic components studied that could be related in any way to a gradient of contamination or disturbance which decreased in intensity away from the rig site. Meiobenthic community structure along the gradient and at the control site was extraordinarily uniform and we have never before observed a failure of replicate samples to cluster. The possible explanations for this could be:

a) the meiobenthos was insensitive to oil-based drilling mud, so the community structure was not affected.

b) the cessation of the drilling activities and the removal of the installations some 18 months prior to sampling provided sufficient time for rapid and complete recovery of the meiofauna.

c) the occurrence of two extremely strong gales in January and February 1990 deposited large quantities of sediments and disrupted and homogenized meiofauna community structure over large areas. The REMOTS-pictures taken by Rumohr and Schomann clearly show fresh deposition of several centimenters of sandy sediment on top of the (probably) old contaminated sediment.

Drilling site-Macrofauna

The univariate measures number of species, biomass and diversity tend to show a minimum value at the site of the oil rig, but the difference is generally not statistically significant, in contrast with earlier observations of Daan (see Section 3). Only in the case of total macrofauna density is there a significant increase away from away from the rig site along the transect to the WNW. Probably most significant is the continued absence of deep burrowing species such as Calliannassa subterranea, Echinocardium cordatum and the polychaete Notomastus latericeus. In three species (Amphiura filiformis, Mysella bidentata and Corbula gibba) length frequency distributions compared along the gradient did not show any difference; it therefore does not appear that some recruitment has taken place close to the drilling site recently in these species.

Classification of the stations resulted in a cluster of the three outermost stations (2,5 and 15 km) being separated from the others. Also the oil rig site itself tends to cluster out. The replicates are frequently clustered in the MDS ordination. It thus appears that community structure in the macrofauna is affected by the contamination gradient along the drilling site transect.

German Bight Transect Meiofauna (copepods only)

The stations along the German Bight Transect can be grouped into three distinct groups. The innermost stations 1-3 are characterized by the species Longipedia helgolandica and several Ectinosomatidae. Abundance and diversity are very low. The stations 5-8 are characterized by the presence of Longipedia coronata, several other Ectinosomatidae and Pseudameira crassicornis. Stations 5-6 have the highest abundance and diversity and are almost similar to the drilling site stations. At stations 7-8 some species from 5-6 are absent and abundance and diversity are lower. Station 4 is intermediate between both groups. The station 9 on the Dogger Bank is entirely different from the others and is characterized by the species Rhizothrix minuta and members of the Paramesochridae and Cylindropsillidae. This is a clear indication that the changes in sediment grain size are the main factors responsible for the change in the fauna. Meiofauna density in general is much lower in stations 1-2 than in the other stations. The number of higher taxa is also much lower here and tends to increase along the transect. The Dogger Bank station is characterized by the large abundance of Gastrotrichs.

German Bight-Macrofauna

Along the transect abundance and species number showed an increase towards the outer stations. Three community types are found:

a) the Nucula nitidosa community in the inner German Bight on mud (stations 1-3)
b) the Amphiura filiformis community in the fossil valley of the Elbe on muddy sands (stations 4-8)
c) a transitional Amphiura filiformis-Tellina fabula community at the edge of the Dogger Bank on fine sands with a low mud content.

The ABC-curves show stressed communities with a shift towards higher numbers of short lived species with low biomass.

The advantages of Benthic Community Analysis for monitoring are the detection of change at a stage at which it matters, in that individuals have been removed from the population. However, it is by then too late for any preventative action and there is little indication of the likely causes. Natural biotic and physico-chemical factors besides those related to contamination. While the benthos will provide little information on causes of community change, their sedentary habits ensures they reflect environmental conditions where they were sampled.

Conclusions Both meio- and macrofauna data show differences between stations along the German Bight Transect, with a group of coastal stations (1-3) and the Dogger Bank (9) clearly delineated from the other stations. These differences are correlated with differences in sediment composition.

The response of the benthic fauna along the Drilling Site transect was interesting. The meiofauna did not show trends along the gradient, agreeing with the absence of trends in the superficial sediments and the measured contaminants in them. The fauna is remarkably uniform over a large area, reflecting well mixed sediment deposited by the passage of two storms. Not only did they import new superficial sediments, but the storms destroyed the typical small-scale patchiness found in benthic communities due to biological processes. The longer-lived and more deeply burrowing macrofaunal species were less affected by the storms, and their community structure reflected the contamination gradient that still exists in the underlying sediments. While the samples for sediment chemistry were taken from the superficial sediments, the Remots images and the tissue burdens of hydrocarbons in burrowing forms (Aphrodite) indicate that the underlying contamination still exists 18 months after drilling ceased.

General Conclusions

1. Benthic fauna is a good tool for describing changes in space (with application in point source pollution monitoring) and time (with application in describing long-term changes in marine systems). A whole battery of simple to very sophisticated statistical techniques is available to describe and compare the structure of benthic communities.

2. The use benthic communities in monitoring is based on the size distribution and the abundance of the species present in a certain delimited environment. To increase predictive power, the causal mechanisms leading to changes in size and abundance should therefore be better understood at several levels of organisation.

3. Basic information on genetics, biochemistry and physiology is lacking for most benthic species. Data on energy flow (food uptake, growth and reproduction, respiration and excretion) and life-cycle strategies, as determined by natural selection, do not exist for many macrofauna species and for nearly all meiofauna.

4. The combination of benthic community data with sediment chemistry and sediment bioassays in the triad approach is thought to be promising and requires further study.

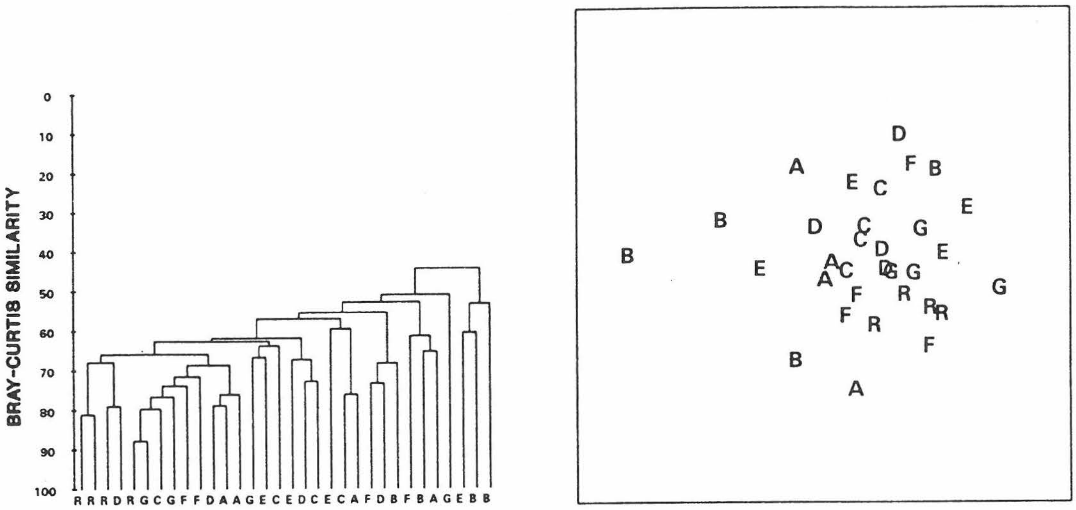
5. The methodology of sampling and sorting of samples should be improved. Sampling should be based on box-corers (macrofauna) or multiple corers (meiofauna) or taken by ROVs and the additional information on the sedimentary environment should be as complete as possible. For this the use of imaging methods such as Video, Stills Photography, REMOTS and side-scan sonar should become standard practice.

6. More effort should be put into the automatization of sorting and speeding up of taxonomic analyses, eg by introducing quick sorting of macrofauna on coarse sieves for preliminary assessments.

7. Analyses of the data should be based on a broad variety of univariate and multivariate measures and no method alone should be used to provide answers (eg. k-dominance curves or ABC-method alone).

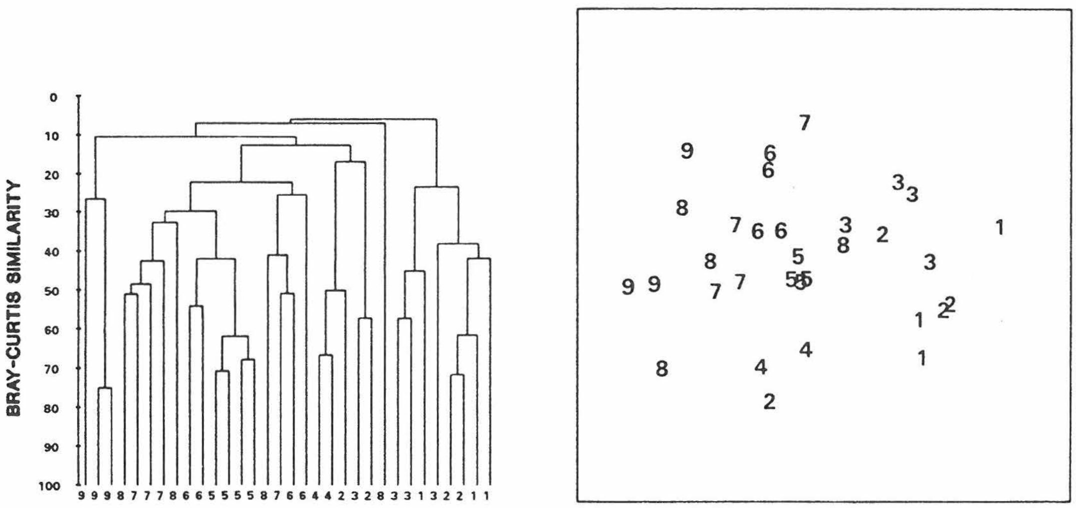
M. Gee

Oil Platform Copepod Abundance

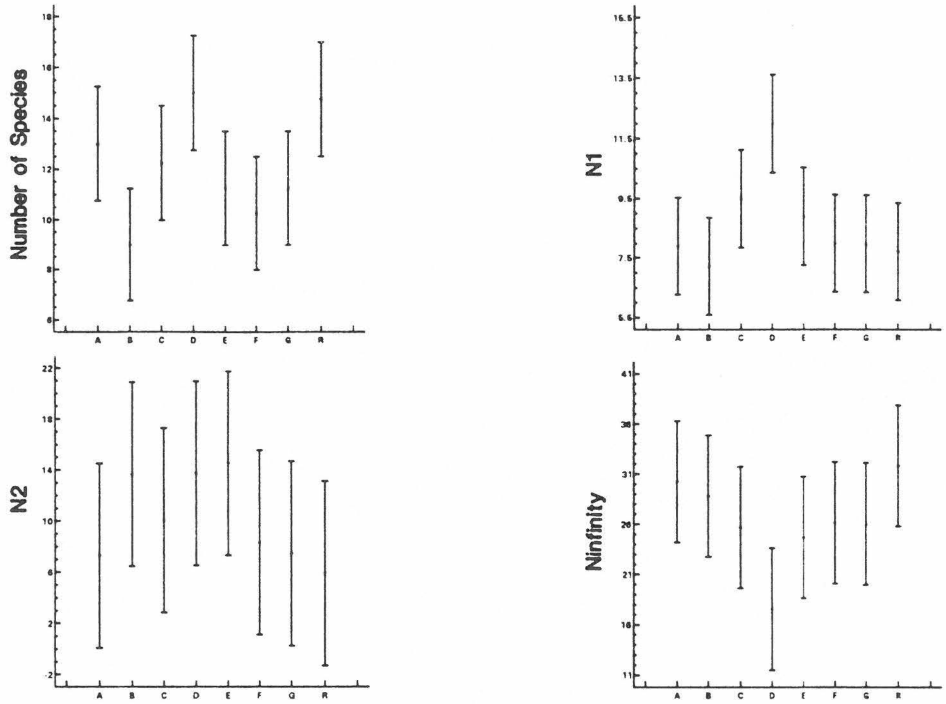


M. Gee

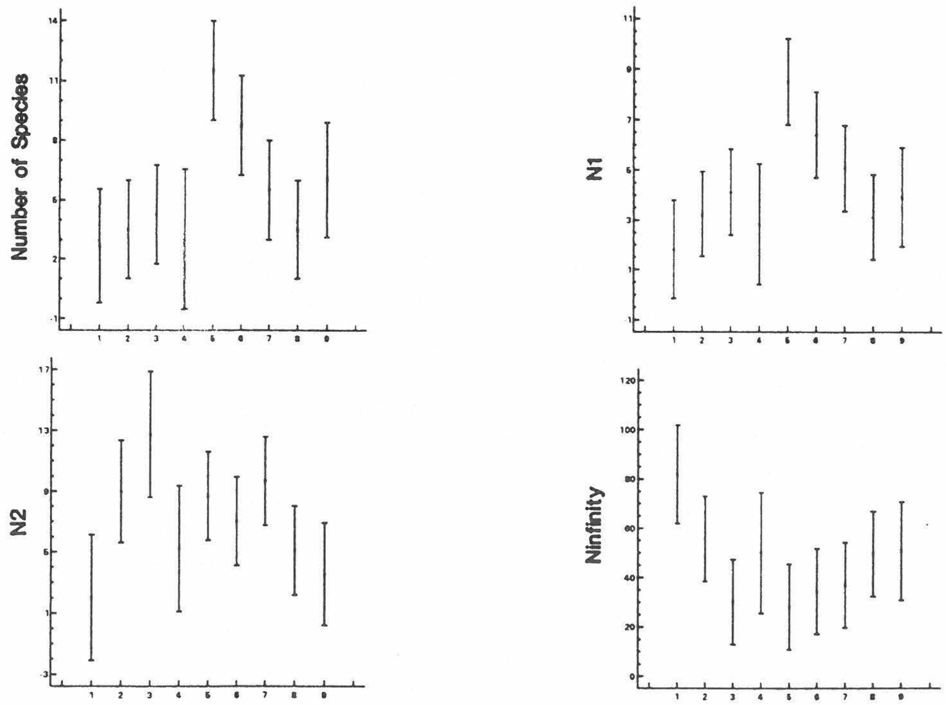
German Bight Copepod Abundance



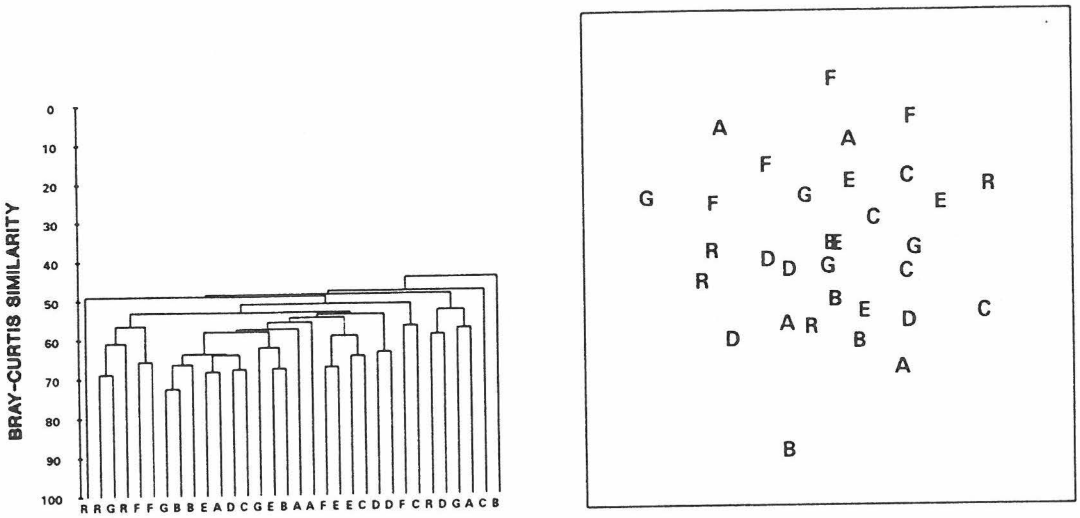
M. Gee
Oil Platform Copepods



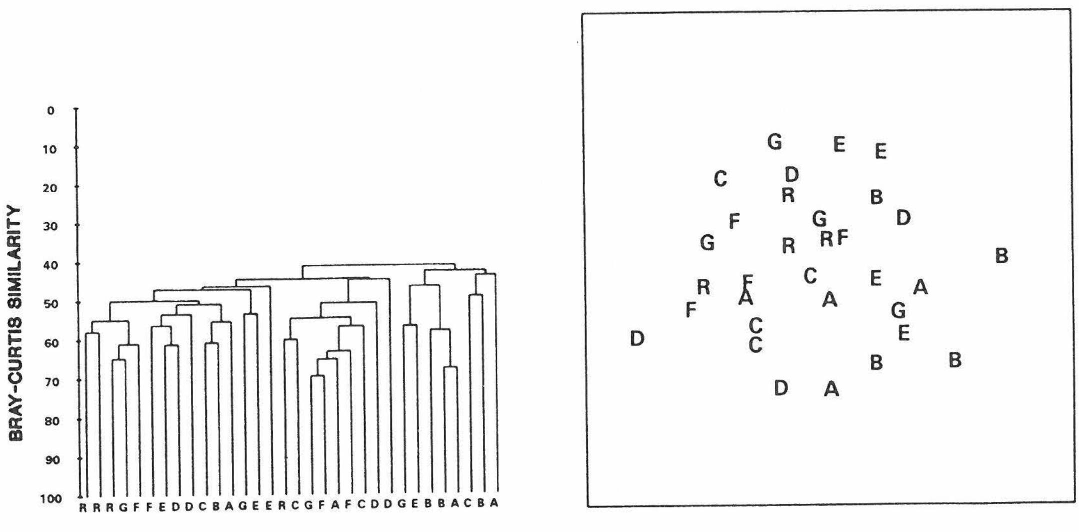
M. Gee
German Bight Copepods



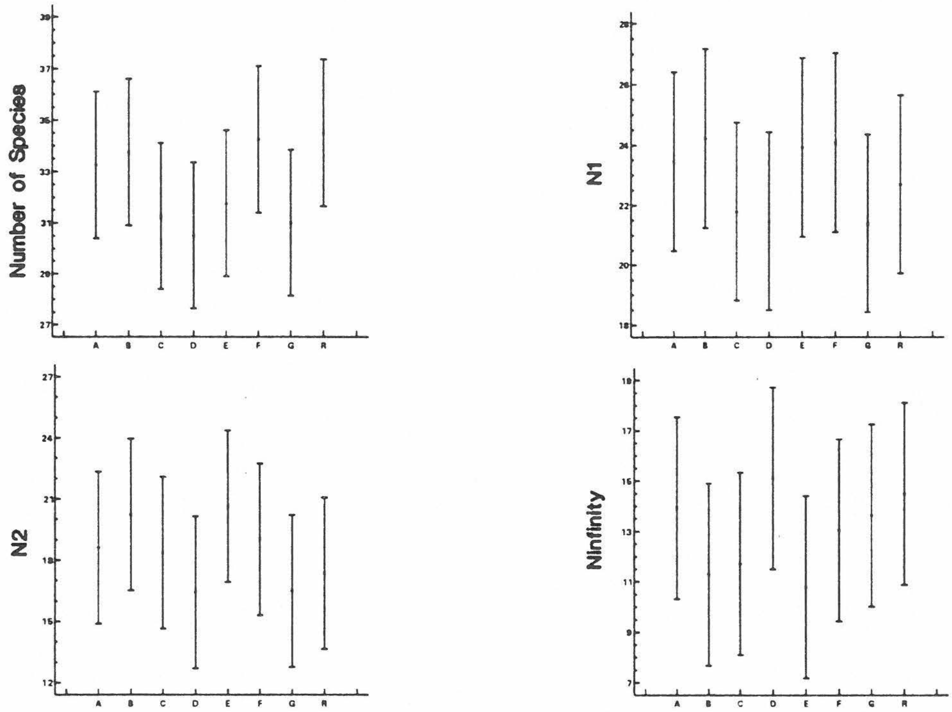
Warwick et al
Oil Platform Nematode Abundance (0-2 cm)



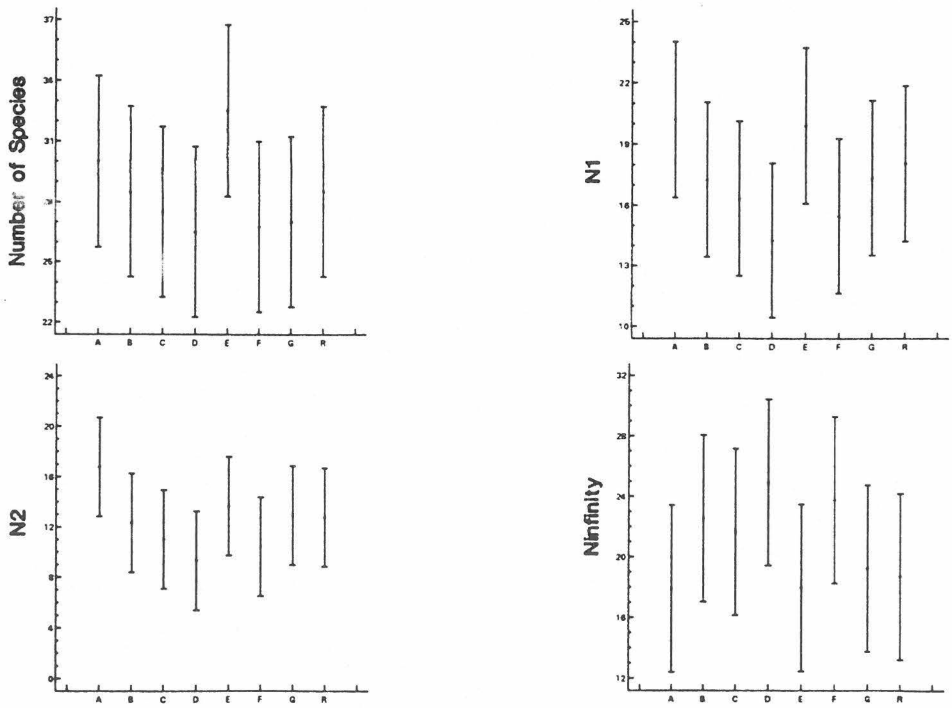
Warwick et al
Oil Platform Nematode Abundance (2-10 cm)



Warwick et al
Oil Platform Nematodes (0-2 cm)

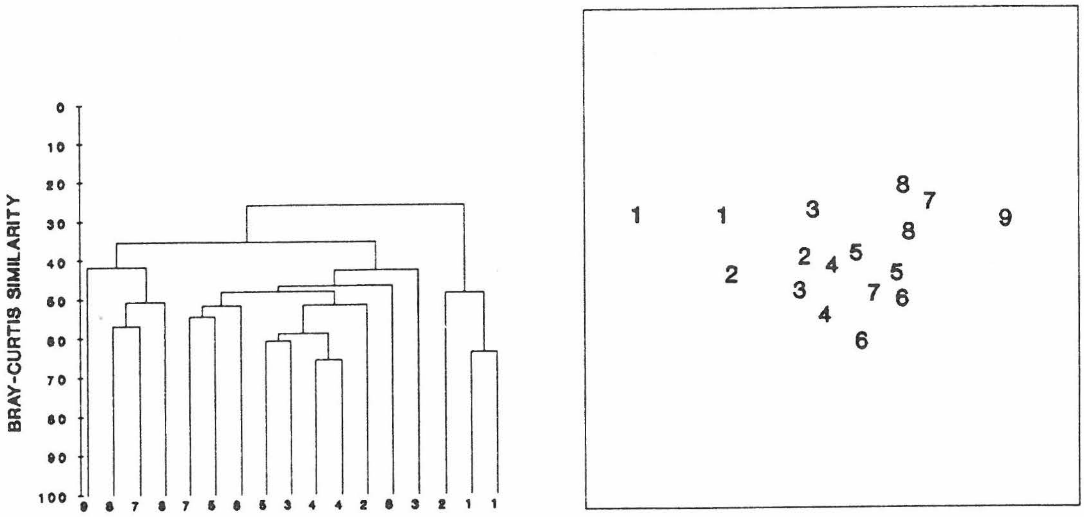


Warwick et al
Oil Platform Nematodes (2-10 cm)



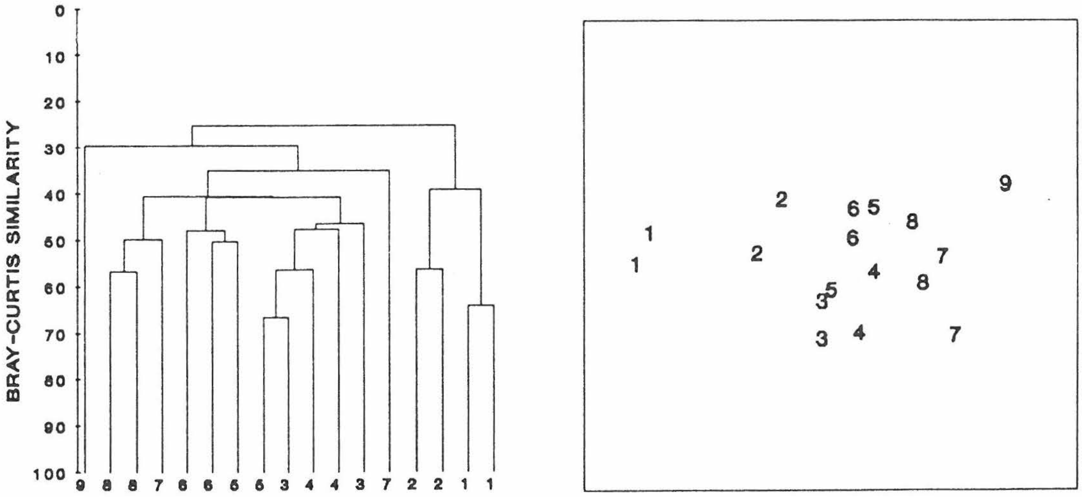
M. Vincx

German Bight Nematode Abundance (0-2 cm)



M. Vincx

German Bight Nematode Abundance (2-10 cm)



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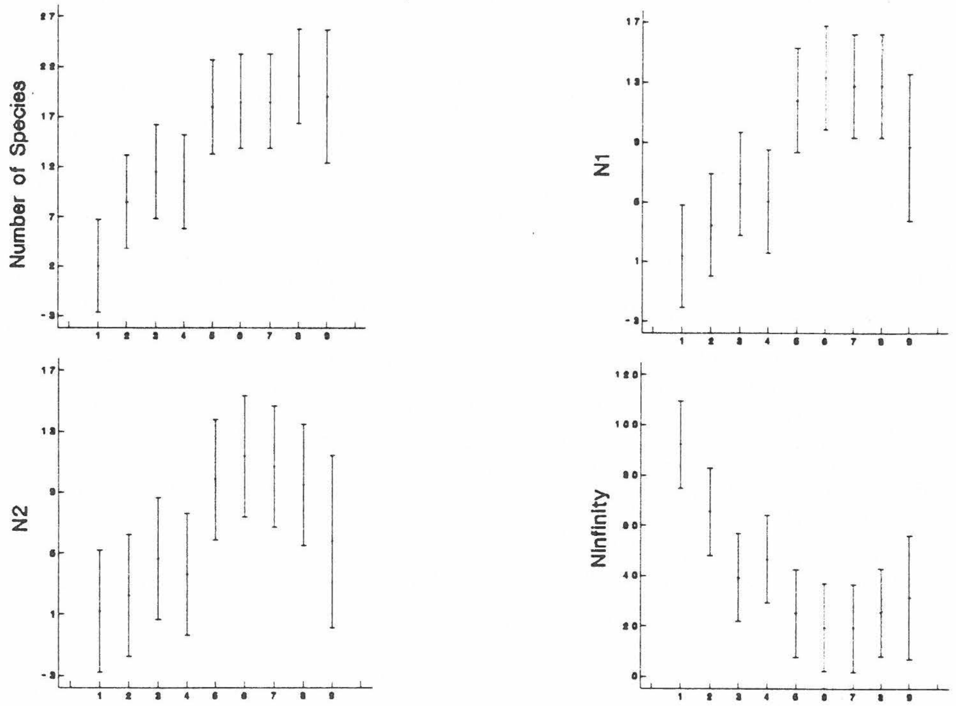
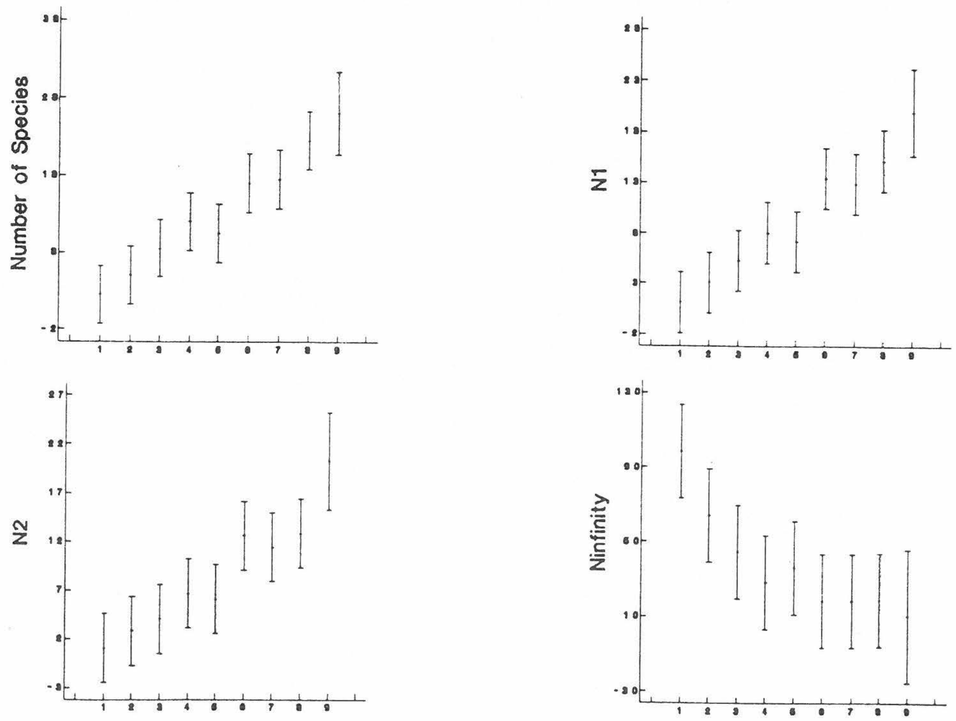
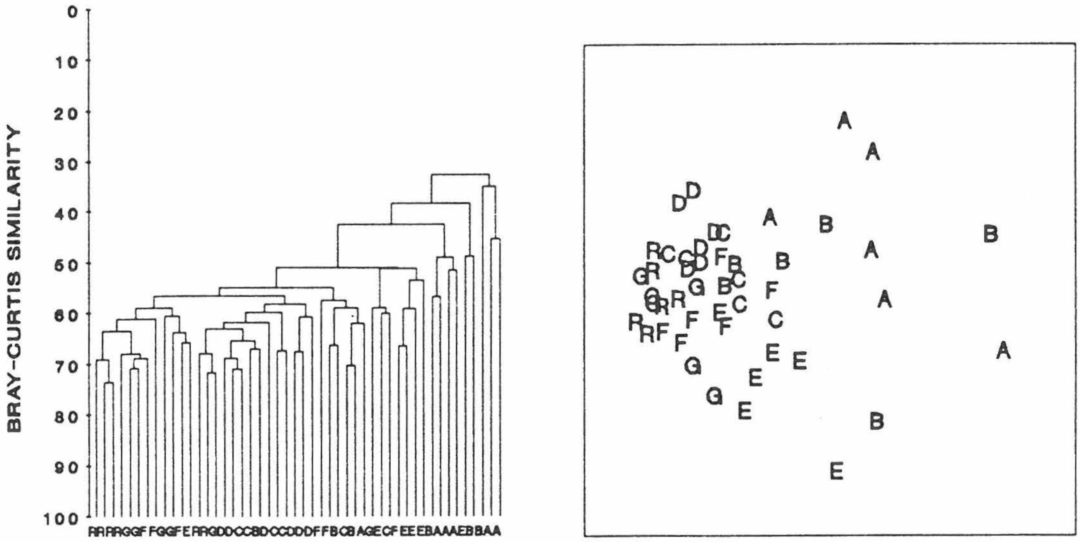
German Bight Nematodes (0-2 cm)



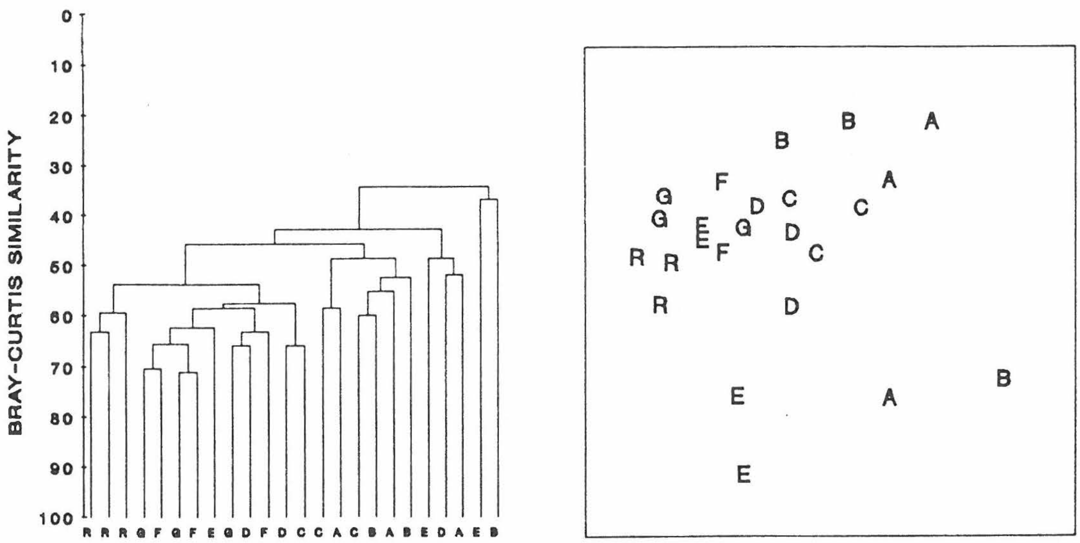
M. Vincx
German Bight Nematodes (2-10 cm)



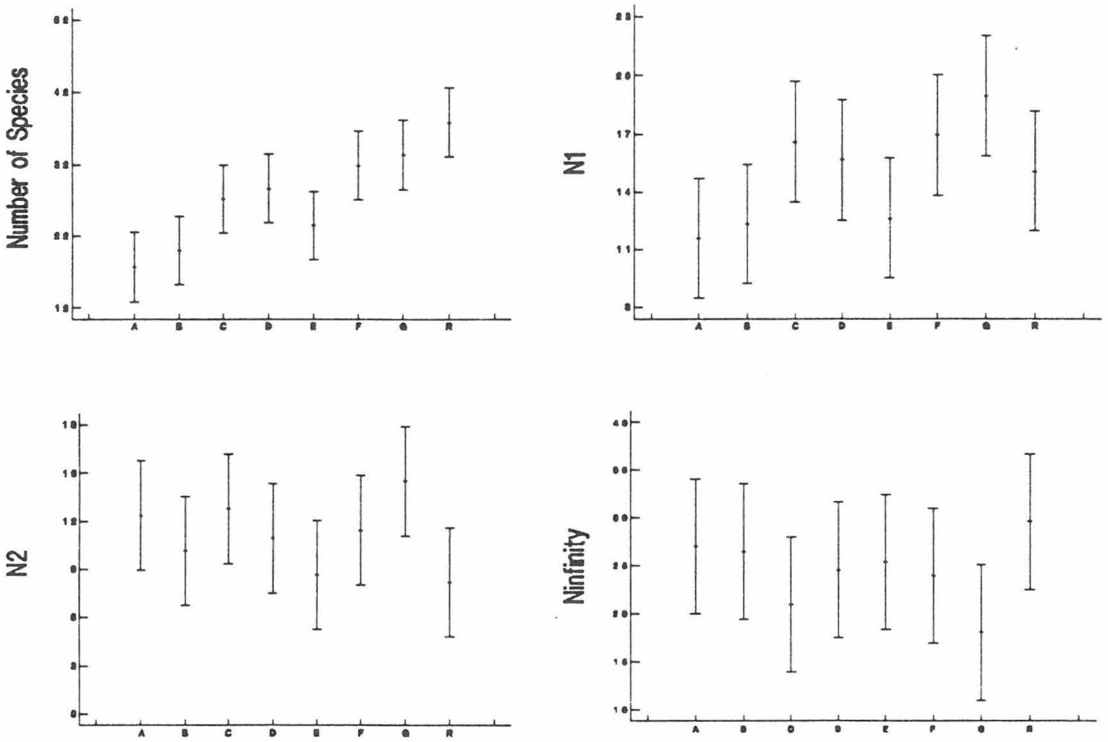
Kroncke,Raak and Duineveld
Oil Platform Macrofauna Abundance



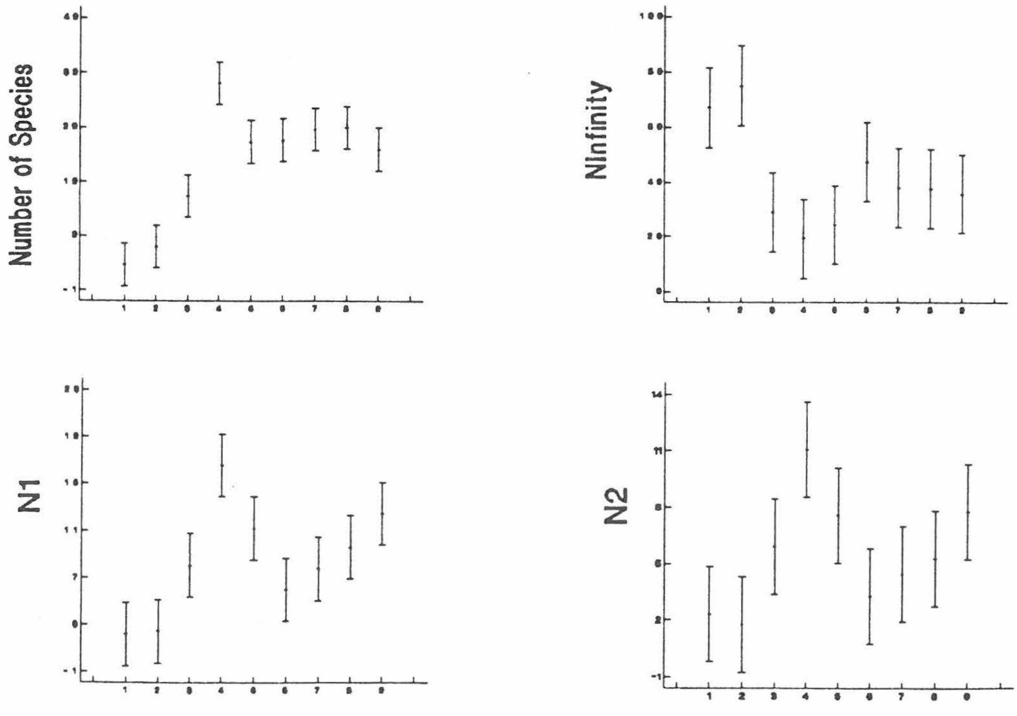
Kroncke,Raak and Duineveld
Oil Platform Macrofauna Biomass



Kroncke,Raak and Duineveld
Oil Platform Macrofauna



Kronke and Rachor
German Bight Macrofauna



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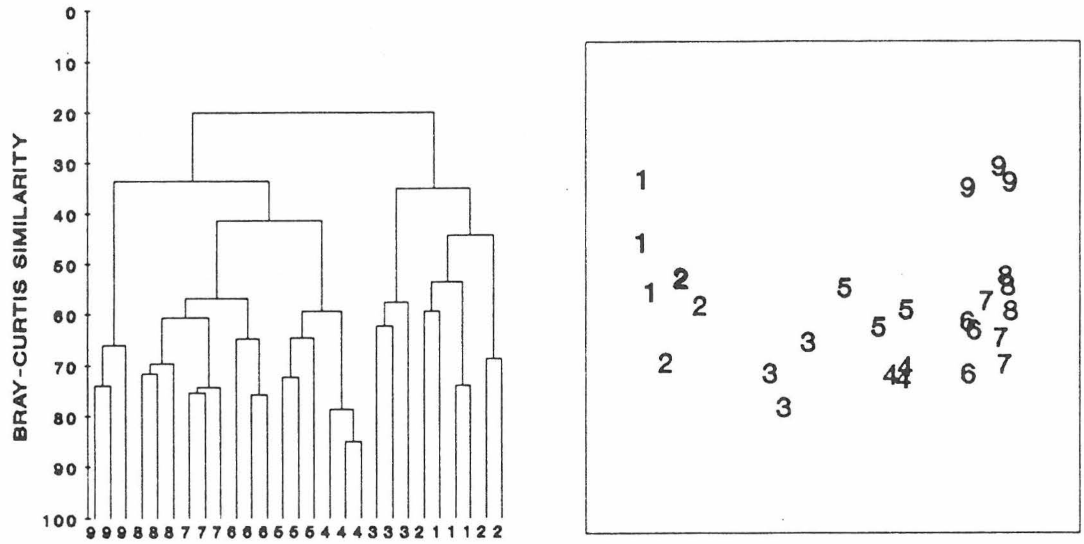
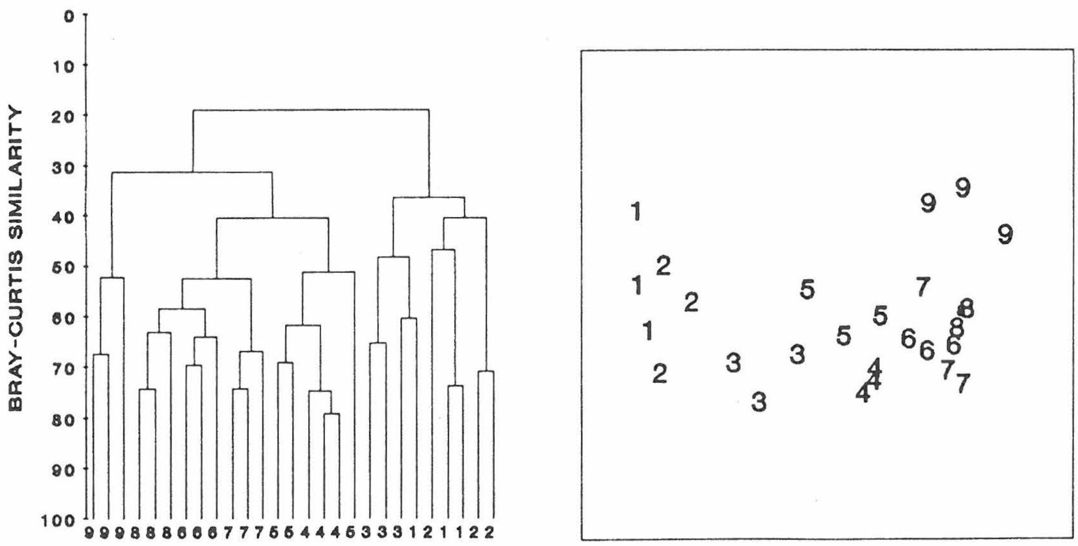
German Bight Macrofauna Abundance



Kroncke and Rachor
German Bight Macrofauna Biomass



12. SEDIMENT QUALITY TRIAD (Coordinator: P. Chapman)

Sediment methods focused on toxicity testing, chemical analysis and measures of *in situ* benthic community structure. Together these methods copmrise the Sediment Quality Triad. Using the Triad in a burden of evidence approach, sediments nearest the Elbe are moderately polluted, with a decreasing pollution gradient leading to unpolluted sediments on the Dogger Bank. Sediments nearest the Drilling Site show relatively low level toxicity and benthic community alteration. Specific, limited chemical measurements of the superficial sediments did not indicate contamination; laboratory toxicity and alterations in the benthic macrofaunal (but not the meiofaunal) community structure may be due to contamination of the underlying sediments. It appears that the process of "natural capping" of the drilling site after 18 months was due to storms preceding the workshop, and differences in the various responses indicates that, while capped, contamination of the underlying sediments by oil-based drilling muds persists. The range of techniques deployed on both transects provides an interpretation of events, as well as an integrated measure essential for assessing the status of the sediments for managers and regulators.

13. QUESTIONS REQUIRING FURTHER RESEARCH - Coordinators.

Cholinesterase inhibition AChE inhibition is usually believed to indicate exposure to organophosphates, which are assumed to be too labile to survive for long in the sea. The levels of inhibition detected offshore indicate that either organophosphates are more stable than has been supposed or that some other agent(s) also have this effect.

Metallothionein distribution Why does the apparent MT and tissue metal distribution vary inversely with that of the dominant trend of decreasing concentrations of contaminants with distance offshore?

Sequence of disease induction There is a cascade of detectable responses and effects, that initially and at the lowest level indicate exposure. These are succeeded by symptoms of the capacity of adaptive systems being exceeded, and the onset of deleterious effects, leading to overt disease at the organismal level. While all the causal links in this hypothetical sequence remain to be understood, it is a necessary research objective to for interpreting biochemical and cellular indices and the organismal and population levels .

Experimental disease induction The most important requirement to demonstrate this sequence of events and the levels of contamination capable of causing disease are laboratory experiments rather than epidemiology.

Chemistry - There are data from the German Bight transect that raise important questions about fluxes and the importance of contaminants in the organic-rich sediment fines in relation to their biological significance, particularly at station 9.

Bioassays and biomarkers There is scope for the integration of biomarkers with the bioassay approach, to link the ease of use of bioassays with some capacity to identify the causes of responses.

Dogger Bank Previous work suggests that contaminants bound to the organic rich fine fraction are deposited on the sea bed in the central North Sea. Workshop data to some extent corroborate such findings, showing elevated concentrations of some contaminants and clear effects in dab at station 9, but more research is needed on the form and flux of toxic contaminants.

14. INTERIM CONCLUSIONS - Coordinators.

a. **Hydrography** - The German Bight transect traversed two water masses, the Continental Coastal Water and the Central North Sea Water; the frontal region that separates them moved offshore during the workshop from Station 4 to 5. This is most likely to be of relevence to those bioassays exposed to water column samples.

b. **Drilling Site** - while there was a clear gradient of contamination by oil-based drilling mud and biological effect on the benthic community in June 1988, Remots images indicate that in the months preceding the workshop the storms deposited about 2 cm of clean coarse sand over the whole area. This event changed the whole character of the site for the purposes of the workshop. Epifaunal and shallow infauna were not exposed to a contamination gradient, while more deeply burried macrofauna were.

c. **Dab migration** - the movement of dab over significant distances is demonstrated using data of different kinds and from various sources. These finding indicate that particular care is required in interpreting epidemiological data for responses, and the acquisition of tissue burdens, which both take some time to develop.

d. **Chemistry** - the data demonstrate some clear gradients on both transects that reflect the expected contamination gradients. Some data may be causally linked to observed effects and will provide important information for the interpretation of biological data, as well as the flux of contaminants in one of the most polluted regions of the North Sea.

The workshop demonstrated clearly the need for collaboration between chemists and biologists from the earliest planning stages, as well as the other interdisciplinary links, since the question of exposure of organisms to toxic contaminants in the environment sits at the interface between the disciplines represented by different Working Groups withion ICES and IOC.

e. **Biochemistry** - MFO activity in dab tended to be fairly consistent between samples and among the various indices of activity. Fish from inshore German Bight stations showed the highest MFO activity and offshore stations the lowest activity. Similarly cholinesterase inhibition was greatest at the inshore stations and least offshore. For both MFO activity and cholinesterase inhibition the site least affected appeared to be Station 8, while the outermost station showed some increase in contamination. Metallothionein measurements showed an opposite trend with activity increasing offshore, positively correlated with liver concentrations of copper and zinc.

f. **Molecular and cellular pathology** - the results demonstrate degenerative changes in liver cells and embryos of the dab from the most contaminated sites. These are most clear in the indices of lysosomal damage, together with higher order lesions such as fatty change and preneoplastic foci are **biomarkers of liver degeneration** in the fish without overt expression of disease used. Increased endoplasmic reticulum, its associated EROD activity and activation of pro-mutagens, can be considered as **biomarkers of exposure** to contaminants, as indicated by the liver PCB data. The most appropriate use of such techniques is as a suite of molecular and cellular biomarkers of contaminant exposure and pathological effects.

g. **Gross pathology and histopathology** The most promising **external disease** for monitoring appears to be epidermal hyperplasia/ papilloma, and the most promising **internal disease** is preneoplastic and neoplastic liver lesions. While the workshop will contribute to the database of correlations between disease and contamination, they can not be expected to demonstrate a cause and effect relationship.

h. **Water quality bioassays** Bioassays demonstrated effects in the sea surface microlayer and nearshore subsurface water samples that apparently relate to the two water masses that the German Bight traversed. Bioassay effects are most marked at interfaces (the sea surface and sea bottom) where contaminants accumulate. Trials during the workshop indicate that cryopreservation techniques will soon be able to provide inherently seasonal material for bioassays all the year round in a readily usable form. Bioassay experiments with environmental samples should incorporate positive as well as negative controls.

i. **Sediment bioassays** The sediments from the German Bight and Drilling Site transects were not adequately toxic to provide an good test of the bioassay techniques, and it would therefore have been instructive to have included positive and negative control samples. Nevertheless on the basis of the workshop data the 10 day infaunal abnormality test and the 48 h oyster embryo abnormality test were effective.

j. **Benthic community analysis** On the Drilling Site transect the meiofauna inhabiting the newly deposited sediments showed no gradient of effect, but the more deeply burrowing macrofauna indicated that significant contamination remains at the site after 18 months. Clear trends in macro and meiofauna along the German Bight transect appear to be most closely related to the changes in sediment granulometry, but the chemical data have not been interpreted yet.

k. **Benthic sediment quality Triad.** An integrated approach to sediment quality, utilising toxicity, chemistry and benthic community analysis provides the kind of comprehensive assessment needed by regulators for management decisions.

15. ACKNOWLEDGEMENTS

Although this Interim Report is inevitably an incomplete account of the workshop results, it is nevertheless the most complete that will precede the Special Volume of Marine Ecology Progress Series dedicated to the workshop. There a full list of sponsors and acknowledgements will be given, but we would like to thank here our major sponsors. The Intergovernmental Oceanographic Commission of Unesco not only supported the workshop but also provided funding for participants to travel to the Concluding Meeting in Copenhagen recently. The International Council for the Exploration of the Sea have supported the workshop since its inception and organised the Concluding Meeting. Substantial funding has also been provided for the workshop and the employment of chemists by the UK Department of the Environment.

ARD Stebbing and V Dethlefsen
23 September 1991